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Insights in clinical nutrition

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Table of contents

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Benefits and Safety of Astaxanthin in the Treatment of Mild-To-Moderate Dry Eye Disease

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Objectives: To evaluate the effect of astaxanthin in the treatment of mild-to-moderate dry eye disease (DED) in middle-aged and elderly patients.

Methods: 120 eyes of 60 middle-aged and elderly patients with mild-to-moderate DED were enrolled in this prospective, one-group, quasi-experimental study. Six milligram Astaxanthin tablets (Weihong Haematococcus Pluvialis Astaxanthin, Hangzhou Xinwei Low Carbon Technology R&D Co., Ltd., China) were administered orally, twice daily for 30 ± 2 days. History of eye diseases, treatment, systemic disease, and medication before the test were recorded. In addition, the ocular surface disease index (OSDI) questionnaire, non-invasive tear break-up time (NIBUT), fluorescein break-up time (FBUT), corneal fluorescein staining (CFS) score, eyelid margin signs, meibomian gland (MG) expressibility, meibum quality, meibomian gland dropout (MGDR), Schirmer I test (SIt), tear meniscus height (TMH), bulbar conjunctiva congestion degree, blink frequency, incomplete blink rate, and thickness of tear film lipid layer were collected before treatment, 2 weeks after the initiation of treatment, and at the end of treatment. Visual acuity (VA), intraocular pressure (IOP), anterior segment, fundus, discomfort symptoms and other adverse reactions were also monitored throughout the study to assess the safety.

Results: OSDI score, NIBUT, BUT, CFS score, eyelid margin signs, MG expressibility, meibum quality, and blink frequency improved significantly to varying degrees after treatment compared with those before the treatment ($P < 0.05$), while TMH, SIt, conjunctival congestion, the thickness of tear film lipid layer, MGDR, incomplete blink rate, VA and IOP did not differ ($P > 0.05$).

Conclusions: Oral administration of astaxanthin improves the symptoms and signs of middle-aged and elderly patients with mild-to-moderate DED.

Keywords: astaxanthin, dry eye disease, ocular surface, antioxidant, oxidative stress

INTRODUCTION

According to the TFOS DEWS II published in 2017, dry eye disease (DED) is a multifactorial disease of the ocular surface characterized by the loss of homeostasis of the tear film and ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles (1). DED can cause a variety of ocular symptoms, such as dryness, burning, and blurred vision. If not treated properly, it causes ocular surface damage, leading to corneal complications and permanent vision loss (2). With the improvement in people's life and accelerated population aging, the prevalence of DED increases gradually. Currently, the global prevalence of DED ranges from 5 to 50% (3), and it is about 21–58% in China (4, 5). As a common ocular disease, DED affects patients' health and quality of life, and has even been associated with increased prevalence of depression (6).

DED is a disease caused by many factors such as environment, diet, trauma, drugs, local inflammation, living habits, and changes in body hormone levels (7). Among them meibomian gland dysfunction (MGD) is one of the most common causes of DED, resulting in lipid abnormalities of tear and evaporative DED (3). In middle-aged and older people (>40-years-old), the prevalence of MGD can reach 38–68% (3). This is probably due to the increase in oxidative stress with advanced age may lead to the loss of function of ocular surface glands, such as lacrimal glands and meibomian glands, resulting in reduced tear secretion and MGD (8, 9). Decreased tear secretion can lead to aqueous-deficient DED and MGD can lead to evaporative DED (1). The reduced secretion and increased evaporation cause hyperosmosis of tears, which increases the generation of reactive oxygen species (ROS) in corneal epithelial cells, disrupts the balance of oxygenase and antioxidants, and eventually induces oxidative stress (10). ROS cause membrane lipid peroxidation and mitochondrial DNA damage (11). These phenomena lead to mitochondrial dysfunction and increase cell damage in conjunctival epithelial tissue, lacrimal glands, meibomian gland, and other tissues secreting tears, thereby aggravating DED (12). Furthermore, oxidative stress can activate multiple inflammatory pathways and lead to ocular surface inflammation (10, 13). The concentration of inflammatory cytokines in tears of patients with DED is higher than that in healthy individuals and positively correlated with the severity of symptoms and signs of DED (14, 15). Oxidative stress and ocular surface inflammation complement each other in the pathogenesis of DED, suggesting that the oxidative stress pathway may be a new target for DED treatment. Presently, the mechanism of oxidative stress and related therapies of DED has gained increasing attention. Previous studies have shown that the human meibomian gland and conjunctival tissue contain a natural antioxidant system that resists the oxidation of secreted lipids and proteins (16). The imbalance of the local antioxidant system alters the lipid and mucin layers of the tear film, resulting in excessive evaporation and instability of the tear film and eventually DED (13). Some clinical studies have shown that the oral administration of compound preparations containing antioxidants, such as anthocyanin, astaxanthin, and vitamins A, C, and E, can reduce the concentration of ROS in tears, thereby increasing the production of tears and improving the stability of tear film (17, 18).

Astaxanthin is a naturally occurring carotenoid and the highest grade product of carotenoid synthesis that is recognized as one of the strongest antioxidants in nature (19). A series of *in vitro* and *in vivo* cell and animal experiments have demonstrated that astaxanthin increases local antioxidants, inactivates and scavenges oxygen free radicals, and inhibits the rise in age-related oxidative stress markers, such as p53, p21, and p16 (20, 21). Moreover, it downregulates the inflammatory factors, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) (22–24), and its antioxidant and anti-inflammatory effects are dose-dependent (25). These findings suggest that astaxanthin can reduce oxidative stress damage and inhibit the inflammatory response. In an *in vitro* study, astaxanthin was shown to inhibit the increase in the age-related oxidative stress markers in corneal epithelial cells (20) and the ocular surface inflammation by downregulating the expression of high mobility group box 1 protein (HMGB1) and inflammatory cytokines (TNF-α and IL-1β) (26), which has a potential therapeutic effect on DED. However, its clinical effect on DED is yet to be investigated. The present study aims to investigate the efficacy of astaxanthin in the treatment of mild-to-moderate DED through a single-group pretest-posttest study.

MATERIALS AND METHODS

Study Design, Patients, and Ethics Approval

A prospective, single-center, one-group, pretest-posttest quasi-experimental study was conducted in 60 middle-aged and elderly patients with mild-to-moderate DED admitted to the Ophthalmology Department of Beijing Tongren Hospital, Capital Medical University from March 2021 to May 2021. A total of 120 eyes were assessed, including 18 eyes of 9 males and 102 eyes of 51 females with an average age of 60 (range: 51 to 72 years). The diagnosis of DED was based on the Diagnostic Methodology Report published by the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) in 2017 (2). This study was reviewed and approved by the Ethics Committee of Beijing Tongren hospital and registered in the Chinese Clinical Trial Registry (Clinical Trial Registration No. ChiCTR2100044243). The patients and their families were informed of the examination content, treatment items, and matters needing attention before treatment, and all patients signed the informed consent.

Inclusion Criteria

The patients in this study conformed to all the following inclusion criteria: (1) voluntarily participated in the clinical study and signed the informed consent; (2) no gender limitation, aged 50–80 years; (3) presence of clinical signs and symptoms of DED, and the clinical examination conformed to the diagnostic criteria for mild-to-moderate DED; (4) no staining or spotty staining of corneal fluorescein $\leq$ 30 points or no more than two quadrants; (5) tear film break-up time $\geq$ 2 s; (6) Schirmer I test (SIt) $>$ 0

mm/5 min; (7) tear meniscus height (TMH) > 0 mm; (8) had not participated in clinical trials of other drugs within the past 2 weeks; and (9) had not taken any other medication for DED, or had stopped taking any other medication for > 2 weeks.

Exclusion Criteria

Patients with a diagnosis not consistent with DED or any of the following conditions were excluded: (1) allergic to any ingredient in the test drug; (2) the patients were presumed to be at the active stage of fungal, bacterial, or viral keratitis and conjunctivitis; (3) the total corneal staining points were >30 points or more than two quadrants, with patchy staining fusion or filamentous material; (4) tear film break-up time < 2 s; (5) Schirmer I test (SIt) = 0 mm/5 min; (6) tear meniscus height (TMH) = 0 mm; (7) severe MGD patients (grade 4); (8) prolonged systemic or ocular medication may affect the study evaluation of patients; (9) Sjögren's syndrome, systemic diseases, and other ocular diseases that affect the evaluation of efficacy; and (10) any other condition that affecting the ocular surface and was not appropriate for inclusion in the study such as eye surgery, abnormal eyelid structure and so on.

Treatment and Follow-Up

A total of 120 eyes of 60 middle-aged and elderly patients with mild-to-moderate DED who met the standards of enrollment were enrolled. The patients' history of eye diseases, treatment, systemic diseases, medication, subjective symptoms and objective signs before the test were recorded. Six milligram Astaxanthin tablets (6 mg astaxanthin/tablet, Weihong Haematococcus Pluvialis Astaxanthin, Hangzhou Xinwei Low Carbon Technology R&D Co., Ltd., China) were administered orally, twice daily for 30 ± 2 days. Then, the subjective symptoms and objective signs 2 weeks after the initiation and at the end of the treatment were recorded. All the objective checks were performed by the same experienced operator using the same equipment.

Evaluation of Symptoms and Signs

Ocular Surface Disease Index (OSDI) Questionnaire

The OSDI questionnaire was used to evaluate the subjective ocular symptoms of patients in the past week (2). The questionnaire consisted of 12 questions, with a total score of 0–100, and assessed the severity of subjective symptoms of DED from three aspects: ocular symptoms, visual function, and environmental triggers. The higher the score, the more severe the dry eye symptoms.

Eyelid Margin Signs

The eyelid margin was observed and scored under the slit lamp based on the severity of signs. DED can cause inflammation of the eyelid margins (27) and the increase of inflammatory factor concentration in eyelid margin can lead to blood vessel dilation, new capillary formation, proliferation and keratosis of epithelial cells (28). This results in redness, thickening and blunt round eyelid margins. Moreover, meibum will also easily coagulate and be mixed with keratinized duct epithelial cells, blocking meibomian gland orifices (29). Eyelid margin signs were closely related to meibomian gland dropout (30), leading to MGD and eventually DED. The composite eyelid score was used to evaluate the eyelid margin signs (31), including blunt rounding shape of the eyelid margin, thickened lid margin, hyperkeratinization of the lid margin, congestion of the anterior lid margin, and vascularity and telangiectasia around meibomian gland orifices. Each clinical symptom was allocated a score of 1 point, on a scale of 0–5 points. The higher the score, the more severe the inflammation of eyelids and the more severe the MGD.

Meibomian Gland (MG) Expressibility

The central five glands of the lower eyelids were pressed with Meibomian Gland Evaluator (MGE; Tear Science, Inc., Milpitas, USA), and the amount of meibum extruded was observed to judge its expressibility (32). Meibum expressibility was thought to reflect meibomian gland function (2). The scoring criteria were as follows: 0, all glands expressible; 1, 3–4 glands expressible; 2, 1–2 glands expressible; 3, no glands expressible. The higher the score, the worse the MG expressibility.

Meibum Quality

Meibum was extracted from the central five meibomian glands by gently pressing 1–2 mm below the eyelid margin. The properties of meibum were observed and scored according to a previously proposed method (33). Meibum quality was also used to assess meibomian gland function (2). The scoring criteria were as follows: 0, clear fluid; 1, cloudy fluid; 2, cloudy particulate fluid; 3, inspissated, toothpaste-like discharge. The total score is between 0 and 15. The higher the score, the worse the quality of meibum.

Meibomian Gland Dropout (MGDR)

The meibomian gland was photographed by the Meibo-Scan of Oculus Keratograph 5M (K5M, Oculus Optikgerate GmbH, Germany). In infrared light, the meibomian gland showed white lines, while the other parts showed dark gray background. Blockage of the meibomian gland orifices leads to increased pressure in the meibomian gland, degenerative dilation, and eventual loss (30). The loss of the glands was divided into four grades (34): grade 0, no loss of meibomian glands; grade 1, loss of meibomian glands below 1/3rd of the total area; grade 2, loss of meibomian glands accounting for 1/3rd−2/3rd of the total area; and grade 3, loss of meibomian glands accounting for 2/3rd or more of the total area. The higher the score, the greater the meibomian gland loss area.

Tear Meniscus Height (TMH)

The white light tool of Oculus Keratograph 5M was used to measure the TMH image of the patient. TMH was formed by the tear gathered at the upper and lower eyelid margin, representing the amount of tear secretion and lacrimal gland function (35). The central TMH data of the lower eyelid was acquired by the measuring tool in the system and recorded in millimeters (mm). The higher the TMH value, the more tear secretion.

Schirmer I Test (SIt)

After the tears gathered at the lower eyelid margin were wiped, a 35 mm × 5 mm filter paper strip was placed in the conjunctival

sac under the patient's eyes without topical anesthesia. SIt was mainly used to measure the basal and reflex secretions amount of the main and accessory lacrimal glands (36). The wetted length of the filter paper strip was observed after 5 min, and the tear secretion of the left and right eyes was recorded in mm. The longer the wetted paper, the more the tear secretion.

Corneal Fluorescein Staining (CFS)

One to two percentage fluorescein sodium dye was dropped into the conjunctival sac of the patient's lower eyelid. After 1 min, the cornea was observed under the cobalt blue light of a slit lamp microscope. The corneal epithelial defect site showed yellowish-green staining, and the fluorescein break-up time (FBUT) was recorded in second (s) for the unit. The corneal fluorescence staining was scored according to the American NEI scale (37). The cornea was divided into five regions and graded based on dye distribution. The CFS score was between 0 and 15, ranging from 0 to 3 in each region as follows: 0, no staining; 1, 1–30 punctate staining; 2, punctate staining >30; and 3, diffuse staining, filaments, and ulcer. The higher the score, the more severe the corneal epithelial defect. The longer the FBUT time, the more stable the tear film.

Noninvasive Tear Break-Up Time (NIBUT)

NIBUT was recorded using Oculus Keratograph 5M. After blinking twice, the patients were instructed to keep their eyes open. The system automatically recorded NIBUT and showed broken parts according to the inspection procedures for the formula to calculate the average NIBUT, with second (s) as the measuring unit (38). The longer the NIBUT time, the more stable the tear film.

Degree of Conjunctival Congestion

The conjunctival images of the patients were taken by the white light tool of Oculus Keratograph 5M, and the conjunctival congestion was evaluated and recorded by the image analysis technique. Inflammation is an important factor in the occurrence and development of DED, and conjunctival congestion is one of the most significant signs of ocular surface inflammation. The higher the grade, the more serious the congestion.

Lipid Layer Thickness (LLT) and Blink Analysis

The LipiView was used to measure LLT, blink frequency and incomplete blink proportion of eyes in 20 s. LLT reflects the ability of meibomian gland to secrete lipids (39). Blink abnormality is closely related to the occurrence and development of DED (40). The color unit of the interference image was converted to the thickness of the lipid layer of the tear film on the eye surface, the number of blinks in 20 s, and the proportion of incomplete blinks.

Safety Assessment

Visual acuity (VA) was measured using a standard logarithmic eye chart and intraocular pressure (IOP) was measured using a non-contact tonometer. Both eyes in all patients were monitored and recorded at the first and last follow-up. At the same time, the patients' anterior segment, fundus, discomfort symptoms and other adverse reactions were questioned and recorded.

TABLE 1 | Changes in OSDI score before and after treatment.

Time	Number	OSDI score
Before	60	52 ± 19
14 days later	60	42 ± 15^{a}
30 days later	60	30 ± 16^{ab}
P-value		<0.001

$^{a}P < 0.05$ vs. before treatment; $^{b}P < 0.05$ vs. 14 days after treatment.
Data are mean and standard deviation (SD).
OSDI, ocular surface disease index.

Statistical Analysis

SPSS 20.0 software was used for statistical analysis. In this study, the total OSDI score, NIBUT, FBUT, TMH, SIT, degree of conjunctival congestion, and LLT conformed to a normal distribution by W test, and the data are represented by mean ± standard deviation ($\overline{X} \pm s$). Before treatment, at 2 weeks after the initiation of treatment, and the end of treatment, no correlation was established between the OSDI score and the left and right eye data. One-way repeated-measures analysis of variance (ANOVA) was used for overall comparison, and Least Significant Difference (LSD)-*t*-test was used for pairwise comparison at different time. NIBUT, FBUT, TMH, SIt, degree of conjunctival congestion, LLT, and other data involving the correlation between the left and right eyes were collected bilaterally, and thus, a mixed-effect model was used for analysis. The corneal fluorescence staining score, eyelid margin changes, meibomian gland expressibility, meibum quality, meibomian gland dropout, blink frequency, incomplete blink proportion, VA and IOP did not conform to a normal distribution by W test and are represented by median (the first quartile value, the third quartile value) as M (Q1, Q3). Before treatment, 2 weeks after the initiation of treatment, and at the end of the treatment, the corneal fluorescence staining score, eyelid margin changes, meibomian gland expressibility, meibum quality, meibomian gland dropout, blink frequency, incomplete blink proportion, VA and IOP data were collected bilaterally, which were analyzed by mixed-effects model. $P < 0.05$ indicated statistical significance.

RESULTS

Changes in OSDI Score, NIBUT, FBUT, TMH, SIt, LLT, and Severity of Conjunctival Congestion Before and After Treatment

The overall OSDI scores before and after treatment showed a statistically significant difference (**Table 1**). Moreover, a statistically significant difference was detected in the overall NIBUT, FBUT of patients before and after treatment (**Table 2**). Before and after treatment, no statistically significant difference

TABLE 2 | Changes in NIBUT, FBUT, TMH, SIt, LLT, and conjunctival congestion degree before and after treatment.

Time	Number	NIBUT (s)	FBUT (s)	TMH (mm)	SIt (mm)	Conjunctival congestion degree	LLT (nm)
Before	120	7.18 ± 3.28	3.94 ± 1.58	0.23 ± 0.11	7.74 ± 6.33	1.34 ± 0.44	71.90 ± 23.77
14 days later	120	8.31 ± 3.91[a]	4.53 ± 2.29[a]	0.23 ± 0.09	7.70 ± 6.42	1.42 ± 0.45	74.55 ± 25.27
30 days later	120	8.34 ± 3.44[a]	4.69 ± 2.12[a]	0.23 ± 0.07	6.98 ± 6.61	1.39 ± 0.46	75.00 ± 24.03
P-value		0.016	0.011	0.880	0.458	0.397	0.567

[a]P < 0.05 vs. before treatment.
Data are mean and standard deviation (SD).
NIBUT, noninvasive tear break-up time; FBUT, fluorescein break-up time; TMH, tear meniscus height; SIt, Schirmer I test; LLT, lipid layer thickness.

TABLE 3 | Changes in CFS score, eyelid margin changes, MG expressibility, meibum quality, MGDR, blink frequency, and incomplete blink proportion before and after treatment.

Time	Number	CFS score	Eyelid margin signs	MG expressibility	Meibum quality	MGDR (upper eyelid)	MGDR (lower eyelid)	Blink frequency (per minute)	Incomplete blink proportion
Before	120	1 (0, 1)	1 (1, 1)	1 (1, 2)	1 (1, 1)	1 (1, 2)	1 (1, 2)	27 (12, 33)	0.75 (0.22, 1.00)
14d later	120	1 (0, 1)	1 (1, 1)	1 (1, 2)	1 (0, 1)[a]	1 (1, 2)	1 (1, 2)	18 (12, 27)[a]	0.88 (0.50, 1.00)
30 d later	120	0 (0, 2)[ab]	1 (0, 1)[ab]	1 (1, 1)[ab]	1 (0, 1)[a]	1 (1, 2)	1 (1, 1)	18 (9, 27)[a]	0.88 (0.50, 1.00)
P-value		<0.001	<0.001	<0.001	<0.001	0.183	0.407	0.020	0.086

[a]P < 0.05 vs. before treatment; [b]P < 0.05 vs. 14 days after treatment.
Data are median, the first quartile and the third quartile.
CFS, corneal fluorescein staining; MG, meibomian gland; MGDR, meibomian gland dropout.

TABLE 4 | Changes in VA and IOP before and after treatment.

Time	Number	VA	IOP (mmHg)
Before	120	0.3 (0.6, 0.8)	12.5 (10.8, 14.9)
30 days later	120	0.3 (0.7, 0.9)	13.0 (11.8, 14.8)
P-value		0.812	0.387

Data are median, the first quartile and the third quartile.
VA, visual acuity; IOP, intraocular pressure.

was observed in the TMH, SIt, conjunctival congestion degree, and LLT of the tear film (all $P > 0.05$) (**Table 2**).

Changes in CFS Score, Eyelid Margin Changes, MG Expressibility, Meibum Quality, MGDR, Blink Frequency, and Incomplete Blink Proportion Before and After Treatment

The overall comparison of CFS score, eyelid margin signs, MG expressibility, meibum quality, blink frequency before and after treatment showed a statistically significant difference (**Table 3**). The mean blink frequency was 24.9, 21.3, and 20.4 per minute before, 14 and 30 days after the initiation of treatment, respectively. Before and after treatment, no statistical significance was detected in the MGDR and incomplete blink proportion (all $P > 0.05$) (**Table 3**).

Changes in VA, IOP, and Other Safety Assessment Before and After Treatment

There were no significant changes in visual acuity and intraocular pressure before and after treatment (all $P > 0.05$) (**Table 4**). The anterior segment and fundus in all patients also had no significant changes. None of the patients complained of other adverse reactions.

DISCUSSION

With the deepening of the understanding of the etiology and pathogenesis of DED, the promotion of inflammation and oxidative stress on the occurrence and development of DED and their interaction has been under intensive focus (13, 41, 42). Currently, anti-inflammatory drugs, including glucocorticoid, cyclosporine A, and tacrolimus, have been marketed for the treatment of DED (43). Only a few clinical studies have addressed the application of antioxidants in DED. A study found that oxidative stress promotes the production of reactive oxygen molecules, induces ocular surface epithelial damage, and promotes the occurrence and development of DED (44). Therefore, it is crucial to explore the effects of oxidative stress and antioxidant therapy on DED to elucidate the pathogenesis of DED and explore novel therapeutic targets. Astaxanthin has good antioxidant properties and can be industrially produced. Presently, the main industrial production method of astaxanthin is through the culture of *Rhodococcus* (45). Some studies have shown that the accumulation of astaxanthin in the ocular tissues of rats fed with astaxanthin-rich *Rhodococcus* can reach the maximum level within 6 h (46), indicating good bioavailability

and the potential of achieving an effective blood concentration and playing a role in resisting oxidative stress in the eye. Therefore, astaxanthin from *Rhodiococcus* was selected to explore its therapeutic effect on mild to moderate middle-aged and elderly patients with DED.

This prospective, single-center, one-group pretest-posttest quasi-experimental study was conducted to observe the improvement in subjective symptoms and objective signs of mild-to-moderate DED in middle-aged and elderly patients with different etiologies after a daily supplement of 12 mg astaxanthin for 14 and 30 days. The results showed that OSDI score, CFS score, NIBUT, FBUT, meibum quality, and blink frequency were significantly improved on day 14 after daily astaxanthin supplementation compared with that before treatment. Both OSDI score and CFS score showed a tendency to improve with the duration of treatment. NIBUT, FBUT, and blink frequency were significantly improved 14 days after the initiation of the treatment. Although MG expressibility and eyelid margin signs were not significantly improved on day 14, they were significantly improved on day 30 compared with that before administration. Also, no significant differences were detected in the MGDR, TMH, SIt, LLT, conjunctival congestion degree, and incomplete blink.

OSDI is the score representing patients' subjective symptoms related to the severity of DED signs. In the current study, the patients' OSDI scores continued to decline, which might be attributable to astaxanthin blocking oxidative stress injury by increasing the level of antioxidant substances in the eye and inactivating and scavenging oxygen free radicals while downregulating inflammatory factors, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α) (26). Therefore, astaxanthin may alleviate local inflammatory response, reduce the severity of DED signs and relieve the symptoms.

Corneal fluorescence staining score reflects the degree of the corneal epithelial defect and is an objective index to measure the severity of DED. The results of this study showed that after oral astaxanthin tablets, the CFS score of patients showed a downward trend compared with that before the treatment, and the corneal epithelial defect was improved continuously, which was greater at 30 days than at 14 days after oral administration. Some animal experiments have shown that astaxanthin improved the corneal fluorescence staining and reduced the expression of age-related markers in the dry eyes of rats (47). Thus, this phenomenon may be related to the fact that astaxanthin is an effective antioxidant, reduces local oxidative damage and inflammatory response, and provides a local microenvironment for the repair of corneal epithelial cells. It usually takes a while before the local microenvironment becomes suitable for corneal epithelial proliferation and hence, the speed of corneal epithelial repair is slow in the initial 14 days. Well-controlled inflammation and a local microenvironment suitable for cell proliferation could explain the marked improvement of corneal epithelial defect at day 30.

NIBUT and FBUT mainly reflect the stability of the tear film. The tear film stability is related to several factors, such as the changes in tear composition, tear secretion, and mucin secretion (7). In the current study, NIBUT and FBUT showed an overall upward trend, and the increase was obvious in the first 14 days, which might be related to the improvement in the secretion properties of the ocular glands. Since the control of inflammation in the first 14 days improved the structure and composition and greatly increased the stability of the tear film, the NIBUT and FBUT were prolonged. The level of oxidative stress in conjunctival goblet cells in middle-aged and elderly individuals with DED is high (48). The antioxidant and anti-inflammatory effects of astaxanthin may improve the inflammatory response of conjunctival goblet cells, thereby promoting mucin secretion, playing the role of mucin anchor, and increasing tear film stability.

The eyelid margin signs, MG expressibility, meibum quality, and MGDR could reflect the degree of eyelid margin inflammation and the function of the meibomian gland. Compared with before treatment, all three parameters were improved continuously, and the improvement in meibum quality was significant at 14 days after the initiation of treatment, while the changes in eyelid margin signs and the enhanced MG expressibility were more obvious at 30 days. The meibomian gland of DED patients is in a state of high oxidative stress (16, 41). Astaxanthin could improve the surrounding environment of meibomian gland cells, reduce oxidative stress and inflammation, which would help improve the amount and quality of meibum secretion. However, the increase of MG expressibility was a slow process, and hence, its improvement lagged slightly behind that of meibum quality. Astaxanthin improved the oxidative stress and inflammation of the eyelids, which helped reduce inflammation edema, inhibit angiogenesis, vasodilatation and keratosis. Therefore, the eyelid margin signs score decreased. However, the improvement of eyelid margin was not obvious in the first 14 days, which was believed to be the reason that keratosis of the eyelid margin and congested vessels needed a period of time to be absorbed. No significant difference was detected in MGDR before and after treatment, which was related to the irreversibility.

LLT is affected by the amount and quality of meibum secreted by the meibomian glands (49). The lipid layer of the tear film is composed of meibum secreted by the meibomian glands. The decrease in meibum secretion can directly lead to the decrease of LLT (50). Reduced meibum quality can increase tear film instability, resulting in uneven distribution of tear film on the ocular surface, and thereby affecting the LLT value. LLT increased after the treatment, albeit not significantly. Moreover, this phenomenon could also be accounted for a low baseline level of tear film lipid layer thickness in patients with mild-to-moderate DED. The improvement in the secretion capacity of the meibomian gland was not obvious in the first 14 days, and it would result in less increase in the thickness of the lipid layer of the tear film.

Change in the blink characteristics is a major contributing factor to DED, mainly including blink frequency and incomplete blink proportion. These two characteristics are affected by many factors, such as mental state, attention, physical activity, eye contact, and environment (40). Under normal circumstances, the average number of blinks per minute is 15–20 (7), i.e., the number of blinks per 20 s is about 5–7. In the current study, the number

of blinks in 20 s was measured. According to the results, the blink frequency decreased gradually. Pretreatment blink frequency was higher than the normal value, which might be related to ocular surface symptoms and signs of patients with DED. Patients with poor tear film stability, shortened BUT, and severe DED symptoms, could only rely on increased blink frequency to achieve an optimal visual effect. However, after treatment, the blink frequency decreased gradually, albeit in some individuals, it was still higher than normal levels. Combined with the BUT, the time also increased but was still shorter than normal, which could be attributed to the blink frequency limited by the short BUT. In addition, the patients' OSDI score decreased but was higher than normal, indicating that the patients' symptoms had not disappeared completely, and long-term efficacy of astaxanthin is yet to be observed. Incomplete blinks are usually associated with long-term use of video terminals or other factors that impede eyelid closure (51, 52). Incomplete blink proportion had no significant change before and after treatment. This indicated that astaxanthin could not affect the method of blinking.

Conjunctival congestion degree is a major indicator of ocular surface inflammation. In this study, the degree of conjunctival congestion in patients was not significantly improved compared with that before the treatment, which might be due to the fact that the local anti-inflammatory effect of astaxanthin takes a long time to effectuate, the degree of ocular surface inflammation in patients with mild-to-moderate DED was mild or the short test time and small sample size.

The TMH and SIt reflected the lacrimal gland secretion capacity. In this study, TMH and SIt of patients before and after treatment did not improve significantly, which is similar to the results of previous animal experiments (47). This phenomenon could be attributed to the lack of a promoting effect on lacrimal gland secretion or failure to achieve an effective concentration of astaxanthin in the local lacrimal gland, thereby resulting in an inadequate improvement of lacrimal eye inflammation. It may also be related to the short test time and small sample size.

There were no significant changes in visual acuity, intraocular pressure, anterior segment and fundus before and after treatment. During the experiment, the subjects showed adaptability and no adverse reactions or complications. Given astaxanthin is administered orally, there is no ocular irritation. These results indicated that astaxanthin tablets could exert antioxidant, anti-inflammatory, ocular surface repair functions without causing discomfort. Additionally, the patients showed high medication compliance. Therefore, oral astaxanthin could be considered a safe method for the treatment of DED.

Nevertheless, the present study has some shortcomings. First, this study was a single-group design and had no control group. Although the changes in patients' conditions before and after astaxanthin intervention could be obtained, the placebo effect was ignored. Second, the observation time of this study was only about 1 month, and hence, it is impossible to predict the long-term efficacy of astaxanthin on DED. Third, the tear composition of the patients was not tested and systemic and local oxidative stress was not measured, rendering it impossible to observe whether astaxanthin reduces the oxidation in the tear. However, a range of measures were taken to improve the credibility of our study. The authors ensured that all patients were examined by the same experienced ophthalmologist, explained the questions before completing the OSDI questionnaire and allowed adequate time to answer it. Herein, the authors would propose a follow-up study to set up a reasonable control group, prolonged observation time for the observation of long-term curative effect and adverse reactions, in which case the sample size would be increased and the tear composition detection would be added, so that the efficacy of the astaxanthin in patients of different gender and age groups could be observed.

CONCLUSIONS

In conclusion, as a comfortable option with fewer adverse reactions, oral administration of astaxanthin could serve as an effective treatment of DED by improving the tear film stability, the repair of corneal and conjunctival epithelial cells and the secretion function of the meibomian gland, improving subjective symptoms of DED. However, its effect on promoting lacrimal gland secretion was observed to be limited. The single-group design and lack of molecular biological detection constitute limitations of the current study, thus further studies with more robust methodology, such as randomized controlled trials and tests for inflammatory factors and oxidative stress are needed. Astaxanthin is of the potential to be a new choice for the treatment of mild to moderate DED.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Tongren hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LT contributed to the conception of the study. LT and YWe prepared the first draft. YWe, SL, PZ, YWa, and JW performed the experiment and recorded the data. KC and LD contributed significantly to the data analysis. NW and YJ provided constructive suggestions regarding the manuscript preparation, the data analysis and the interpretation of the results, and made critical revisions to the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II definition and classification report. *Ocul Surf.* (2017) 15:276–83. doi: 10.1016/j.jtos.2017.05.008
2. Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II diagnostic methodology report. *Ocul Surf.* (2017) 15:539–74. doi: 10.1016/j.jtos.2017.05.001
3. Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, et al. TFOS DEWS II epidemiology report. *Ocul Surf.* (2017) 15:334–65. doi: 10.1016/j.jtos.2017.05.003
4. Song P, Xia W, Wang M, Chang X, Wang J, Jin S, et al. Variations of dry eye disease prevalence by age, sex and geographic characteristics in China: a systematic review and meta-analysis. *J Glob Health.* (2018) 8:020503. doi: 10.7189/jogh.08.020503
5. Zhang S, Hong J. Risk factors for dry eye in mainland China: a multi-center cross-sectional hospital-based study. *Ophthalmic Epidemiol.* (2019) 26:393–9. doi: 10.1080/09286586.2019.1632905
6. Zheng Y, Wu X, Lin X, Lin H. The prevalence of depression and depressive symptoms among eye disease patients: a systematic review and meta-analysis. *Sci Rep.* (2017) 7:46453. doi: 10.1038/srep46453
7. Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, et al. TFOS DEWS II pathophysiology report. *Ocul Surf.* (2017) 15:438–510. doi: 10.1016/j.jtos.2017.05.011
8. Rocha EM, Alves M, Rios JD, Dartt DA. The aging lacrimal gland: changes in structure and function. *Ocul Surf.* (2008) 6:162–74. doi: 10.1016/S1542-0124(12)70177-5
9. Borchelt DR, Ibrahim OMA, Dogru M, Matsumoto Y, Igarashi A, Kojima T, et al. Oxidative stress induced age dependent meibomian gland dysfunction in Cu, Zn-superoxide dismutase-1 (Sod1) knockout mice. *PLoS ONE.* (2014) 9:e99328. doi: 10.1371/journal.pone.0099328
10. Chen Y, Li M, Li B, Wang W, Lin A, Sheng M. Effect of reactive oxygen species generation in rabbit corneal epithelial cells on inflammatory and apoptotic signaling pathways in the presence of high osmotic pressure. *PLoS ONE.* (2013) 8:e72900. doi: 10.1371/journal.pone.0072900
11. Slimen IB, Najar T, Ghram A, Dabbebi H, Ben Mrad M, Abdrabbah M. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *Int J Hyperthermia.* (2014) 30:513–23. doi: 10.3109/02656736.2014.971446
12. Deng R, Hua X, Li J, Chi W, Zhang Z, Lu F, et al. Oxidative stress markers induced by hyperosmolarity in primary human corneal epithelial cells. *PLoS ONE.* (2015) 10:e0126561. doi: 10.1371/journal.pone.0126561
13. Dogru M, Kojima T, Simsek C, Tsubota K. Potential role of oxidative stress in ocular surface inflammation and dry eye disease. *Invest Ophthalmol Vis Sci.* (2018) 59:DES163–8. doi: 10.1167/iovs.17-23402
14. Lee SY, Han SJ, Nam SM, Yoon SC, Ahn JM, Kim TI, et al. Analysis of tear cytokines and clinical correlations in Sjögren syndrome dry eye patients and non-Sjögren syndrome dry eye patients. *Am J Ophthalmol.* (2013) 156:247–53.e1. doi: 10.1016/j.ajo.2013.04.003
15. Aragona P, Aguennouz M, Rania L, Postorino E, Sommario MS, Roszkowska AM, et al. Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. *Ophthalmology.* (2015) 122:62–71. doi: 10.1016/j.ophtha.2014.07.048
16. Nezzar H, Mbekeani JN, Noblanc A, Chiambaretta F, Drevet JR, Kocer A. Investigation of antioxidant systems in human meibomian gland and conjunctival tissues. *Exp Eye Res.* (2017) 165:99–104. doi: 10.1016/j.exer.2017.09.005
17. Huang JY, Yeh PT, Hou YC. A randomized, double-blind, placebo-controlled study of oral antioxidant supplement therapy in patients with dry eye syndrome. *Clin Ophthalmol.* (2016) 10:813–20. doi: 10.2147/OPTH.S106455
18. Kojima T, Dogru M, Kawashima M, Nakamura S, Tsubota K. Advances in the diagnosis and treatment of dry eye. *Prog Retinal Eye Res.* (2020) 78:100842. doi: 10.1016/j.preteyeres.2020.100842
19. Higuera-Ciapara I, Félix-Valenzuela L, Goycoolea FM. Astaxanthin: a review of its chemistry and applications. *Crit Rev Food Sci Nutr.* (2006) 46:185–96. doi: 10.1080/10408690590957188
20. Shimokawa T, Yoshida M, Fukuta T, Tanaka T, Inagi T, Kogure K. Efficacy of high-affinity liposomal astaxanthin on up-regulation of age-related markers induced by oxidative stress in human corneal epithelial cells. *J Clin Biochem Nutr.* (2019) 64:27–35. doi: 10.3164/jcbn.18-27
21. Sudharshan SJ, Dyavaiah M. Astaxanthin protects oxidative stress mediated DNA damage and enhances longevity in Saccharomyces cerevisiae. *Biogerontology.* (2021) 22:81–100. doi: 10.1007/s10522-020-09904-9
22. Fang Q, Guo S, Zhou H, Han R, Wu P, Han C. Astaxanthin protects against early burn-wound progression in rats by attenuating oxidative stress-induced inflammation and mitochondria-related apoptosis. *Sci Rep.* (2017) 7:41440. doi: 10.1038/srep41440
23. Yeh PT, Huang HW, Yang CM, Yang WS, Yang CH. Astaxanthin inhibits expression of retinal oxidative stress and inflammatory mediators in streptozotocin-induced diabetic rats. *PLoS ONE.* (2016) 11:e0146438. doi: 10.1371/journal.pone.0146438
24. Chang MX, Xiong F. Astaxanthin and its effects in inflammatory responses and inflammation-associated diseases: recent advances and future directions. *Molecules.* (2020) 25:5342. doi: 10.3390/molecules25225342
25. Otsuka T, Shimazawa M, Inoue Y, Nakano Y, Ojino K, Izawa H, et al. Astaxanthin protects against retinal damage: evidence from *in vivo* and *in vitro* retinal ischemia and reperfusion models. *Curr Eye Res.* (2016) 41:1465–72. doi: 10.3109/02713683.2015.1127392
26. Li H, Li J, Hou C, Li J, Peng H, Wang Q. The effect of astaxanthin on inflammation in hyperosmolarity of experimental dry eye model *in vitro* and *in vivo*. *Exp Eye Res.* (2020) 197:108113. doi: 10.1016/j.exer.2020.108113
27. Rynerson JM, Perry HD. DEBS - a unification theory for dry eye and blepharitis. *Clin Ophthalmol.* (2016) 10:2455–67. doi: 10.2147/OPTH.S114674
28. Amescua G, Akpek EK, Farid M, Garcia-Ferrer FJ, Lin A, Rhee MK, et al. American academy of ophthalmology preferred practice pattern, and p. External disease, blepharitis preferred practice pattern(R). *Ophthalmology.* (2019) 126:P56–93. doi: 10.1016/j.ophtha.2018.10.019
29. Bernardes TF, Bonfioli AA. Blepharitis. *Semin Ophthalmol.* (2010) 25:79–83. doi: 10.3109/08820538.2010.488562
30. Ha M, Kim JS, Hong SY, Chang DJ, Whang WJ, Na KS, et al. Relationship between eyelid margin irregularity and meibomian gland dropout. *Ocul Surf.* (2021) 19:31–7. doi: 10.1016/j.jtos.2020.11.007
31. Yan X, Hong J, Jin X, Chen W, Rong B, Feng Y, et al. The efficacy of intense pulsed light combined with meibomian gland expression for the treatment of dry eye disease due to meibomian gland dysfunction: a multicenter, randomized controlled trial. *Eye Contact Lens.* (2021) 47:45–53. doi: 10.1097/ICL.0000000000000711
32. Pflugfelder SC, Tseng SC, Sanabria O, Kell H, Garcia CG, Felix C, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea.* (1998) 17:38–56. doi: 10.1097/00003226-199801000-00007
33. Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. *Eye.* (1991) 5(Pt. 4):395–411. doi: 10.1038/eye.1991.65
34. Srinivasan S, Menzies K, Sorbara L, Jones L. Infrared imaging of meibomian gland structure using a novel keratograph. *Optom Vis Sci.* (2012) 89:788–94. doi: 10.1097/OPX.0b013e318253de93
35. Tung CI, Perin AF, Gumus K, Pflugfelder SC. Tear meniscus dimensions in tear dysfunction and their correlation with clinical parameters. *Am J Ophthalmol.* (2014) 157:301–10 e1. doi: 10.1016/j.ajo.2013.09.024
36. Brott NR, Ronquillo Y. *Schirmer Test*. Treasure Island, FL: StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC (2021).
37. Amparo F, Wang H, Yin J, Marmalidou A, Dana R. Evaluating corneal fluorescein staining using a novel automated method. *Invest Ophthalmol Vis Sci.* (2017) 58:BIO168–73. doi: 10.1167/iovs.17-21831
38. Lee R, Yeo S, Aung HT, Tong L. Agreement of noninvasive tear break-up time measurement between Tomey RT-7000 auto refractor-keratometer and oculus keratograph 5M. *Clin Ophthalmol.* (2016) 10:1785–90. doi: 10.2147/OPTH.S110180
39. Li J, Ma J, Hu M, Yu J, Zhao Y. Assessment of tear film lipid layer thickness in patients with Meibomian gland dysfunction at different ages. *BMC Ophthalmol.* (2020) 20:394. doi: 10.1186/s12886-020-01667-8
40. Tsubota K, Hata S, Okusawa Y, Egami F, Ohtsuki T, Nakamori K. Quantitative videographic analysis of blinking in normal subjects and patients with dry eye. *Arch Ophthalmol.* (1996) 114:715–20. doi: 10.1001/archopht.1996.01100130707012

41. Seen S, Tong L. Dry eye disease and oxidative stress. *Acta Ophthalmol.* (2018) 96:e412–20. doi: 10.1111/aos.13526
42. Yamaguchi T. Inflammatory response in dry eye. *Invest Ophthalmol Vis Sci.* (2018) 59:Des192–9. doi: 10.1167/iovs.17-23651
43. Jones L, Downie LE, Korb D, Benitez-Del-Castillo JM, Dana R, Deng SX, et al. TFOS DEWS II management and therapy report. *Ocul Surf.* (2017) 15:575–628. doi: 10.1016/j.jtos.2017.05.006
44. Uchino Y, Kawakita T, Ishii T, Ishii N, Tsubota K. A new mouse model of dry eye disease: oxidative stress affects functional decline in the lacrimal gland. *Cornea.* (2012) 31(Suppl. 1):S63–7. doi: 10.1097/ICO.0b013e31826a5de1
45. Ambati RR, Phang SM, Ravi S, Aswathanarayana RG. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications–a review. *Mar Drugs.* (2014) 12:128–52. doi: 10.3390/md12010128
46. Ranga Rao Raghunath Reddy RL, Baskaran V, Sarada R, Ravishankar GA. Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model. *J Agric Food Chem.* (2010) 58:8553–9. doi: 10.1021/jf101187k
47. Shimokawa T, Fukuta T, Inagi T, Kogure K. Protective effect of high-affinity liposomes encapsulating astaxanthin against corneal disorder in the *in vivo* rat dry eye disease model. *J Clin Biochem Nutr.* (2020) 66:224–32. doi: 10.3164/jcbn.19-102
48. de Souza RG, Yu Z, Hernandez H, Trujillo-Vargas CM, Lee A, Mauk KE, et al. Modulation of oxidative stress and inflammation in the aged lacrimal gland. *Am J Pathol.* (2021) 191:294–308. doi: 10.1016/j.ajpath.2020.10.013
49. Finis D, Pischel N, Borrelli M, Schrader S, Geerling G. Einflussfaktoren auf die messung der lipidschichtdicke des tränenfilms mittels interferometrie. *Klin Monatsblätter Augenheilkunde.* (2014) 231:603–10. doi: 10.1055/s-0034-1368536
50. Chou YB, Fan NW, Lin PY. Value of lipid layer thickness and blinking pattern in approaching patients with dry eye symptoms. *Can J Ophthalmol.* (2019) 54:735–40. doi: 10.1016/j.jcjo.2019.03.005
51. Talens-Estarelles C, Garcia-Marques JV, Cervino A, Garcia-Lazaro S. Use of digital displays and ocular surface alterations: a review. *Ocul Surf.* (2021) 19:252–65. doi: 10.1016/j.jtos.2020.10.001
52. Park J, Baek S. Dry eye syndrome in thyroid eye disease patients: the role of increased incomplete blinking and Meibomian gland loss. *Acta Ophthalmol.* (2019) 97:e800–6. doi: 10.1111/aos.14000

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Raw and Cooked Vegetable Consumption and Risk of Cardiovascular Disease: A Study of 400,000 Adults in UK Biobank

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Objectives: Higher levels of vegetable consumption have been associated with a lower risk of cardiovascular disease (CVD), but the independent effect of raw and cooked vegetable consumption remains unclear.

Methods: From the UK Biobank cohort, 399,586 participants without prior CVD were included in the analysis. Raw and cooked vegetable intakes were measured with a validated dietary questionnaire at baseline. Multivariable Cox regression was used to estimate the associations between vegetable intake and CVD incidence and mortality, adjusted for socioeconomic status, health status, and lifestyle factors. The potential effect of residual confounding was assessed by calculating the percentage reduction in the likelihood ratio (LR) statistics after adjustment for the confounders.

Results: The mean age was 56 years and 55% were women. Mean intakes of raw and cooked vegetables were 2.3 and 2.8 tablespoons/day, respectively. During 12 years of follow-up, 18,052 major CVD events and 4,406 CVD deaths occurred. Raw vegetable intake was inversely associated with both CVD incidence (adjusted hazard ratio (HR) [95% CI] for the highest vs. lowest intake: 0.89 [0.83–0.95]) and CVD mortality (0.85 [0.74–0.97]), while cooked vegetable intake was not (1.00 [0.91–1.09] and 0.96 [0.80–1.13], respectively). Adjustment for potential confounders reduced the LR statistics for the associations of raw vegetables with CVD incidence and mortality by 82 and 87%, respectively.

Conclusions: Higher intakes of raw, but not cooked, vegetables were associated with lower CVD risk. Residual confounding is likely to account for much, if not all, of the observed associations. This study suggests the need to reappraise the evidence on the burden of CVD disease attributable to low vegetable intake in the high-income populations.

Keywords: vegetable intake, raw vegetable, cooked vegetable, cardiovascular diseases, UK biobank, cardiovascular mortality

INTRODUCTION

There exists a large body of research evidence to suggest that a high vegetable intake may protect against a wide range of health outcomes, including cardiovascular disease (CVD) (1, 2). Although dietary guidelines have consistently recommended a high consumption of vegetables to the general population (3, 4) as a source of beneficial macronutrients and micronutrients, such as dietary fiber, vitamins, and phytochemicals (5), it is estimated that inadequate vegetable consumption accounts for about 1.5 million premature deaths from CVD alone each year (6).

However, little is known about the independent effects of cooked vegetables and raw vegetables on health outcomes. Previous epidemiological studies have demonstrated inconsistent findings. The EPIC study (7) of 450,000 participants recruited across Europe found that both cooked and raw vegetable intake was associated with lower CVD mortality and all-cause mortality. The PURE study (8) of 135,000 participants reported an inverse association with all-cause mortality for raw vegetable intake, but not for cooked vegetable intake, and neither cooked nor raw vegetable intake was associated with CVD incidence. An Australian cohort study (9) of 150,000 participants reported that only cooked vegetable intake was associated with lower overall mortality, but did not investigate cardiovascular outcomes. The reason for the discrepancies in these findings is unclear, but may reflect variation in the dietary patterns between populations and also methodological differences, such as dietary assessment methods and insufficient adjustment for potential confounders.

The UK Biobank is a cohort of half-million participants with over a decade of follow-up (10). A wide range of participant characteristics were measured at baseline using standardized methods, minimizing measurement error and allowing for adjustment for a broad set of potential confounders. During follow-up, a large number of incident CVD and CVD deaths have been recorded, allowing for well-powered epidemiological investigations on cardiovascular outcomes (11). The objective of this study was to examine the effect of vegetable intake, and specifically the independent effects of raw and cooked vegetable intake, on CVD incidence and mortality in UK Biobank.

METHODS

Study Design and Participants

The UK Biobank is a population-based prospective cohort study (10). Between 2006 and 2010, half-million participants aged 40–69 years were recruited across England, Wales, and Scotland. Participants attended assessment centers, during which time they completed a touchscreen questionnaire that collected information on sociodemographic characteristics, lifestyle, health status, medication use, reproductive history, and environmental factors. In addition, anthropometric and other physical measures were taken using standardized procedures, and blood, urine, and saliva samples were collected.

The health of the participants was followed-up *via* linkage to hospitalization databases (the National Health Service [NHS] Hospital Episode Statistics for participants in England; the Scottish Morbidity Record for participants in Scotland; and the Patient Episode Database for participants in Wales) and national death registries (NHS Information Center for participants in England and Wales; and NHS Central Registry for participants in Scotland). UK Biobank was approved by the North West Multicenter Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. All the participants provided the informed consent.

This study excluded participants that withdrew their consents during follow-up, had missing data on vegetable intake, had prior CVDs, had conditions likely to change dietary patterns (e.g., pregnancy and cancer). Furthermore, 5,885 participants had missing data on other key covariates (body mass index [BMI], meat consumption, and Townsend deprivation index), and were excluded. In total, 399,586 participants were included in the analysis (**Figure 1**).

Measurement of Exposures and Outcomes

Information was collected at baseline on the total daily intake of raw vegetables and cooked vegetables. Participants were asked in the dietary questionnaire "*On average how many heaped tablespoons of salad or raw vegetables would you eat per day? (including lettuce, tomato in sandwiches)*" and "*On average how many heaped tablespoons of cooked vegetables would you eat per day? (do not include potatoes)*". Total vegetable intake was calculated as the sum of raw and cooked vegetable intakes. Vegetable intake was categorized into four levels, using cutoff values of 0, 1–2, 3–4, and $\geq$ 5 tablespoons/day for raw and cooked vegetable intake, and cutoff values of 0–1, 2–3, 4–7, and $\geq$ 8 for total vegetable intake. Previous analyses have demonstrated high repeatability and validity of vegetable consumption measured in this baseline dietary questionnaire: repeatability over a 4-year period is 82% for cooked vegetables and 72% for raw vegetables, with high agreement also observed when compared with 24-h dietary assessment (12).

The primary outcomes were CVD incidence and mortality. The secondary outcomes were incident myocardial infarction (MI), incident stroke, and all-cause mortality. Incident CVD was defined as hospitalization or death from MI or stroke (13). CVD mortality was defined as death due to any CVD. For analyses of disease incidence, participants were censored at the date of hospitalization, date of death, or last date of follow-up (March 31, 2021 for participants from England and Scotland, and February 28, 2018 for participants from Wales), whichever occurred first. In the mortality analysis, participants were censored at the date of death or last date of follow-up (February 28, 2021), whichever occurred first. Health outcomes were defined using the International Classification of Disease (ICD) codes. The exact ICD codes used are shown in **Supplementary Table 1**.

Statistical Analysis

Cox proportional hazard models were used to yield hazard ratios (HR) and 95% CI for the associations between health outcomes and vegetable intake. Models were adjusted by age ($<$50, 50–60, $\geq$ 60 years), sex, ethnicity, and region, and adjusted for educational attainment, Townsend deprivation index (continuous), hypertension, diabetes, physical activity

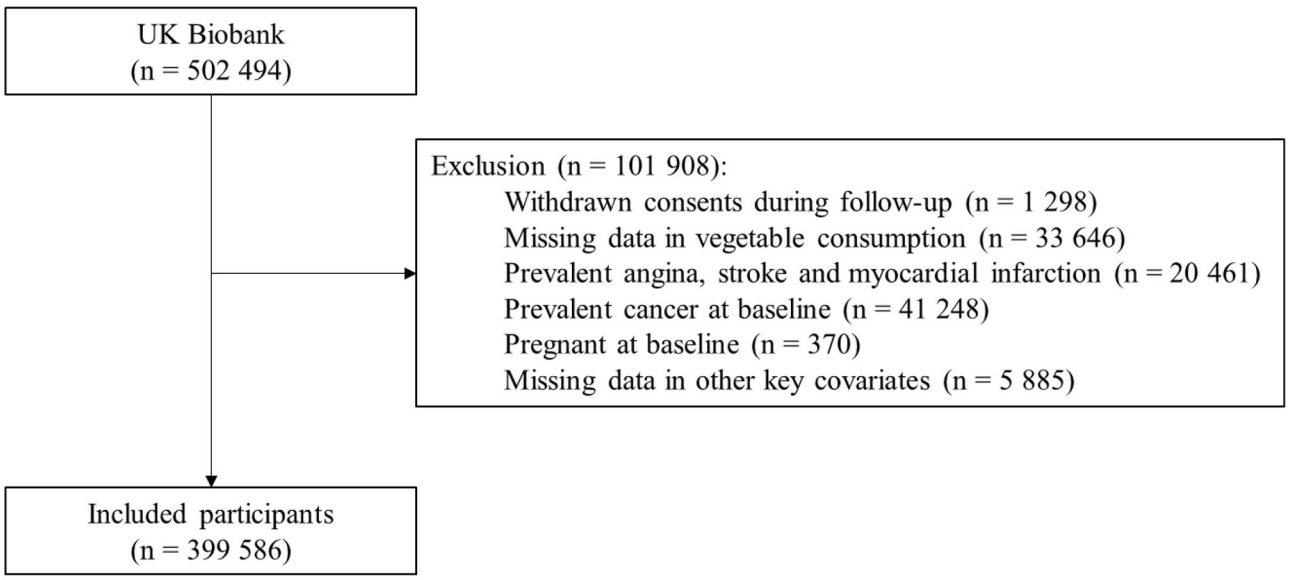

FIGURE 1 | Flowchart of participant included in the main analysis.

level, smoking, alcohol consumption, BMI (continuous), use of mineral supplements, use of vitamin supplements, aspirin/ibuprofen, antihypertensive drugs, statins, insulin treatment, intake of fresh fruits, red meat, processed meat, oily fish, and non-oily fish. The definition and measurement of the covariates are shown in the **Supplementary Materials**. The lowest intake level was used as the referent group. Test of the linear trend was obtained by fitting the mean values of each vegetable intake level. The proportional hazards assumption was assessed using scaled Schoenfeld residuals (no violation was found in this study). Raw and cooked vegetable intake were mutually adjusted when investigating their independent effects. Variance inflation factor values were used to examine potential multicollinearity.

We calculated the increase in the likelihood ratio (LR) chi-squared statistics on the addition of the vegetable intake term (raw, cooked, and total) to the Cox models with various levels of adjustment of potential confounders. This provides a quantitative measure of the extent to which vegetable intake improves risk prediction for the outcome in different models. Comparisons of the changes in the LR chi-squared statistic between a model with minimal adjustments (e.g., age, sex, ethnicity, and region) to those with a more comprehensive set of confounders ("fully-adjusted" models) is therefore measure of the extent to which the confounders account for minimally adjusted associations between vegetable intake and the outcome of interest. Furthermore, given that many confounders are measured imperfectly, the proportional change in this LR chi-squared statistic is a semiquantitative method of assessing for residual confounding, as models with perfectly measured confounders would be expected to further reduce the LR chi-squared statistic in fully adjusted models (14). More details are shown in the **Supplementary Materials**.

For sensitivity analysis, we first excluded participants who developed the outcomes of interest during the first 2 years of follow-up, to minimize reverse causation. Second, we investigated the effect of the proportion of raw vegetables in total vegetable intake (raw vegetables divided by total vegetables), conditional on total vegetable intake and other covariates, after excluding the participants with the total vegetable intake of *zero* tablespoon/day (n = 5,304). We conducted subgroup analysis based on ethnicity (White vs. non-White), to examine potential ethnic differences in the associations. All the analysis were performed using R (version 3.6.0; R Development Core Team, Vienna, Austria).

RESULTS

After exclusion, 399,586 participants were included in the main analysis (**Figure 1**). The baseline characteristics of these participants are shown in **Table 1** (**Supplementary Table 2**). The mean age of participants was 56.1 (SD 8.1) years, 55.4% were women, and 90.9% were of White ethnicity. Mean BMI was 27.3 (4.7) kg/m^2, 41.3% reported high levels of physical activity, and 4.7% had a self-reported history of diabetes. Mean intakes of total vegetables, raw vegetables, and cooked vegetables were 5.0 (3.4), 2.3 (2.2), and 2.8 (2.2) tablespoons/day, respectively; the distributions of total, raw and cooked vegetable intakes are shown in **Supplementary Figure 1**.

Participants with higher levels of total vegetable intake were more likely to be women, better educated, and residents of an affluent area, with lower mean BMI and higher levels of physical activity, and less likely to be smokers. Raw and cooked vegetable intake were weakly correlated (Pearson correlation coefficient = 0.30). Variance inflation

TABLE 1 | Baseline characteristics of the 399,586 participants in the main analysis, by total vegetable consumption.

	≤1 tablespoon/day (*n* = 15 902)	2–3 tablespoons/day (*n* = 109 536)	4–7 tablespoons/day (*n* = 216 499)	≥8 tablespoons/day (*n* = 57 649)	Overall (*n* = 399 586)
Female (%)	6 174 (38.8)	54 948 (50.2)	126 375 (58.4)	33 997 (59.0)	221 494 (55.4)
Age (years)	54.0 (8.1)	55.3 (8.2)	56.5 (8.0)	56.4 (8.0)	56.1 (8.1)
Total vegetable intake (tablespoons/day)	0.7 (0.5)	2.6 (0.5)	5.1 (1.0)	10.7 (5.0)	5.0 (3.4)
Raw vegetable intake (tablespoons/day)	0.1 (0.3)	0.9 (0.6)	2.2 (1.1)	5.5 (3.5)	2.3 (2.5)
Cooked vegetable intake (tablespoons/day)	0.5 (0.5)	1.7 (0.6)	2.8 (1.0)	5.3 (3.4)	2.8 (1.9)
White ethnicity (%)	14 782 (93.3)	104 731 (95.9)	206 372 (95.6)	52 143 (90.9)	378 028 (94.9)
Townsend Deprivation index*	−0.2 (3.5)	−1.4 (3.0)	−1.5 (2.9)	−1.1 (3.1)	−1.4 (3.0)
University educated (%)	3,321 (21.3)	37,040 (34.3)	73,733 (34.6)	19,483 (34.5)	133,577 (34.0)
Body mass index (kg/m^2)	28.0 (5.2)	27.2 (4.7)	27.3 (4.7)	27.4 (4.8)	27.3 (4.7)
Current smoker (%)	3 485 (22.0)	11 828 (10.8)	19 427 (9.0)	5 506 (9.6)	40 246 (10.1)
Current drinker (%)	13 817 (87.1)	101 873 (93.1)	201 964 (93.3)	52 123 (90.5)	369 777 (92.6)
High physical activity level (%)†	3 971 (32.2)	31 459 (35.0)	75 528 (42.5)	24 328 (51.0)	135 286 (41.3)
Self-reported hypertension (%)	4 172 (26.2)	26 482 (24.2)	55 071 (25.4)	15 131 (26.2)	100 856 (25.2)
Self-reported diabetes (%)	994 (6.2)	4 859 (4.4)	9 904 (4.6)	3 009 (5.2)	18 766 (4.7)
Regular use of aspirin/ibuprofen (%)	4 065 (25.6)	26 039 (23.8)	53 667 (24.8)	14 394 (25.0)	98 165 (24.6)
Regular use of mineral supplement (%)	2 869 (18.0)	25 789 (23.5)	61 980 (28.6)	17 955 (31.1)	108 593 (27.2)
Regular use of vitamin supplement (%)	1 760 (11.1)	13 223 (12.1)	30 534 (14.1)	9 756 (16.9)	55 273 (13.8)
Use of antihypertensive drugs (%)	938 (5.9)	8 133 (7.4)	20 449 (9.4)	5 628 (9.8)	35 148 (8.8)
Use of statin (%)	696 (4.4)	5 426 (4.9)	13 443 (6.2)	3 822 (6.6)	23 387 (5.9)
Use of insulin (%)	58 (0.4)	375 (0.3)	855 (0.4)	275 (0.5)	1 563 (0.4)
Fruit intake ≥5 pieces/day (%)	684 (4.3)	4 659 (4.3)	15 781 (7.3)	10 076 (17.5)	31 200 (7.8)
Oily fish intake >1 times/week (%)	3 267 (20.7)	39 335 (36.0)	88 514 (41.0)	21 682 (37.7)	72 515 (18.2)
Non-oily fish intake >1 times /week (%)	1 565 (9.9)	13 441 (12.3)	37 587 (17.4)	13 698 (23.8)	66 291 (16.6)
Processed meat intake ≥2 times/week (%)	6 949 (43.8)	38 331 (35.0)	62 278 (28.8)	14 132 (24.5)	121 690 (30.5)
Red meat intake (times/week)	2.0 (1.6)	2.1 (1.4)	2.1 (1.4)	2.0 (1.6)	2.1 (1.4)

Data are mean (SD) or frequency (percentage). Vegetable consumption was self-reported in number of heaped tablespoons/day. People with baseline angina, stroke, myocardial infarction, cancer, pregnancy, and missing data on vegetable consumption and other important covariates were excluded.

**Area-level measure of material deprivation.*

†High physical activity defined based on International Physical Activity Questionnaire and WHO guideline.

factor values for raw and cooked vegetable intake were 1.32 and 1.29, respectively, indicating very low collinearity (< 10). **Supplementary Tables 3**, **4** showed the baseline characteristics of the participants across different raw vegetable intake levels and the cooked vegetable intake levels, respectively. The distributions of baseline characteristics by raw and cooked vegetable intake were similar to the distributions by total vegetable intake.

During a median follow-up of 12.1 years for CVD incidence outcomes, 18,052 participants developed CVD (11,317 MI and 6,969 strokes). There was an inverse association between incident CVD and total and raw vegetable intake, but not cooked vegetable intake (**Figure 2**; **Supplementary Figure 2**). Compared with the lowest level of total vegetable intake, the highest level was associated with 10% lower CVD incidence (HR [95% CI] 0.90 [0.83–0.97]). Higher intake of raw vegetable intake was inversely

associated with incident CVD (HR [95% CI] for the highest vs. lowest intake: 0.89 [0.83–0.95]) and incident MI (0.88 [0.81–0.96]; **Figure 2**), whereas cooked vegetable intake showed null associations with incident CVD (1.00 [0.91–1.09]) or incidence MI (0.97 [0.86–1.08]). We noted a potential inverse association between raw vegetable intake and incident stroke, although this was not statistically significant. No evidence was found for an association between incident stroke and total or cooked vegetable intakes (**Figure 2**).

During a median follow-up of 12.0 years for mortality outcomes, 13,398 participants died, of which 2,589 deaths were due to CVD. Consuming 2 or more heaped tablespoons/day of total vegetables was associated with a lower risk of CVD mortality (HR [95%CI] for the highest vs. lowest intake: 0.83 [0.71–0.96]), but there was little evidence of a trend in risk with higher levels of intake (**Figure 3**). Similarly, there was evidence of an inverse association of CVD mortality with raw vegetable intake (0.85 [0.74–0.97]) but little evidence of a trend ($p = 0.164$), and there was no evidence of an association of CVD mortality with cooked vegetables. For all-cause mortality, there was a strong inverse association with eating some vegetables (1 or more tablespoons of raw or cooked vegetables per day), and strong evidence of a trend with increasing raw vegetable intake ($p < 0.001$) but not cooked vegetables ($p = 0.932$).

Progressive adjustment for potential confounders attenuated HR estimates and substantially reduced the LR chi-squared statistics in adjusted models (**Table 2**). For models of CVD incidence and raw vegetable intake, covariate adjustment attenuated HR (highest vs. lowest intake groups) from 0.79 (0.74–0.84) to 0.88 (0.83, 0.94), with the LR chi-squared statistic declining by 81.9%. This substantial attenuation suggests that were the potential confounders measured perfectly, much, if not all, of the observed association with reported vegetable intake, would be explained by residual confounding, although one cannot rule out the possibility of a true causal protective effect. Similar findings were observed for MI, CVD mortality, and all-cause mortality with both raw and cooked vegetable intake, with the proportional changes in the LR chi-squared statistic of about 80% or more (**Table 2**, **Supplementary Table 5**). Adjustment for socioeconomic (including educational attainment, and Townsend deprivation index) and lifestyle factors (including physical activity, smoking, drinking, use of mineral supplements, use of vitamin supplements, fruit intake, oily fish intake, non-oily fish intake, red meat intake, and processed meat intake) results in most of the reductions in LR chi-squared statistic, while further adjustment for BMI and baseline health status resulted in only slight further reductions (**Supplementary Table 5**), suggesting that the observed associations are likely to be accounted for by residual confounding from socioeconomic status and lifestyle factors.

In the sensitivity analyses, when adjusting for total vegetable intake, a higher proportion of raw vegetable intake in total vegetable intake was associated with lower CVD incidence and all-cause mortality, but not with other outcomes (**Supplementary Table 6**). Furthermore, excluding the participants who had outcome events within the first 2 years of follow-up did not materially change the main findings (**Supplementary Table 7**). Subgroup analyses restricted to White participants ($n = 378{,}028$) showed similar results to the primary analysis (**Supplementary Table 8**); and there was no evidence that the associations differed from those of non-White ethnicity, although there were substantially fewer non-White participants ($n = 21{,}558$) (**Supplementary Table 9**), and as such limited power to assess for heterogeneity.

DISCUSSION

In this large prospective cohort study, total vegetable intake was associated with reduced risks of CVD incidence, CVD mortality, and all-cause mortality. When assessing the independent effect of raw and cooked vegetable intake, only raw vegetable intake showed inverse associations with CVD outcomes, whereas cooked vegetables showed no association. However, given the large reductions in the predictive values of total and raw vegetable intake after adjustment for socioeconomic and lifestyle factors, residual confounding is likely to account for much, if not all, of the remaining associations.

The modest inverse associations of total vegetable intake with CVD outcomes and all-cause mortality in our analyses are consistent with previous large-scale observational evidence. For example, a meta-analysis of 24 cohort studies estimated that high vegetable intake reduced all-cause mortality by about 13% (relative risk 0.87 [0.82–0.92]) (15). Previous systematic reviews showed total vegetable consumption was associated with a risk reduction in CVD incidence by 11 (15) to 18% (16), similar to the ∼10% lower risk in this study. Our findings of the inverse association with MI are also in line with published meta-analyses with effect sizes ranging from 9 to 15% (15–17). Although previous studies have also demonstrated an association with a reduced risk of stroke (15–17), we did not find sufficient evidence for such an association.

In contrast to a large number of studies on total vegetable intake, there is limited evidence on the independent effect of raw and cooked vegetables on all-cause mortality. Aune et al. (15) conducted a meta-analysis that found cooked vegetable was associated with 13% (relative risk 0.87 [0.80–0.94]) lower risk of all-cause mortality, and raw vegetable was associated with 12% (relative risk 0.88 [0.79–0.98]) lower risk of mortality, although the analyses of raw and cooked vegetables were not mutually adjusted. Studies that have attempted to assess the independent effects of raw and cooked vegetable intakes on all-cause mortality have reported conflicting results. Our results are broadly consistent with the EPIC study (7), in which both raw vegetable intake and cooked vegetable intake were associated with reduced risk of all-cause mortality. By contrast, the PURE study (8) reported an inverse association with all-cause mortality for raw vegetable intake, but not for cooked vegetable intake, while an Australian cohort study (9) reported that only cooked vegetable intake was associated with lower overall mortality. The characteristics and main findings of these studies are summarized in **Supplementary Table 10**.

In this study, cooked vegetable intake and raw vegetable intake showed different associations with cardiovascular outcomes. We

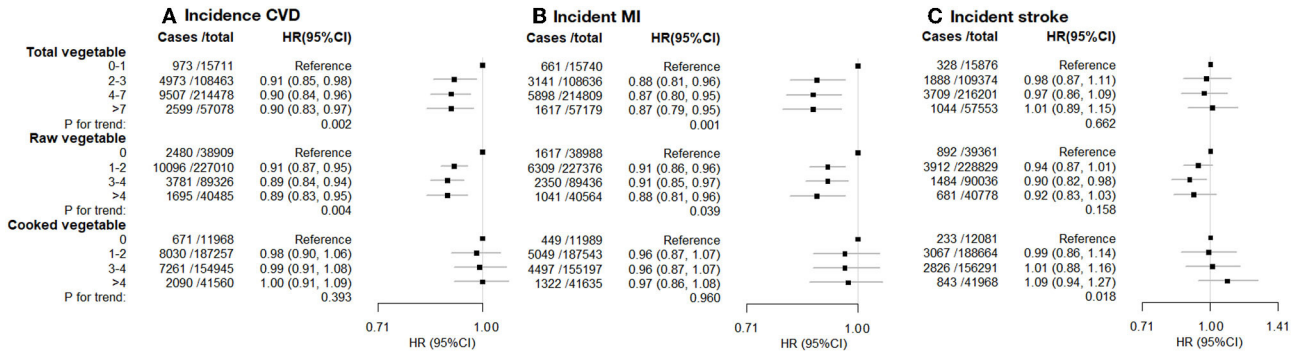

FIGURE 2 | (A) incident CVD. **(B)** incident MI. **(C)** Incident stroke. Incident cardiovascular disease (CVD), myocardial infarction (MI), and stroke vs. vegetable consumption. Hazard ratios (HR; fully adjusted models) and 95% confidence interval (CI) by the level of total, raw, and cooked vegetable consumption (heaped tablespoons/day), relative to the lowest (reference) consumption level. Exclusions as in **Table 1**. The model was stratified by age (<50, 50–60, 60 years), sex, ethnicity, and region, and adjusted for educational attainment, Townsend deprivation index (continuous), hypertension, diabetes, physical activity level, smoking, alcohol consumption, BMI (continuous), use of mineral supplements, use of vitamin supplements, aspirin/ibuprofen, antihypertensive drugs, statins, insulin treatment, intake of fresh fruits, red meat, processed meat, oily fish and non-oily fish.

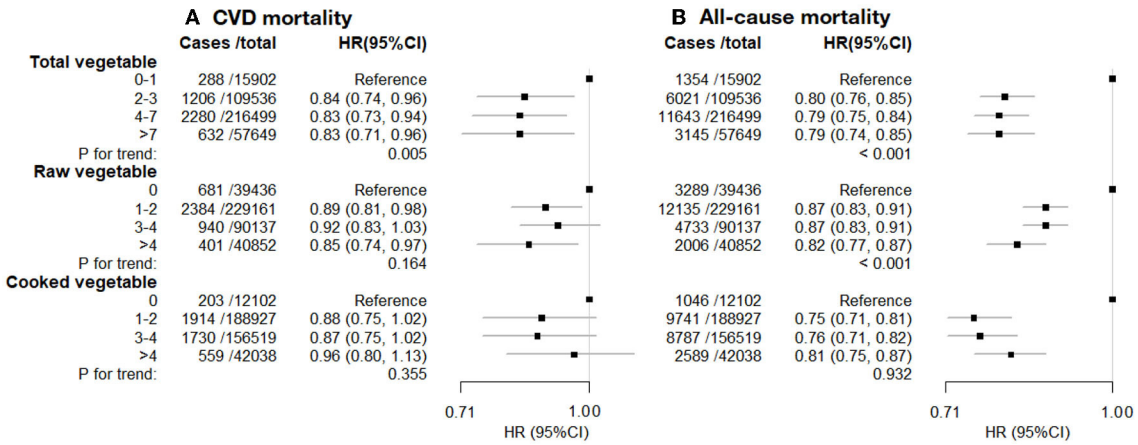

FIGURE 3 | (A) CVD mortality. **(B)** All-cause mortality. Cardiovascular disease (CVD) mortality and all-cause mortality vs. vegetable consumption. Hazard ratios (HR; fully adjusted models) and 95% confidence interval (CI) by the level of total, raw, and cooked vegetable consumption (heaped tablespoons/day), relative to the lowest (reference) consumption level. Exclusions as in **Table 1**. The model was stratified by age (<50, 50–60, 60 years), sex, ethnicity, and region, and adjusted for educational attainment, Townsend deprivation index (continuous), hypertension, diabetes, physical activity level, smoking, alcohol consumption, BMI (continuous), use of mineral supplements, use of vitamin supplements, aspirin/ibuprofen, antihypertensive drugs, statins, insulin treatment, intake of fresh fruits, red meat, processed meat, oily fish and non-oily fish.

found inverse associations of raw vegetables with CVD incidence and mortality, but null associations with cooked vegetables. This is consistent with the MORGEN study, a Dutch cohort (18), in which raw, but not processed, vegetables were associated with a reduced risk of ischemic stroke. In the EPIC cohort (7), there was a stronger inverse association of CVD mortality with raw than cooked vegetables. Whereas the PURE study (8) found no evidence of an association of CVD and raw vegetable intake, and high intakes levels of cooked vegetable was positively associated with CVD incidence.

Previous studies that reported associations of higher levels of vegetable intake with lower risk of CVD have proposed various mechanisms by which these associations might be mediated. For example, it has been suggested that diets high in vegetables have, on average, fewer calories and replace foods that are high in fat, sodium, and glycemic load (15, 19). It has also been hypothesized that the lower risk might be mediated by micronutrients, namely, higher intake of vitamins, polyphenols, and antioxidant compounds (2, 5), which are required for regulating various biological processes, including anti-oxidation, anti-inflammation, lipid metabolism, and endothelial function (20). As for the different associations of raw and cooked vegetables observed in this and other studies, several possible mechanisms have been proposed in previous studies. First, it has been proposed that the kinds of vegetables that are usually consumed cooked (e.g., beans, peas, eggplant) may differ from those typically consumed raw (e.g., lettuce). Second, cooking processes can alter the digestibility of food as well as the

TABLE 2 | Associations between vegetable intake with CVD incidence, myocardial infarction incidence, stroke incidence, CVD mortality and all-cause mortality in basic model and fully-adjusted model.

	Basic model		Fully-adjusted model		Attenuation (% reduction in χ^2)†
	Improvement in fit (χ^2)	HR (95% CI)*	Improvement in fit (χ^2)	HR (95% CI)*	
CVD incidence					
Total vegetable intake	87.8	0.74 (0.69, 0.80)	10.1	0.90 (0.83, 0.97)	88.6
Raw vegetable intake	127.9	0.79 (0.74, 0.84)	23.2	0.89 (0.83, 0.95)	81.9
Cooked vegetable intake	53.0	0.77 (0.71, 0.84)	1.5	1.00 (0.91, 1.09)	97.2
MI incidence					
Total vegetable intake	75.1	0.71 (0.65, 0.78)	11.1	0.87 (0.79, 0.95)	85.2
Raw vegetable intake	88.8	0.78 (0.72, 0.84)	13.3	0.88 (0.81, 0.96)	85.0
Cooked vegetable intake	42.8	0.74 (0.67, 0.83)	0.6	0.97 (0.86, 1.08)	98.5
Stroke incidence					
Total vegetable intake	18.8	0.84 (0.74, 0.95)	2.2	1.01 (0.89, 1.15)	88.1
Raw vegetable intake	31.7	0.85 (0.77, 0.94)	5.6	0.92 (0.83, 1.03)	82.2
Cooked vegetable intake	19.1	0.87 (0.75, 1.01)	6.1	1.09 (0.94, 1.27)	68.3
All CVD mortality					
Total vegetable intake	58.2	0.63 (0.55, 0.73)	8.0	0.83 (0.71, 0.96)	86.3
Raw vegetable intake	63.8	0.74 (0.65, 0.84)	8.2	0.85 (0.74, 0.97)	87.2
Cooked vegetable intake	53.9	0.67 (0.57, 0.78)	6.3	0.96 (0.80, 1.13)	88.4
All-cause mortality					
Total vegetable intake	298.7	0.61 (0.57, 0.65)	57.9	0.80 (0.74, 0.85)	80.6
Raw vegetable intake	352.7	0.69 (0.65, 0.73)	57.3	0.82 (0.77, 0.87)	83.8
Cooked vegetable intake	347.8	0.57 (0.53, 0.61)	72.0	0.81 (0.75, 0.87)	79.3

Improvement in the prediction of relative risk (LR chi-squared statistic) by the addition of the given vegetable intake term to the basic model (in which the relative risk depends only on age, sex, ethnicity, and region), and to the fully-adjusted models with all major potential confounders, including mutual adjustment for raw and cooked vegetables, as described in the Methods.

**Hazard ratio for the highest vs. lowest vegetable intake group.*

† Proportional reduction in chi-squared for the improvement in model fit relative to the basic model, equivalent to the proportion of the association attenuated by the potential confounders.

bioavailability of nutrients (21). For example, Lee et al. found that vitamin C retention after cooking ranged from 0 to 91% for various combinations of cooking methods and vegetable, with higher retention after microwaving and lower retention after boiling (22). Third, the seasoning and oils used in cooking vegetables often increase intake of sodium and fat, which are known risk factors for CVD incidence and mortality (23, 24).

Despite these proposed mechanisms, this study indicates that observed associations of vegetable intakes with CVD risk and all-cause mortality are likely to be mostly accounted for by residual confounding. Studies using Mendelian randomization (which are less susceptible to confounding, and other biases of observational studies) might be particularly useful in reliably assessing the associations of diet on disease risk. For example, a recent Mendelian randomization study that used genetic determinants of plasma vitamin C concentration as a surrogate for vegetable intake reported a null association with ischemic heart disease (odds ratio 0.90 [0.75–1.08]) and all-cause mortality (odds ratio 0.88 [0.72–1.08]), despite strong inverse associations between vitamin C and these outcomes in observational analyses (25).

This study found the observed associations were mainly accounted for by socioeconomic status and lifestyle factors (26). Both the low socioeconomic status and major lifestyle factors, such as smoking and alcohol intake, are established risk factors for CVD, and there is strong evidence that the effect of socioeconomic status is partially mediated by the known lifestyle factors (27). For example, one study reported that an unhealthy lifestyle (including smoking, drinking, obesity, physical inactivity, and others) mediated 34–38% of the association between socioeconomic status and all-cause death (28). Therefore, given the complicated inter-relationship between socioeconomic status, lifestyle, and health outcomes, adjustment of measures of both socioeconomic status and lifestyle factors is likely to be important.

This study has some limitations. First, we did not measure intake of specific types of raw or cooked vegetables, nor were we able to account for differences in cooking methods. Second, vegetable intakes are self-reported in the baseline dietary questionnaire, although the validity and repeatability of the UK Biobank baseline dietary questionnaire have been evaluated and confirmed in previous studies (12). Third, we did not adjust for total calorie intake because such information was not available from the baseline dietary questionnaire, but we did adjusted for physical activity level and BMI, which has been shown as a valid method for isocaloric adjustment (29). Future studies should seek to address these limitations. However, such studies should also be aware of the importance of assessing reliably for

residual confounding using similar methods to this report, or other approaches, such as Mendelian randomization.

Although this report does not find strong evidence of an association between higher vegetable intake and reduced risk of major CVD, the wider literature suggests that increasing vegetable intake is likely to reduce the risk of some other common diseases (4). As such, the public health recommendations on the benefits to health and the environment of a diet that is high in vegetable intake remain.

CONCLUSION

In this study of 0.4 million middle-aged adults with 12-year follow-up, higher intakes of raw but not cooked vegetables were associated with lower CVD risk. However, given the large reductions in the predictive values of raw vegetable intake after adjustment for socioeconomic and lifestyle factors, residual confounding is likely to account for much, if not all, of the remaining associations. This study highlights the need for rigorous assessment for residual confounding in studies of the effects of diet and other lifestyle factors on disease risk and suggests the need to reappraise the evidence on the burden of CVD disease attributable to low vegetable intake in high-income populations.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ukbiobank.ac.uk/.

ETHICS STATEMENT

The studies involving human participants were reviewed and UK Biobank was approved by the North West Multicenter Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QF designed the study and analyzed the data. QF, BL, JK, and MM interpreted results. QF drafted the manuscript. All the coauthors critically reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.831470/full#supplementary-material

REFERENCES

1. Angelino D, Godos J, Ghelfi F, Tieri M, Titta L, Lafranconi A, et al. Fruit and vegetable consumption and health outcomes: an umbrella review of observational studies. *Int J Food Sci Nutr.* (2019) 70:652–67. doi: 10.1080/09637486.2019.1571021
2. Wallace TC, Bailey RL, Blumberg JB, Burton-Freeman B, Chen CYO, Crowe-White KM, et al. Fruits, vegetables, and health: a comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. *Crit Rev Food Sci Nutr.* (2020) 60:2174–211. doi: 10.1080/10408398.2019.1632258
3. US Department of Health and Human Services and US Department of Agriculture. *Dietary guidelines for Americans 2015-2020. 8th edition*. (2015). Available online at: https://health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015 (accessed January 12, 2021).
4. World Health Organization. Healthy diet. *World Health Organization*. (2020) Available online at: https://www.who.int/news-room/fact-sheets/detail/healthy-diet (accessed January 12, 2021).
5. Slavin JL, Lloyd B. Health benefits of fruits and vegetables. *Adv Nutr.* (2012) 3:506–16. doi: 10.3945/an.112.002154
6. Afshin A, Sur PJ, Fay KA, Cornaby L, Ferrara G, Salama JS, et al. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2019) 393:1958–72. doi: 10.1016/S0140-6736(19)30041-8
7. Leenders M, Sluijs I, Ros MM, Boshuizen HC, Siersema PD, Ferrari P, et al. Fruit and vegetable consumption and mortality: European prospective investigation into cancer and nutrition. *Am J Epidemiol.* (2013) 178:590–602. doi: 10.1093/aje/kwt006
8. Miller V, Mente A, Dehghan M, Rangarajan S, Zhang X, Swaminathan S, et al. Fruit, vegetable, and legume intake, and cardiovascular disease and deaths in 18 countries (PURE): a prospective cohort study. *Lancet.* (2017) 390:2037–49. doi: 10.1016/S0140-6736(17)32253-5
9. Nguyen B, Bauman A, Gale J, Banks E, Kritharides L, Ding D. Fruit and vegetable consumption and all-cause mortality: evidence from a large Australian cohort study. *Int J Behav Nutr Phys Act.* (2016) 13:9. doi: 10.1186/s12966-016-0334-5
10. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* (2015) 12:e1001779. doi: 10.1371/journal.pmed.1001779
11. Littlejohns TJ, Sudlow C, Allen NE, Collins R. UK Biobank: opportunities for cardiovascular research. *Eur Heart J.* (2019) 40:1158–66. doi: 10.1093/eurheartj/ehx254

12. Bradbury KE, Young HJ, Guo W, Key TJ. Dietary assessment in UK Biobank: an evaluation of the performance of the touchscreen dietary questionnaire. *J Nutr Sci.* (2018) 7:e6. doi: 10.1017/jns.2017.66
13. Fan M, Sun D, Zhou T, Heianza Y, Lv J, Li L, et al. Sleep patterns, genetic susceptibility, and incident cardiovascular disease: a prospective study of 385 292 UK biobank participants. *Eur Heart J.* (2020) 41:1182–9. doi: 10.1093/eurheartj/ehz849
14. Floud S, Balkwill A, Moser K, Reeves GK, Green J, Beral V, et al. The role of health-related behavioural factors in accounting for inequalities in coronary heart disease risk by education and area deprivation: prospective study of 12 million UK women. *BMC Med.* (2016) 14:145. doi: 10.1186/s12916-016-0687-2
15. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. *Int J Epidemiol.* (2017) 46:1029–56. doi: 10.1093/ije/dyw319
16. Zhan J, Liu YJ, Cai LB, Xu FR, Xie T, He QQ. Fruit and vegetable consumption and risk of cardiovascular disease: a meta-analysis of prospective cohort studies. *Crit Rev Food Sci Nutr.* (2017) 57:1650–63. doi: 10.1080/10408398.2015.1008980
17. Bechthold A, Boeing H, Schwedhelm C, Hoffmann G, Knüppel S, Iqbal K, et al. Food groups and risk of coronary heart disease, stroke and heart failure: a systematic review and dose-response meta-analysis of prospective studies. *Crit Rev Food Sci Nutr.* (2019) 59:1071–90. doi: 10.1080/10408398.2017.1392288
18. Oude Griep LM, Verschuren WMM, Kromhout D, Ocké MC, Geleijnse JM. Raw and processed fruit and vegetable consumption and 10-year stroke incidence in a population-based cohort study in the Netherlands. *Eur J Clin Nutr.* (2011) 65:791–9. doi: 10.1038/ejcn.2011.36
19. Jenkins DJA, Dehghan M, Mente A, Bangdiwala SI, Rangarajan S, Srichaikul K, et al. Glycemic Index, Glycemic Load, and Cardiovascular Disease and Mortality. *N Engl J Med.* (2021) 384:1312–22. doi: 10.1056/NEJMoa2007123
20. Tang GY, Meng X, Li Y, Zhao CN, Liu Q, Li HB. Effects of vegetables on cardiovascular diseases and related mechanisms. *Nutrients.* (2017) 9:857. doi: 10.3390/nu9080857
21. Palermo M, Pellegrini N, Fogliano V. The effect of cooking on the phytochemical content of vegetables. *J Sci Food Agric.* (2014) 94:1057–70. doi: 10.1002/jsfa.6478
22. Lee S, Choi Y, Jeong HS, Lee J, Sung J. Effect of different cooking methods on the content of vitamins and true retention in selected vegetables. *Food Sci Biotechnol.* (2018) 27:333–42. doi: 10.1007/s10068-017-0281-1
23. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ.* (2013) 346:f1325. doi: 10.1136/bmj.f1325
24. Chanita U, Prapimporn S, Daruneewan W, Vijj K, Thakkinstian A. Oil consumption and cardiovascular disease: an umbrella review of systematic reviews and meta-analyses. *Curr Dev Nutr.* (2020) 4:571. doi: 10.1093/cdn/nzaa046_071
25. Kobylecki CJ, Afzal S, Davey Smith G, Nordestgaard BG. Genetically high plasma vitamin C, intake of fruit and vegetables, and risk of ischemic heart disease and all-cause mortality: a Mendelian randomization study. *Am J Clin Nutr.* (2015) 101:1135–43. doi: 10.3945/ajcn.114.104497
26. Tomson J, Emberson J, Hill M, Gordon A, Armitage J, Shipley M, et al. Vitamin D and risk of death from vascular and non-vascular causes in the Whitehall study and meta-analyses of 12,000 deaths. *Eur Heart J.* (2013) 34:1365–74. doi: 10.1093/eurheartj/ehs426
27. Wang J, Geng L. Effects of socioeconomic status on physical and psychological health: lifestyle as a mediator. *Int J Environ Res Public Health.* (2019) 16:281. doi: 10.3390/ijerph16020281
28. Laine JE, Baltar VT, Stringhini S, Gandini M, Chadeau-Hyam M, Kivimaki M, et al. Reducing socio-economic inequalities in all-cause mortality: a counterfactual mediation approach. *Int J Epidemiol.* (2020) 49:497–510. doi: 10.1093/ije/dyz248
29. Jakes RW, Day NE, Luben R, Welch A, Bingham S, Mitchell J, et al. Adjusting for energy intake–what measure to use in nutritional epidemiological studies? *Int J Epidemiol.* (2004) 33:1382–6. doi: 10.1093/ije/dyh181

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GENERAL COMMENTARY
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Commentary: Raw and Cooked Vegetable Consumption and Risk of Cardiovascular Disease: A Study of 400,000 Adults in UK Biobank

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Keywords: vegetable intake, cardiovascular mortality, scientific inference and reasoning, statistical inference abuse, meta-analytic thinking

A Commentary on

Raw and Cooked Vegetable Consumption and Risk of Cardiovascular Disease: A Study of 400,000 Adults in UK Biobank
by Feng, Q., Kim, J. H., Omiyale, W., Bešević, J., Conroy, M., May , M., Yang, Z., Wong, S. Y. S., Tsoi, K. K. F., Allen, N., and Lacey, B. (2022). Front. Nutr. 9:831470. doi: 10.3389/fnut.2022.831470

INTRODUCTION

The purpose of this commentary is to offer a constructive critique on one of the principal findings of this important and interesting study. As described, the objective of the study is to learn more about the independent effects of raw and cooked vegetable consumption on cardiovascular disease (CVD) (1). Associations between vegetable intake and two primary outcomes, namely CVD incidence and CVD mortality, are modeled, and the key quantities of interest are adjusted hazard ratios (HR). The claim is made that cooked vegetable intake and raw vegetable intake showed different associations with both cardiovascular outcomes. In the Discussion section (1), Feng et al. write "When assessing the independent effect of raw and cooked vegetable intake, only raw vegetable intake showed inverse associations with CVD outcomes, whereas cooked vegetables showed no association." The problem with this claim is that, in the case of one of the two primary outcomes, CVD mortality, the evidence does not seem to support such a conclusion.

EVIDENCE AND INTERPRETATION

What does the evidence seem to indicate? Figure 3 presents the HR estimates of the relationship between the level of vegetable intake, relative to the lowest consumption level, and CVD mortality, along with 95% CIs, from the fully adjusted models (1). For both raw vegetables and cooked vegetables, the levels of intake were categorized into four levels (tablespoons/day): 0, 1–2, 3–4, and >4. Using zero as the reference category, the HR estimates for raw vegetables for the three categories above the reference category are 0.89, 0.92, and 0.85. The equivalent HR estimates for cooked vegetables are 0.88, 0.87, and 0.96. On its face, an examination of these HR estimates suggests that raw vegetables and cooked vegetables have similar relationships with CVD mortality. In other words, if it is reasonable here to draw a substantive conclusion that raw vegetable intake showed an inverse association with CVD mortality, then the same ought to be said for cooked vegetables.

Why is there a discrepancy between the evidence offered in Figure 3 and the prose characterization of the results? The reason for the discrepancy is a common error in interpretation.

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The mistake is to conflate a binary statistical declaration with a substantive conclusion. In particular, a declaration of no statistically significant association is conflated with a scientific conclusion that no evidence was found for an association or simply of "no association." The interpretation of the results, as presented, is based on a binary declaration regarding statistical significance, or, equivalently, whether a 95% CI for the HR includes one, rather than on an evaluation of the magnitude of the HR estimates. Feng et al. happen to focus on comparing the highest (>4) vs. the lowest level of vegetable intake, though the aforementioned inappropriate conflation would yield similar interpretational errors if considering other levels of vegetable intake. In short, we shouldn't conclude that there is no association because of a binary statistical decision (e.g., $p >$ 0.05, 95% CI for HR includes one), and we should not conclude that two results are different because of differences in statistical significance (2).

What about uncertainty in the HR estimates? The presentation of 95% CIs in Figure 3 is helpful for quantifying uncertainty in HR estimates. Across all levels of intake for both raw and cooked vegetables, the plausible true values of reduction in risk in CVD mortality, compared to the reference category, range from a high of 20–25% to something close to zero. In other words, if uncertainty is taken into account, the difference in ranges of plausible true HR values that are compatible with the data seem clinically indistinguishable when comparing raw and cooked vegetable intake. Embracing this uncertainty in HR estimates further supports the notion that the evidence is not consistent with an interpretation that raw and cooked vegetables showed different associations with CVD mortality.

How do the findings align with previous studies? Given that both raw vegetable intake and cooked vegetable intake are associated with a reduced risk of CVD mortality (and all-cause mortality), it seems that the findings are entirely consistent with the EPIC study, in this regard. The two other identified prior studies examined the relationship between vegetable intake by type and all-cause mortality. In the case of reviewing the Prospective Urban Rural Epidemiology (PURE) study, Feng et al. mischaracterize the findings. In the Introduction, Discussion, and Table 10, they describe the PURE study as having found an inverse association with all-cause mortality for raw vegetable intake, but not for cooked vegetable intake (1). However, the PURE paper explicitly says that "In the fully adjusted models, both raw and cooked vegetable intakes were inversely associated with total mortality" (3). In the case of reviewing the Australian cohort study, Feng et al. again conflate a statistical declaration with a substantive conclusion, when they write that "only cooked vegetable intake was associated with lower overall mortality" (1). In the Results section of the Australian cohort study paper, the authors note that "The association with raw vegetable consumption showed estimates (and CIs) that were consistent with those for cooked vegetables" (4). Examination of Table 2 suggests that this clinical interpretation is reasonable. Therefore, from a meta-analytic perspective, the findings with regard to all-cause mortality are actually consistent across all four studies.

DISCUSSION

The concern described here might be considered an example of a more general century-old problem of not distinguishing between statistical inference and scientific inference (5). Empirical examinations of the literature in various disciplines suggest that associated interpretational errors happen more often than not (2). For example, the PURE study authors prominently make this sort of mistake in their "Interpretation," as they write that "Higher fruit, vegetable, and legume consumption were associated with a lower risk of non-cardiovascular, and total mortality," explicitly excluding the association with CVD mortality, despite the fact that it was with CVD mortality that the highest reduction in risk was observed (3). While statistics offers useful tools for quantifying some types of uncertainty, generating cumulative knowledge depends on summaries of findings that have fidelity to the evidence.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Feng Q, Kim JH, Omiyale W, Bešević J, Conroy M, May M, et al. Raw and cooked vegetable consumption and risk of cardiovascular disease: a study of 400,000 adults in UK biobank. *Front Nutr.* (2022) 9:831470. doi: 10.3389/fnut.2022.831470
2. Amrhein V, Greenland S, McShane B. Retire statistical significance. *Nature.* (2019) 567:305–7. doi: 10.1038/d41586-019-00857-9
3. Miller V, Mente A, Dehghan M, Rangarajan S, Zhang X, Swaminathan S, et al. Fruit, vegetable, and legume intake, and cardiovascular disease and deaths in 18 countries (PURE): a prospective cohort study. *Lancet.* (2017) 390:2037–49. doi: 10.1016/S0140-6736(17)32253-5
4. Nguyen B, Bauman A, Gale J, Banks E, Kritharides L, Ding D. Fruit and vegetable consumption and all-cause mortality: evidence from a large Australian cohort study. *Int J Behav Nutr Phys Act.* (2016) 13:9. doi: 10.1186/s12966-016-0334-5
5. Wasserstein RL, Schirm AL, Lazar NA. Moving to a WORLD BEYOND "p < 0.05." *Am Stat.* (2019) 73 (sup1):1–19. doi: 10.1080/00031305.2019.1583913

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ORIGINAL RESEARCH
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Prevalence and Prognostic Significance of Malnutrition in Hypertensive Patients in a Community Setting

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Background: Malnutrition is a significantly poor prognostic factor for a variety of cardiovascular diseases. However, its prevalence and prognostic value in hypertensive patients is still unclear. The present study sought to determine the prevalence and prognostic value of malnutrition in hypertensive patients in a community setting.

Methods: We included 9,949 hypertensive patients from the National Health and Nutrition Examination Survey (NHANES) (2005–2014). The Controlling Nutritional Status (CONUT) score, the Nutritional Risk Index (NRI), and the Naples Prognostic Score (NPS) were applied to assess the nutritional status of participants. A Cox regression model was established to examine the association between malnutrition and cardiovascular and all-cause mortality.

Results: In all, 19.9, 3.9, and 82.9% hypertensive patients were considered to have malnutrition as evaluated by the CONUT, NRI, and NPS, respectively. Malnutrition assessed by CONUT and NRI was independently associated with cardiovascular mortality (HR [95% CI]) for mild and moderate-to-severe degree of malnutrition, respectively: 1.41 (1.04–1.91) and 5.79 (2.34–14.29) for CONUT; 2.60 (1.34–5.07) and 3.30 (1.66–6.56) for NRI (all $P < 0.05$), and for all-cause mortality (HR [95% CI]) for mild and moderate-to-severe degree of malnutrition, respectively: 1.48 (1.30–1.70) and 4.87 (3.40–6.98) for CONUT; 1.72 (1.24–2.39) and 2.60 (1.96–3.44) for NRI (all $P < 0.01$). Naples Prognostic Score could only independently predict all-cause mortality.

Conclusions: Malnutrition was common among hypertensive patients and was closely associated with both long-term cardiovascular and all-cause mortality.

Keywords: malnutrition, hypertension, Controlling Nutritional Status (CONUT) score, Nutritional Risk Index (NRI), Naples Prognostic Score, cardiovascular mortality, all-cause mortality

INTRODUCTION

The effect of nutritional state on a variety of cardiovascular diseases is now the subject of increasing concern, as it is modifiable compared to other clinical variables (1). Most previous studies were focused on overnutrition and the results suggested that it was a significant risk factor for cardiovascular disease (2). However, recent studies have reported that malnutrition is a significantly poor prognostic factor of acute coronary artery disease, heart failure, atrial fibrillation, and valvular heart disease (3–5).

Hypertension, one of the most commonly occurring diseases worldwide (6), contributes to the risk of developing coronary heart disease, stroke, and other cardiovascular disease (7). The aged-standardized prevalence of hypertension reported on 2015 was 24.1% for men and 20.1% for women globally (8). Moreover, nutritional factors such as nutrient intake, blood lipids, and high Body Mass Index (BMI) have been shown to be associated with blood pressure control and mortality (9, 10). However, less attention has been paid to the prevalence and prognostic value of malnutrition among hypertensive patients.

Thus, we aimed to determine the prevalence and prognostic value of malnutrition among hypertensive patients in a community setting by using three nutritional screening tools (NSTs), namely Controlling Nutritional Status (CONUT), Nutritional Risk Index (NRI), and Naples Prognostic Score (NPS).

METHODS

Study Population

This retrospective observational study was based on National Health and Nutrition Examination Survey (NHANES) (2005–2014) (11)—a large nationwide survey on the civilian US population conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention. All NHANES study protocols survey protocol was approved by the Ethics Review Committee of NCHS of the Centers for Disease Control and Prevention. All participants had provided written, informed consent for the use of their data. All procedures in this study were conducted in accordance with all the relevant guidelines. We included participants aged ≥18 years with hypertension. However, individuals with missing data on lymphocyte count ($n = 888$), serum albumin ($n = 1{,}060$), serum total cholesterol ($n = 1{,}017$), and height and weight ($n = 532$) was excluded, leaving 9,949 participants for the final analysis (**Figure 1**).

Baseline Assessment

The data on physical examination, questionnaires, and laboratory examination was obtained from NHANES, which were performed in a standardized manner. Covariates including sociodemographic information; current smoking status; current alcohol drinking, medical history (congestive heart disease, coronary heart disease, diabetes, stroke, emphysema, liver disease, and malignant tumor); BMI; hemoglobin; albuminuria; and estimated glomerular filtration rate (eGFR) were assessed. Body Mass Index was defined as the weight in kilograms divided by the square of height in meters. Albuminuria was defined by urinary albumin creatine ratio ≥30 mg/g and eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

Definition of Hypertension

Hypertension and antihypertensive medication history were collected by questionnaires. Blood pressure was obtained with a mercury sphygmomanometer with an appropriately sized cuff by a trained physician. Blood pressure measurement was performed three times and the average value of the three measurements was defined as the systolic blood pressure (SBP) and diastolic blood pressure (DBP). Hypertension was defined as having a self-reported hypertension history or using antihypertensive medications or SBP ≥140 mmHg or DPB ≥90 mmHg.

Nutrition Status Assessment

The CONUT score (12), calculated based on the levels of serum albumin, total cholesterol, and lymphocytes, was developed as a screening tool for early detection of malnutrition. We categorized the scores into three groups: normal, 0–1; mild, 2–4; and moderate-to-severe, 5–12.

The NRI (13), a popular nutrition screening tool in recent years, was originally defined as $1.519 \times$ serum albumin (g/l) $+ 41.7 \times$ (current body weight [kg] /usual body weight [kg]). The actual body weight was usually replaced by ideal body weight which is defined as height (cm) – 100 – ([height (cm) – 150]/2.5) for women and height (cm) – 100 – ([height (cm) – 150]/4) for men. Patients were categorized into three groups according to their NRI: no nutritional risk (NRI ≥ 100), mild nutritional risk ($97.5 \leq$ NRI < 100), and moderate-to-severe nutritional risk (NRI < 97.5).

The NPS (14), a tool to access the nutritional and inflammatory status of patients, is often used among patients with malignancies. The NPS takes into account serum albumin (mg/dl), total cholesterol (mg/dl), the neutrophil:lymphocyte ratio, and lymphocyte:monocyte ratio. A score of 0 is considered normal; scores of 1–2 and 3–4 reflect mild and moderate-to-severe malnutrition, respectively.

Outcomes

The endpoints were long-term cardiovascular or all-cause mortality. The mortality status of participants was obtained by data matching with death certificates in the National Death Index until December 31, 2015. Cardiovascular death was determined based on the International Classification of Diseases, 10th Edition, Clinical Modification System codes (I00–I09, I11, I13, I20–I51).

Statistical Analysis

Baseline characteristics were expressed as a median with interquartile range (25^{th}-75^{th} percentiles) for continuous variables and with categorical data expressed as n (%). Venn diagrams were used to illustrate the relationship

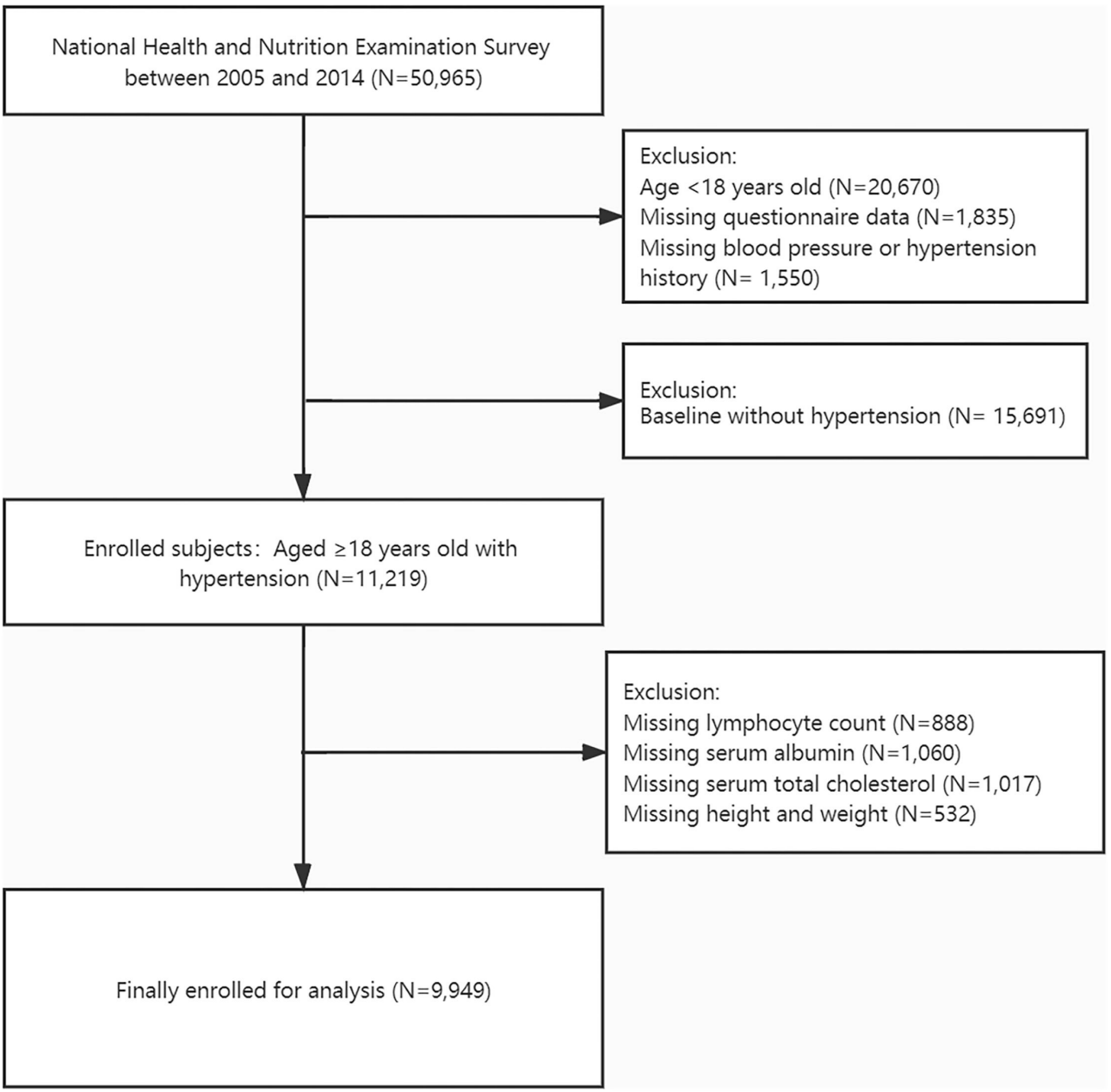

FIGURE 1 | Flow diagram for the selection of the study population.

between the three malnutritional indices. Survival analysis was performed with standardized Kaplan–Meier curves and the log-rank test. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for cardiovascular and all-cause mortality.

All statistical analyses were performed using SPSS v25.0 (IBM Corporation, Armonk, NY, USA) and eulerAPE v3 (15). A two-sided $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Baseline Characteristics of the Study Population

The baseline characteristics of participants are summarized in **Table 1**. The analysis included 9,949 hypertensive patients with a mean age of 49.1 ± 17.8 years; 50.1% subjects were female. Overall, 12.8% participants died during the survey with a mean follow-up time of 5.48 years; of these, 244 (2.5%) participants died from cardiovascular causes.

TABLE 1 | Baseline clinical characteristics of included patients.

Variables	Total (n = 9,949)
Age, years	59.64 ± 15.20
Female gender, *n* (%)	4,992 (50.2)
Ethnicity, *n* (%)	
Non-white	4,750 (47.7)
White	5,199 (52.3)
BMI	30.6 ± 7.2
BMI classification	
Underweight	97 (1.0)
Normal	1,908 (19.2)
Overweight	3,251 (32.7)
Obesity	4,693 (47.2)
Current smoke, *n* (%)	1,896 (19.1)
Diabetes mellitus, *n* (%)	2,136 (22.1)
Stroke, *n* (%)	710 (7.2)
Emphysema, *n* (%)	321 (3.2)
Liver disease, *n* (%)	486 (4.9)
Malignant tumor, *n* (%)	1,367 (13.8)
Congestive heart failure, *n* (%)	608 (6.1)
Coronary heart disease, *n* (%)	763 (7.7)
Albuminuria, *n* (%)	1,949 (19.9)
Hemoglobin (g/L)	13.98 ± 1.58
Serum albumin (g/L)	41.84 ± 3.32
Lymphocyte count (10^9/L)	2.12 ± 1.14
Total cholesterol (mmol/L)	5.06 ± 1.12
eGFR(ml/min/1.73 m^2)	79.11 ± 23.00
Follow-up time, years	5.47 ± 2.82
Long-term mortality*, *n* (%)	
All-cause	1,254 (12.6)
Cardiovascular	241 (2.7)

BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate.

Prevalence and Clinical Feature of Malnutrition

The percentage of malnutrition was 19.9, 3.9, and 82.9% as evaluated by CONUT, NRI, and NPS, respectively. Moreover, the percentage of moderate-to-severe malnutrition varied from 0.7% with CONUT, 2.2% with NRI, and 15.4% with NPS (**Table 2**). The correlation between three NSTs was weak but significant (CONUT vs. NRI: $r = 0.137$, $P < 0.01$; CONUT vs. NPS: $r = 0.226$, $P < 0.01$; NRI vs. NPS: $r = 0.063$, $P < 0.01$ **Figure 2**). Patients with malnutrition assessed by any of the three NSTs were older, with lower BMI and hemoglobin level, and worse renal function and more comorbidities than those with normal nutritional status. The former group of patients also had higher all-cause and cardiovascular mortality (**Table 3**).

Malnutrition Score, All-Cause Mortality and Cardiovascular Mortality

As shown by univariate Cox proportional hazard regression (**Tables 4**, **5**) and Kaplan–Meier survival curves (**Figure 3**), compared to normal nutritional status, worse nutritional status evaluated by any of three NSTs in both continuous form and categorical form tended to have a higher cardiovascular mortality and all-cause mortality. After adjusting for variables such as age, sex, renal insufficiency, and other diseases that could have influenced long-term mortality in univariate Cox regression analyses (**Table 4**), malnutrition evaluated by NPS was not associated with higher incidence of cardiovascular death (mild: adjusted HR = 1.08, 95% CI: 0.69–1.73, $P = 0.76$; moderate-to-severe: adjusted HR = 1.54, 95% CI: 0.91–2.63, $P = 0.11$ **Table 5**; **Figure 4**), but it was still significant for all-cause mortality prediction (mild: adjusted HR = 1.65, 95% CI: 1.30–2.08, $P < 0.01$; moderate-to-severe: adjusted HR = 2.90, 95% CI: 2.24–3.74, $P < 0.01$). Expect for NPS, both CONUT and NRI could independently predict cardiovascular mortality (adjusted HR (95%CI) for mild and moderate-to-severe degree of malnutrition, respectively: 1.41 (1.04–1.91) and 5.79 (2.34–14.29) for CONUT; 2.60 (1.34–5.07) and 3.30 (1.66–6.56) for NRI, all $P < 0.05$) and all-cause mortality (adjusted HR (95%CI) for mild and moderate-to-severe degree of malnutrition, respectively: 1.48 (1.30–1.70) and 4.87 (3.40–6.98) for CONUT; 1.72 (1.24–2.39) and 2.60 (1.96–3.44) for NRI, all $P < 0.01$) and further adjustment for educational level, family income, current smoking, alcohol intake, and diet health also showed a similar result (**Supplementary Tables 1A,B**). By further stratified by age, chronic disease, and blood pressure control, NRI could only independently predict cardiovascular mortality and all-cause mortality in hypertensive patient with aged over 60 or comorbidities (**Supplementary Tables 2A,B**) and had advantages on predicting cardiovascular mortality among hypertensive patients with controlled blood pressure (**Supplementary Table 2C**).

TABLE 2 | Prevalence of malnutrition according to three nutritional screening tools.

	Nutritional indices	Total (n = 9,949)
CONUT	Normal, *n* (%)	7,950 (79.7)
	Mild, *n* (%)	1,934 (19.4)
	Moderate to severe, *n* (%)	65 (0.7)
NRI	Normal, *n* (%)	9,566 (96.2)
	Mild, *n* (%)	164 (1.6)
	Moderate to severe, *n* (%)	219 (2.2)
NPS	Normal, *n* (%)	1,691 (17.0)
	Mild, *n* (%)	6,700 (67.3)
	Moderate to severe, *n* (%)	1,558 (15.7)

CONUT, Controlling Nutritional Status score; NRI, Nutritional Risk Index; NPS, Naples Prognostic Score.

DISCUSSION

In this study, we reported the prevalence and prognostic value of malnutrition among 9,949 hypertensive patients in a community setting by three NSTs. Our research showed that malnutrition as

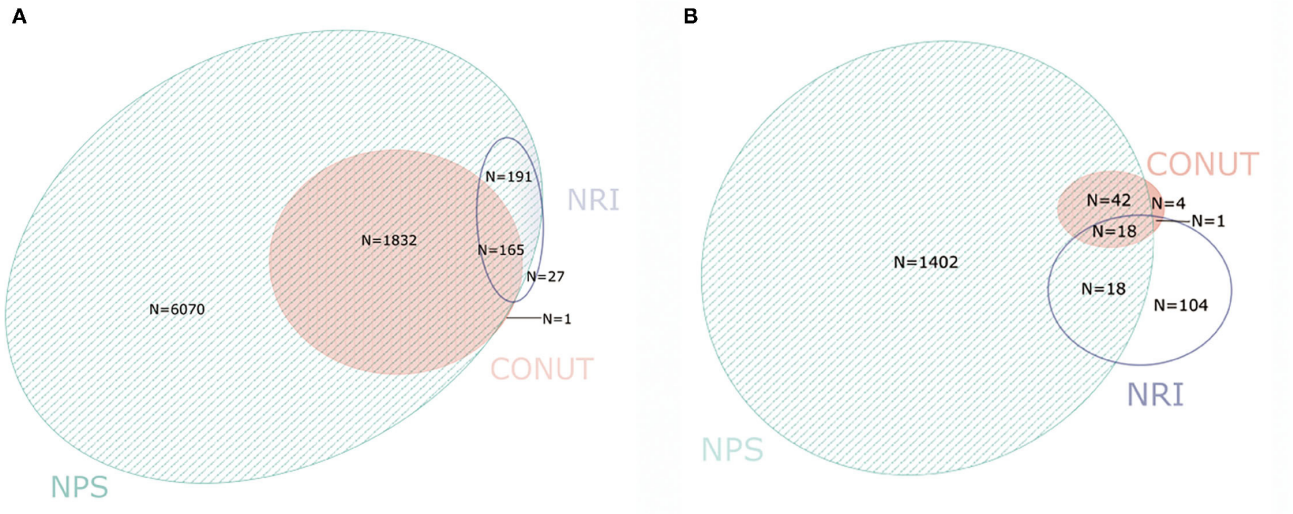

FIGURE 2 | Venn diagram. The numbers in circles indicate the cumulative frequency of malnutrition [any degree **(A)** vs. moderate-severe **(B)**] according to each malnutrition screening tool. The overlapping area of the circles indicate the patient which diagnosed with malnutrition by more than one malnutrition screening tool. CONUT, Controlling Nutritional Status score; NRI, Nutritional Risk Index; NPS, Naples Prognostic Score.

TABLE 3 | Comparison of characteristics of study population by different nutritional status.

Variables	COUNT			NPS			NRI		
	Normal	Malnutrition	*P*	Normal	Malnutrition	*P*	Normal	Malnutrition	*P*
Age, years	58.5 ± 14.9	64.3 ± 15.4	<0.01	55.5 ± 13.7	60.5 ± 15.4	<0.01	59.3 ± 15.1	67.2 ± 14.8	<0.01
Female gender, *n* (%)	4,226 (53.2)	766 (38.3)	<0.01	1,040 (61.5)	3,952 (47.9)	<0.01	4,785 (50.0)	207 (54.0)	0.14
Ethnicity(white), *n* (%)	3,703 (46.6)	1,047 (52.4)	<0.01	579 (34.2)	4,171 (50.5)	<0.01	4,569 (47.8)	181 (47.3)	0.88
BMI	30.8 ± 7.1	30.0 ± 7.3	<0.01	30.4 ± 6.8	30.7 ± 7.2	0.11	31.0 ± 7.0	21.0 ± 2.8	<0.01
Diabetes mellitus, *n* (%)	1,508 (19.7)	615 (31.9)	<0.01	282 (17.3)	1,841 (23.2)	<0.01	2,064 (22.4)	59 (25.6)	<0.01
Stroke, *n* (%)	492 (6.2)	281 (10.9)	<0.01	79 (4.7)	631 (7.7)	<0.01	657 (6.9)	53 (13.9)	<0.01
Emphysema, *n* (%)	221 (2.8)	100 (5.0)	<0.01	32 (1.9)	289 (3.5)	<0.01	287 (3.0)	34 (8.9)	<0.01
Liver disease, *n* (%)	370 (4.7)	116 (5.8)	0.04	66 (3.9)	420 (5.1)	0.04	467 (4.9)	19 (5.0)	1.00
Malignant tumor, *n* (%)	944 (11.9)	423 (21.2)	<0.01	134 (7.9)	1,233 (14.9)	<0.01	1,293 (13.5)	74 (19.3)	<0.01
Congestive heart failure, *n* (%)	370 (4.7)	238 (12.0)	<0.01	48 (2.8)	560 (6.8)	<0.01	576 (6.0)	32 (8.4)	0.07
Coronary heart disease, *n* (%)	475 (6.0)	288 (14.6)	<0.01	52 (3.1)	711 (8.7)	<0.01	737 (7.8)	26 (6.9)	0.59
Albuminuria, *n* (%)	1,425 (18.1)	524 (21.7)	<0.01	248 (14.7)	1,701 (21.0)	<0.01	1,833 (19.4)	116 (32.0)	<0.01
Hemoglobin (g/L)	14.1 ± 1.5	13.5 ± 1.7	<0.01	14.1 ± 1.4	14.0 ± 1.6	<0.01	14.0 ± 1.6	13.0 ± 1.6	<0.01
eGFR(ml/min/1.73m^2)	80.9 ± 22.0	71.9 ± 25.5	<0.01	85.3 ± 19.9	77.8 ± 23.4	<0.01	79.5 ± 22.8	70.4 ± 26.7	<0.01
Long-term mortality, (%)									
All-cause	801 (10.1)	453 (22.7)	<0.01	90 (5.3)	1,164 (14.1)	<0.01	1,119 (11.7)	135 (35.2)	<0.01
Cardiovascular	159 (2.2)	82 (5.0)	<0.01	24 (1.5)	217 (3.0)	<0.01	216 (2.5)	25 (9.2)	<0.01

BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; CONUT, Controlling Nutritional Status score; NRI, Nutritional Risk Index; NPS, Naples Prognostic Score.

assessed by NSTs was common among hypertensive patients and associated with both cardiovascular and all-cause mortality.

Hypertension is considered a nutritional factor-related disease (16, 17). Few studies have analyzed the prevalence of malnutrition in hypertensive patients. Sun et al. (18) reported that the prevalence of malnutrition in elderly patients with hypertension was 52.4 and 27.1% for mild malnutrition and moderate-to-severe malnutrition, respectively, using the CONUT scoring system in a cohort of 336 patients aged ≥80 years. In our study of hypertensive patients, the percentage of individuals with malnutrition assessed by CONUT, NRI, and NPS was 19.9, 3.9, and 82.9%, respectively. Moreover, 0.7, 2.2, and 15.4% of individuals with hypertension were classified as having moderate-to-severe malnutrition assessed by CONUT, NRI, and NPS, respectively. The remarkable difference in malnutrition prevalence among the three screening tools

TABLE 4 | Univariate Cox regression for long-term mortality.

Variables	Cardiovascular death			All cause death		
	HR	95%CI	*P*	HR	95%CI	*P*
Age	1.10	1.09–1.12	<0.01	1.08	1.08–1.09	<0.01
Female gender	0.59	0.46–0.77	<0.01	0.79	0.70–0.88	<0.01
White race	1.79	1.38–2.32	<0.01	1.62	1.45–1.82	<0.01
BMI (continuous)	0.95	0.93–0.97	<0.01	0.95	0.94–0.96	<0.01
Current smoke	1.04	0.75–1.43	0.83	1.08	0.93–1.24	0.31
Diabetes mellitus	1.74	1.32–2.30	<0.01	1.61	1.42–1.82	<0.01
Stroke	3.28	2.34–4.60	<0.01	2.97	2.56–3.44	<0.01
Emphysema	3.44	2.13–5.57	<0.01	3.64	3.00–4.41	<0.01
Liver disease	0.72	0.36–1.46	0.34	1.21	0.94–1.54	0.14
Malignant tumor	1.95	1.43–2.65	<0.01	2.07	1.81–2.36	<0.01
Congestive heart failure	7.12	5.32–9.52	<0.01	3.69	3.18–4.29	<0.01
Coronary heart disease	4.49	3.34–6.04	<0.01	2.48	2.13–2.89	<0.01
Albuminuria	3.45	2.65–4.49	<0.01	3.16	2.81–3.54	<0.01
Hemoglobin	0.83	0.77–0.90	<0.01	0.81	0.78–0.84	<0.01
eGFR < 60 ml/min/1.73 m^2	4.28	3.33–5.52	<0.01	3.55	3.18–3.97	<0.01
CONUT (continuous)	1.54	1.41–1.68	<0.01	1.51	1.46–1.58	<0.01
NRI (continuous)	0.96	0.95–0.96	<0.01	0.96	0.95–0.96	<0.01
NPS (continuous)	1.72	1.52–1.94	<0.01	1.71	1.62–1.81	<0.01

BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; CONUT, Controlling Nutritional Status score; NRI, Nutritional Risk Index; NPS, Naples Prognostic Score.

TABLE 5 | Univariate and multivariate Cox regression of three nutritional screening tools for cardiovascular death and all-cause death.

Variables	Cardiovascular death				All-cause death			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	HR (95%CI)	*P*	HR (95%CI)	*P*	HR (95%CI)	*P*	HR (95%CI)	*P*
CONUT Normal	/		/		/		/	
Mild	2.52 (1.90–3.34)	<0.01	1.41 (1.04–1.91)	0.03	2.51 (2.22–2.84)	<0.01	1.48 (1.30–1.70)	<0.01
Moderate to severe	9.01 (3.70–21.98)	<0.01	5.79 (2.34–14.29)	<0.01	8.35 (5.89–11.84)	<0.01	4.87 (3.40–6.98)	<0.01
NPS Normal	/		/		/		/	
Mild	1.94 (1.23–3.07)	<0.01	1.08 (0.67–1.73)	0.76	2.40 (1.91–3.02)	<0.01	1.65 (1.30–2.08)	<0.01
Moderate to severe	4.69 (2.86–7.70)	<0.01	1.54 (0.91–2.63)	0.11	6.31 (4.96–8.01)	<0.01	2.90 (2.24–3.74)	<0.01
NRI Normal	/		/		/		/	
Mild	3.89 (2.17–6.96)	<0.01	2.60 (1.34–5.07)	<0.01	2.64 (1.96–3.54)	<0.01	1.72 (1.24–2.39)	<0.01
Moderate to severe	3.88 (2.17–6.94)	<0.01	3.30 (1.66–6.56)	<0.01	4.28 (3.42–5.35)	<0.01	2.60 (1.96–3.44)	<0.01

**Adjusted with age, sex, white race, BMI, diabetes mellitus, stroke, emphysema, malignant tumor, congestive heart failure, coronary heart disease, hemoglobin, eGFR (estimated Glomerular Filtration Rate), abuminuria.*

might because of different parameters included or different thresholds for the same parameter. The poor concordance in identifying malnutrition among the three screening tools suggest that they are not interchangeable. The nutritional status of hypertensive patients evaluated by CONUT and NPS suggest that malnutrition was quite common among these patients. On the one hand, it might be owing to the physiological interrelationship between hypertension and inflammation (4, 19). Previous studies have reported that hypertensive patients had higher plasma concentrations of proinflammatory cytokines and acute phase proteins (20). The activation of inflammatory pathways might increase the catabolic demands and result in malnutrition. On the other hand, vitamin D deficiency was prevalent among malnourished patients (21) and its deficiency was highly associated with incidence of metabolic syndrome (22) and cardiovascular disease including hypertension (23), while vitamin D could act on endothelial cells and smooth muscle cells to regulate blood pressure (24).

The relationship between nutritional status and prognosis has been confirmed in some cardiovascular diseases. In a study including 5,062 acute coronary syndrome patients with

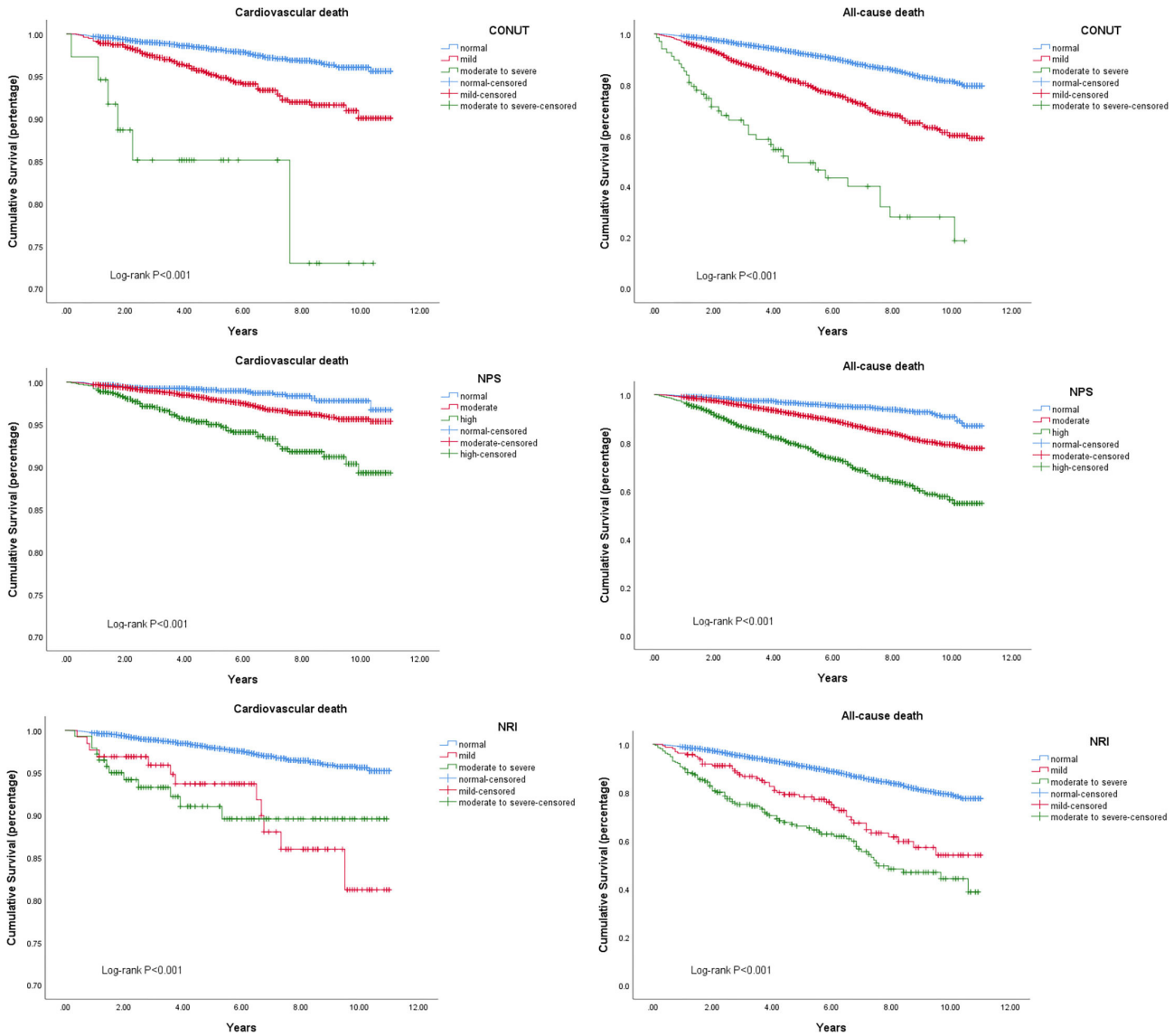

FIGURE 3 | Kaplan–Meier curves of long-term cardiovascular and all-cause mortality for different nutritional status assessed by three nutritional screening tools.

a median age of 66.2 years, multivariate cox proportional hazard regression analysis indicated that malnutrition assessed by CONUT and NRI was an independent factor for all-cause mortality and cardiovascular events (25). Another study involving 336 elderly hypertensive patients confirmed that poor nutritional status assessed by the CONUT score was significantly associated with all-cause mortality (18). In the present study, malnutrition evaluated by different NSTs was also significantly associated with both cardiovascular and all-cause mortality in hypertensive patients. The possible underlying mechanism might be explained by the following facts. First, tumor necrosis factor-alpha (TNF-α), a key inflammatory mediator (26), was found to be higher in patients with moderate-to-severe malnutrition. The underlying inflammation process could involve the pathogenesis and progress of some cardiovascular diseases (27, 28) such as coronary artery disease (29, 30) and heart failure (31), thereby supplementing the cardiovascular risk brought on by hypertension (32) and finally leading to cardiovascular events. Second, as mentioned previously, malnutrition patient always comorbidity with vitamin D deficiency (21, 33). The deficiency of vitamin D was reported associated with higher risk of uncontrolled BP in hypertensive patients (34) which was associated premature vascular death and CVD mortality (35). Third, all three NSTs include serum albumin as a parameter, while hypoalbuminemia was confirmed to be associated with extremely poor prognosis and cardiac cachexia (36, 37).

Given that malnutrition assessed by NSTs is now a common occurrence among hypertensive patients and proved to be associated with higher cardiovascular and all-cause

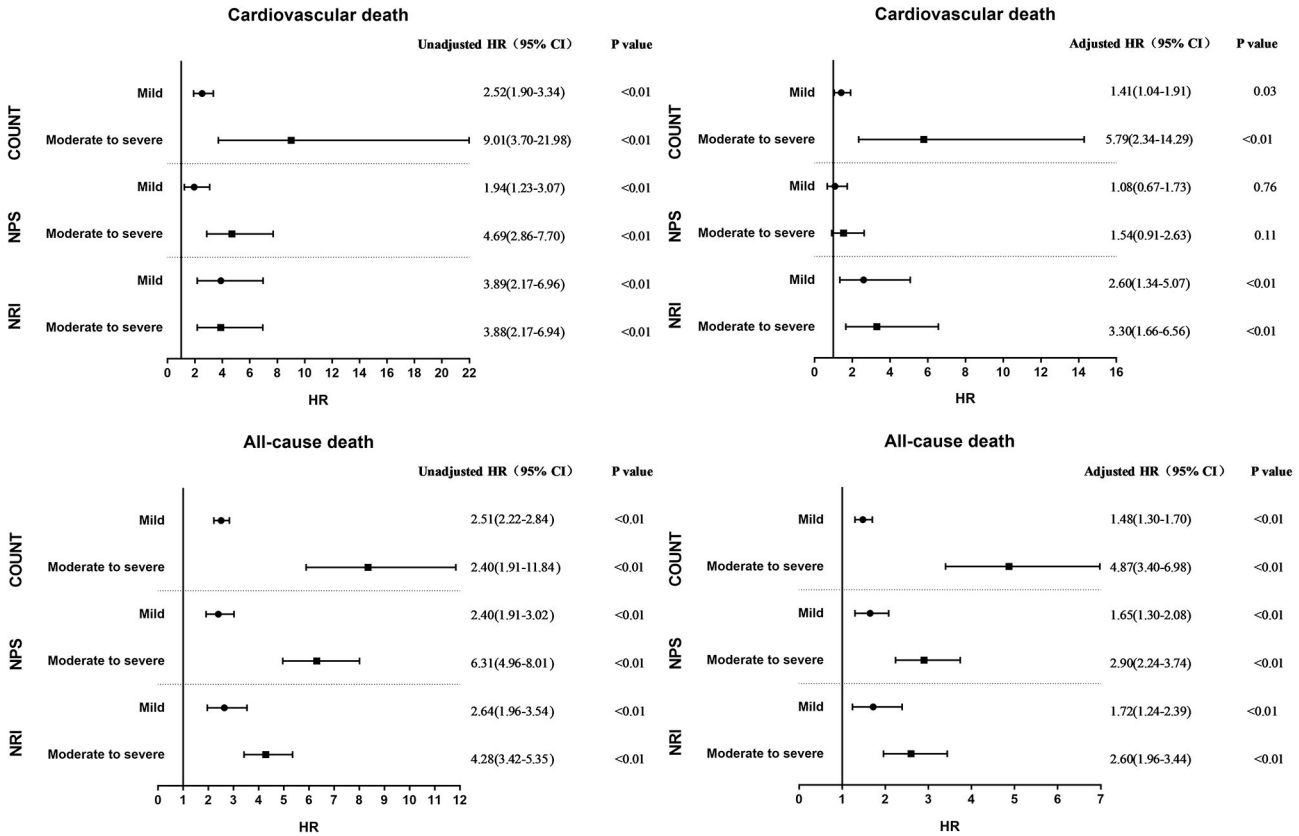

FIGURE 4 | Unadjusted and adjusted hazard ratio for cardiovascular death and all-cause death by degree of malnutrition according to the three nutritional screening tools.

mortality, the question remains, which NST is suitable for clinicians to identify hypertensive patients with malnutrition. Our study showed that NPS was not suitable for predicting long-term cardiovascular mortality, while nutritional status evaluated by NPS was significantly associated with all-cause mortality but not cardiovascular mortality after adjustment for potential confounding factors. This phenomenon might be due to the threshold setting of its parameters. In the NPS scoring system (14), patients with serum albumin <40 g/L were considered to have malnutrition, while the threshold for serum albumin was 35 g/L in the CONUT scoring system (12), which led to its poor ability to distinguish patients with and without malnutrition. Although nutritional status assessed by NRI was significantly associated with both all-cause mortality and cardiovascular mortality, it showed poorer performance than CONUT in differentiating patients with mild and moderate-to-severe malnutrition in terms of mortality risk; this could be explained by the small number of patients with malnutrition evaluated by NRI. Moreover, NRI was recommended for identify malnutrition among elderly hypertensive patients or with comorbidities, while the NRI had advantages on predicting both cardiovascular mortality and all-cause mortality among elderly hypertensive patients or with comorbidities. Overall, CONUT was a more suitable NST than NPS and NRI to identify malnutrition among all hypertensive patients.

LIMITATION

There were some limitations to this study that should be noted. First, the proportion of patients who were classified with moderate-to-severe malnutrition was low leading to the limited value of this study for those hypertensive patients with extremely poor nutritional status. Second, because of the retrospective study design, our findings should be interpreted with caution. Finally, although the models were adjusted for potential risk factors using multiple regression analysis techniques, there may have been some residual confounding factors.

CONCLUSION

Malnutrition evaluated by NSTs was common among hypertensive patients and was closely associated with both long-term cardiovascular mortality and all-cause mortality. Clinicians should make additional efforts for the early identification and management of malnutrition.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Review Committee of NCHS of the Centers for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Z-wY, X-bW, D-qY, and J-yC contributed to the conception or design of the study. Z-wY contributed to the acquisition, analyses, and interpretation of data. Z-wY, X-bW, and B-qF drafted the manuscript. D-qY revised the manuscript critically, had all access to the data, and is responsible for the overall content as guarantor. All authors contributed to refinement of the study protocol and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.822376/full#supplementary-material

REFERENCES

1. Freeman AM, Morris PB, Barnard N, Esselstyn CB, Ros E, Agatston A, et al. Trending cardiovascular nutrition controversies. *J Am Coll Cardiol.* (2017) 69:1172–87. doi: 10.1016/j.jacc.2016.10.086
2. Saxton SN, Clark BJ, Withers SB, Eringa EC, Heagerty AM. Mechanistic links between obesity, diabetes, and blood pressure: role of perivascular adipose tissue. *Physiol Rev.* (2019) 99:1701–63. doi: 10.1152/physrev.00034.2018
3. Goldfarb M, Lauck S, Webb JG, Asgar AW, Perrault LP, Piazza N, et al. Malnutrition and mortality in frail and non-frail older adults undergoing aortic valve replacement. *Circulation.* (2018) 138:2202–11. doi: 10.1161/circulationaha.118.033887
4. Sze S, Pellicori P, Kazmi S, Rigby A, Cleland JGF, Wong K, et al. Prevalence and prognostic significance of malnutrition using 3 scoring systems among outpatients with heart failure: a comparison with body mass index. *JACC Heart Fail.* (2018) 6:476–86. doi: 10.1016/j.jchf.2018.02.018
5. Raposeiras-Roubín S, Abu-Assi E, Paz RC, Rosselló X, Barreiro Pardal C, Piñón Esteban M., et al. Impact of malnutrition in the embolic-haemorrhagic trade-off of elderly patients with atrial fibrillation. *Europace.* (2020) 22:878–87. doi: 10.1093/europace/euaa017
6. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. *Circulation.* (2016) 134:441–50. doi: 10.1161/circulationaha.115.018912
7. Zhou D, Xi B, Zhao M, Wang L, Veeranki SP. Uncontrolled hypertension increases risk of all-cause and cardiovascular disease mortality in US adults: the NHANES III linked mortality study. *Sci Rep.* (2018) 8:9418. doi: 10.1038/s41598-018-27377-2
8. Zhou B, Perel P, Mensah GA, Ezzati M. Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. *Nat Rev Cardiol.* (2021) 18:785–802. doi: 10.1038/s41569-021-00559-8
9. Craig LS, Gage AJ, Thomas AM. Prevalence and predictors of hypertension in Namibia: a national-level cross-sectional study. *PLoS ONE.* (2018) 13:e0204344. doi: 10.1371/journal.pone.0204344
10. Muntner P, Hardy ST, Fine LJ, Jaeger BC, Wozniak G, Levitan EB, et al. Trends in blood pressure control among US adults with hypertension, 1999–2000 to 2017–2018. *Jama.* (2020) 324:1190–200. doi: 10.1001/jama.2020.14545
11. Centers for Disease Control and Prevention, NCHS. *National Health and Nutrition Examination Survey Data.* (2020). Available online at: https://www.cdc.gov/nchs/nhanes/ (accessed September 1, 2020).
12. Wada H, Dohi T, Miyauchi K, Doi S, Konishi H, Naito R, et al. Prognostic impact of nutritional status assessed by the Controlling Nutritional Status score in patients with stable coronary artery disease undergoing percutaneous coronary intervention. *Clin Res Cardiol.* (2017) 106:875–83. doi: 10.1007/s00392-017-1132-z
13. Bouillanne O, Morineau G, Dupont C, Coulombel I, Vincent JP, Nicolis I, et al. Geriatric nutritional risk index: a new index for evaluating at-risk elderly medical patients. *Am J Clin Nutr.* (2005) 82:777–83. doi: 10.1093/ajcn/82.4.777
14. Galizia G, Lieto E, Auricchio A, Cardella F, Mabilia A, Podzemny V, et al. Naples prognostic score, based on nutritional and inflammatory status, is an independent predictor of long-term outcome in patients undergoing surgery for colorectal cancer. *Dis Colon Rectum.* (2017) 60:1273–84. doi: 10.1097/dcr.0000000000000961
15. Micallef L, Rodgers P. eulerAPE: drawing area-proportional 3-Venn diagrams using ellipses. *PLoS ONE.* (2014) 9:e101717. doi: 10.1371/journal.pone.0101717
16. Savica V, Bellinghieri G, Kopple JD. The effect of nutrition on blood pressure. *Annu Rev Nutr.* (2010) 30:365–401. doi: 10.1146/annurev-nutr-010510-103954
17. Lennon SL, DellaValle DM, Rodder SG, Prest M, Sinley RC, Hoy MK, et al. 2015 evidence analysis library evidence-based nutrition practice guideline for the management of hypertension in adults. *J Acad Nutr Diet.* (2017) 117:1445.e17–58.e17. doi: 10.1016/j.jand.2017.04.008
18. Sun X, Luo L, Zhao X, Ye P. Controlling Nutritional Status (CONUT) score as a predictor of all-cause mortality in elderly hypertensive patients: a prospective follow-up study. *BMJ Open.* (2017) 7:e015649. doi: 10.1136/bmjopen-2016-015649
19. Nakagomi A, Kohashi K, Morisawa T, Kosugi M, Endoh I, Kusama Y, et al. Nutritional status is associated with inflammation and predicts a poor outcome in patients with chronic heart failure. *J Atheroscler Thromb.* (2016) 23:713–27. doi: 10.5551/jat.31526
20. Guzik TJ, Touyz RM. Oxidative stress, inflammation, and vascular aging in hypertension. *Hypertension.* (2017) 70:660–7. doi: 10.1161/hypertensionaha.117.07802
21. Merker M, Amsler A, Pereira R, Bolliger R, Tribolet P, Braun N, et al. Vitamin D deficiency is highly prevalent in malnourished inpatients and associated

with higher mortality: a prospective cohort study. *Medicine (Baltimore).* (2019) 98:e18113. doi: 10.1097/md.0000000000018113
22. Wieder-Huszla S, Jurczak A, Szkup M, Barczak K, Dołegowska B, Schneider-Matyka D, et al. Relationships between vitamin D3 and metabolic syndrome. *Int J Environ Res Public Health.* (2019) 16:175. doi: 10.3390/ijerph16020175
23. Latic N, Erben RG. Vitamin D and cardiovascular disease, with emphasis on hypertension, atherosclerosis, and heart failure. *Int J Mol Sci.* (2020) 21:6483. doi: 10.3390/ijms21186483
24. de la Guía-Galipienso F, Martínez-Ferran M, Vallecillo N, Lavie CJ, Sanchis-Gomar F, Pareja-Galeano H. Vitamin D and cardiovascular health. *Clin Nutr.* (2021) 40:2946–57. doi: 10.1016/j.clnu.2020.12.025
25. Raposeiras Roubín S, Abu Assi E, Cespón Fernandez M, Barreiro Pardal C, Lizancos Castro A, Parada JA, et al. Prevalence and prognostic significance of malnutrition in patients with acute coronary syndrome. *J Am Coll Cardiol.* (2020) 76:828–40. doi: 10.1016/j.jacc.2020.06.058
26. Tonet E, Campo G, Maietti E, Formiga F, Martinez-Sellés M, Pavasini R, et al. Nutritional status and all-cause mortality in older adults with acute coronary syndrome. *Clin Nutr.* (2020) 39:1572–9. doi: 10.1016/j.clnu.2019.06.025
27. Golia E, Limongelli G, Natale F, Fimiani F, Maddaloni V, Pariggiano I, et al. Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. *Curr Atheroscler Rep.* (2014) 16:435. doi: 10.1007/s11883-014-0435-z
28. Liu HH, Cao YX, Sun D, Jin JL, Zhang HW, Guo YL, et al. High-sensitivity C-reactive protein and hypertension: combined effects on coronary severity and cardiovascular outcomes. *Hypertens Res.* (2019) 42:1783–93. doi: 10.1038/s41440-019-0293-8
29. Ali L, Schnitzler JG, Kroon J. Metabolism: The road to inflammation and atherosclerosis. *Curr Opin Lipidol.* (2018) 29:474–80. doi: 10.1097/mol.0000000000000550
30. Wang H, Liu Z, Shao J, Lin L, Jiang M, Wang L, et al. Immune and inflammation in acute coronary syndrome: molecular mechanisms and therapeutic implications. *J Immunol Res.* (2020) 2020:4904217. doi: 10.1155/2020/4904217
31. Adamo L, Rocha-Resende C, Prabhu SD, Mann DL. Reappraising the role of inflammation in heart failure. *Nat Rev Cardiol.* (2020) 17:269–85. doi: 10.1038/s41569-019-0315-x
32. Kjeldsen SE. Hypertension and cardiovascular risk: general aspects. *Pharmacol Res.* (2018) 129:95–9. doi: 10.1016/j.phrs.2017.11.003
33. Agarwal A, Gupta SK, Sukumar R. Hyperparathyroidism and malnutrition with severe vitamin D deficiency. *World J Surg.* (2009) 33:2303–13. doi: 10.1007/s00268-009-0044-0
34. Del Pinto R, Wright JT, Monaco A, Pietropaoli D, Ferri C. Vitamin D and blood pressure control among hypertensive adults: results from NHANES 2001–2014. *J Hypertens.* (2020) 38:150–8. doi: 10.1097/hjh.0000000000002231
35. Dan H, Kim J, Kim O. Effects of gender and age on dietary intake and body mass index in hypertensive patients: analysis of the Korea national health and nutrition examination. *Int J Environ Res Public Health.* (2020) 17:4482. doi: 10.3390/ijerph17124482
36. Rahman A, Jafry S, Jeejeebhoy K, Nagpal AD, Pisani B, Agarwala R. Malnutrition and cachexia in heart failure. *JPEN J Parenter Enteral Nutr.* (2016) 40:475–86. doi: 10.1177/0148607114566854
37. Corsetti, G., Pasini, E., Romano, C., Chen-Scarabelli, C., Scarabelli, T. M., Flati, V., et al. (2021). How can malnutrition affect autophagy in chronic heart failure? Focus and perspectives. *Int J Mol Sci* 22:3332. doi: 10.3390/ijms22073332

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CORRECTION
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Corrigendum: Prevalence and Prognostic Significance of Malnutrition in Hypertensive Patients in a Community Setting

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Keywords: malnutrition, hypertension, Controlling Nutritional Status (CONUT) score, Nutritional Risk Index (NRI), Naples Prognostic Score, cardiovascular mortality, all-cause mortality

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A Corrigendum on

Prevalence and Prognostic Significance of Malnutrition in Hypertensive Patients in a Community Setting

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In the published article, there was an error in affiliation 1. Instead of "Department of Clinical Medicine, Shantou University Medical College, Shantou, China," it should be "Shantou University Medical College, Shantou, China."

In the published article, there was also an error in affiliation 2. Instead of "Guangdong Provincial Key Laboratory of Coronary Heart Disease Prevention, Division of Cardiology, Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China," it should be "Division of Cardiology, Guangdong Cardiovascular Institute, Guangdong Provincial Key Laboratory of Coronary Heart Disease Prevention, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China."

In the published article, there was also an error in affiliation 3. Instead of "Division of Critical Care Medicine, Geriatrics Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China," it should be "Division of Geriatrics Intensive Medicine, Guangdong Provincial Geriatrics Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Vegetarian Diet Was Associated With a Lower Risk of Chronic Kidney Disease in Diabetic Patients

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Introduction: Diabetes mellitus (DM) is a pathological hyperglycemic state related to the dysregulation of insulin. Chronic kidney disease (CKD) is a common chronic complication in diabetic patients. A vegetarian diet could be one of the preventive strategies for the occurrence of CKD in patients with diabetes mellitus. However, it is still unknown whether a vegetarian diet lowers the occurrence of CKD in DM patients.

Research Design and Methods: This retrospective study was conducted at Taipei Tzu Chi Hospital from 5 September 2005 to 31 December 2016. Subjects with an HbA1c level > 6.5% or previous history of diabetes mellitus elder than 40 years were grouped based on self-reported dietary habits (vegetarians, lacto-ovo vegetarians and omnivores) in the structured questionnaire. Structural equation modeling (SEM) was applied to estimate the direct and indirect effects of variables on the occurrence of chronic kidney disease.

Results: Among these 2,797 subjects, the participants were grouped into dietary habits as vegans ($n = 207$), lacto-ovo vegetarians ($n = 941$) and omnivores ($n = 1,649$). The incidence of overall CKD was higher in the omnivore group [36.6% vs 30.4% (vegans) and 28.5% (lacto-ovo vegetarian), $p < 0.001$]. In the SEM model, after adjusting for age and sex, the lacto-ovo vegetarian [OR: 0.68, 95% confidence interval (CI): 0.57–0.82] and vegan groups (OR 0.68, 95% CI: 0.49–0.94) were both associated with a lower risk of CKD occurrence than the omnivore group. The vegan diet and lacto-ovo diet lowered the risk related to a high BMI (OR: 0.45, $p < 0.001$, OR: 0.58, $p < 0.001$) and hyperuricemia (OR: 0.53, $p < 0.001$; OR: 0.55, $p < 0.001$) for the occurrence of CKD.

Conclusion: Vegetarian dietary habits were associated with a lower occurrence of CKD in DM patients.

Keywords: diabetes mellitus, chronic kidney disease, vegan diet, lacto-ovo vegetarian diet, obesity, hyperuicemia

INTRODUCTION

Diabetes mellitus (DM) is the pathological hyperglycemic state induced by insulin deficiency or resistance. A chronic hyperglycemic status could contribute to multiple organ dysfunction, including cardiovascular disease, peripheral neuropathy, retinopathy and nephropathy (1). The complications of diabetes mellitus influence patient survival and pose an economic burden for health expenditures; therefore, pharmacologic intervention and lifestyle modifications are important for controlling diabetes mellitus and its complications (2). Beyond pharmacologic strategies such as insulin or oral hyperglycemic agents, lifestyle behavior changes play an important adjunctive role in controlling hyperglycemic status. The Diabetes Prevention Program involving body weight loss and maintaining weekly physical activities has been advocated as the cornerstone for managing diabetic control (2018;3). Dietary counseling also plays an important role in preventing the development of DM. An adequate reduction in calories and fat helps lower the incidence of DM (4), and specific eating habits, such as the Mediterranean-style, Dietary Approaches to Stop Hypertension (DASH) or plant-based diet, are important for the prevention of DM (3, 5,6).

Chronic kidney disease (CKD) is characterized by a progressive decline in glomerular filtration rate or persistent proteinuria for more than 3 months (7). Diabetes mellitus, either type 1 or type 2, is a major metabolic etiology that contributes to CKD (8). At the same time, CKD itself disturbs insulin sensitivity by hyperactivity of the sympathetic tone, renin-angiotensin-aldosterone system and chronic inflammation (9–12). Hyperglycemic status enhances the hyperfiltration of the glomerulus and therefore worsens glomerular hypertrophy and sequential glomerular fibrosis (12). To lessen glomerular hypertrophy and downstream glomerular fibrosis, protein restriction is the main dietary intervention (13). As mentioned in the previous section, a Mediterranean-style diet or plant-based diet is suggested because the reduced protein content might provide benefits in relieving glomerular hypertrophy (14,15).

A vegetarian diet is one strategy for lowering protein ingestion. A vegetarian diet, which is composed of plant-based food, involves the consumption of grains, fruit, vegetables and unsaturated fat. Fish, meat and poultry products are excluded. In the lacto-ovo vegetarian diet, milk, dairy products and eggs are included. In the vegetarian diet, soy, wheat and nuts serve as the major sources of protein without an excessive reduction in calories. Previous studies indicate that a vegetarian-based diet is safe for CKD patients (16), and it plays several protective roles in delaying the initiation of renal replacement therapy (17). Previous cohort studies also provided evidence that a vegetarian diet influenced blood pressure control in CKD patients. Liu et al. demonstrated that a lacto-ovo dietary habit was associated with better blood pressure control in patients with proteinuria (18). Lacto-ovo vegetarian habits also provided better phosphate and lipid control in moderate CKD patients (19).

Based on the evidence above, a vegetarian diet might provide a protective role in CKD patients when protein restriction is the cornerstone of daily care. From our previous study, the vegan and lacto-ovo vegetarian habits provided a protective role in lowering the incidence of CKD (20). However, the role of vegetarian dietary habits in protecting against the occurrence of CKD in DM patients is unknown. The aim of the study was to investigate whether healthy dietary habits, especially vegetarian-based diets, are associated with the occurrence of chronic kidney disease in DM patients.

RESEARCH DESIGN AND METHOD

Study Participants

This retrospective study was conducted at Taipei Tzu Chi Hospital from 5 September 2005 to 31 December 2016 in Taiwan. The database was composed of individuals receiving self-paid health exams at the health checkup center in Taipei Tzu Chi Hospital (New Taipei City, Taiwan). The inclusion criteria were (1) subjects older than 40 years old and (2) subjects with serum hemoglobin A1c (HbA1c) levels $> 6.5\%$ or previous history of diabetes mellitus reported by the subjects. The exclusion criteria included participants without correct identification numbers or insufficient biochemical data. The study was approved by the institutional board of Taipei Tzu-Chi Hospital based on the Declaration of Helsinki (06-XD12-033). Further written informed consent were waived in this retrospective study by the ethical committee of Taipei Tzu-Chi Hospital.

Clinical Assessment

We used the structured questionnaire applied in the studies by Chiu et al. except the food questionnaire (21) from Tzu-Chi medical system. After enrollment, a comprehensive health examination would be performed. Trained research nurse interviewed the participants with the questionnaire with gender, medical history, age, lifestyle habits (including smoking, alcohol and physical activities) and dietary habit. The subjects were grouped based on self-reported dietary habits: vegans, lacto-ovo vegetarian and omnivore. The lacto-ovo vegetarian was defined as an individual who consumed eggs or dairy products or both but no other animal products; a vegan was defined as one who consumed only plant-based foods; an omnivore was defined as one who consumed both plant- and animal-based foods.

An automatic electronic meter (SECA GM-1000, Seoul, South Korea) was used to measure height and weight. The body mass index (BMI, kg/m^2) was calculated based on the measured body weight and height by a well-trained nurse. Blood pressure was measured by an automatic blood pressure machine (Welch Allyn 53000, NJ, United States).

Venous blood was drawn after patients had fasted for at least 12 h. Measurements included levels of serum uric acid, total cholesterol (TCH), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) (Dimension RXL Max integrated chemistry system, Siemens, Erlangen, Germany). Serum creatinine was measured using the alkaline picrate (Jaffe) method. The estimated glomerular filtration rate calculation was based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) from serum creatinine (22).

Hyperuricemia was defined if the serum uric acid level was higher than 7 mg/dL in males and 6 mg/dL in females (23).

Urine protein was determined by an automated urine analyzer (Arkray 4030, Tokyo, Japan) analyzing a single dipstick. The severity of proteinuria was graded into six categories: absent (less than 10 mg/dL), trace (±) (10 to 20 mg/dL), 1 + (30 mg/dL), 2 + (100 mg/dL), 3 + (300 mg/dL) or 4 + (1,000 mg/dL). Patients with trace levels, 1 + level and above were defined as having proteinuria. The presence of CKD was defined as either the presence of proteinuria or an estimated glomerular filtration rate (eGFR) $\leq$ 60 mL/min per 1.73 m^2 (7).

Statistics

To compare the normal and continuous variables between the three groups (vegan, ovo-lacto vegetarian, or omnivore), chi-square and one-way ANOVA were applied. When there were fewer than 5 observed values or the data did not conform to a categorical distribution, Fisher's exact test and the Kruskal–Wallis test were used instead. A multivariable logistic model was used to calculate the adjusted odds ratio (OR). Four separate logistic regression models were applied: an unadjusted model; a crude model (Model 1); a model adjusted for age and gender (Model 2); and a model adjusted for all the other parameters [full model (Model 3)]. The stepwise backward and likelihood ratio test were chosen as the approach for model selection.

Structural Equation Modeling Model

Structural equation modeling (SEM) with Bernoulli distribution in a logistic regression model estimated the direct and indirect effects of the vegetarian, lacto-ovo vegetarian and omnivore diets and other research factors on CKD risk in diabetic patients. We assessed the relationships at two levels, including (1) the direct effects of CKD risk factors on CKD and (2) the indirect effects of vegetarian, lacto-ovo vegetarian and omnivore diets on CKD risk factors. We showed the adjusted odds ratios and 95% confidence intervals and p values.

Variables Assessed in Structural Equation Modeling

We estimated the association with several factors and CKD. In addition, the model also assessed the biochemical values and relative index mediated by the vegetarian and lacto-ovo vegetarian diets compared to the omnivore diet in our SEM model. The biochemical values and relative indices were SBP, HbA1c level, BMI greater than 27, TG over HDL ratio, and high uric acid level. The two-tailed test was used for statistical significance testing, and a p-value < 0.05 was considered significant. When we used the Bonferroni adjustment to assess the difference between the variables, the value was still less than 0.05 for vegetarian versus both subgroups. The study assumes that the statistical significance level was 95% and power was 80%, and the proportion of CKD in diabetic patients was 40%. If the odds ratio of vegetarians to CKD was 0.8, the required sample was 2,774. We assessed the adequacy of the sample size based on the above calculations. All statistical analyses were executed with SAS software version 9.4 (SAS Institute, Inc., Cary, NC, United States) and STATA15.1 (Stata Corp, College Station, TX, United States).

RESULTS

Figure 1 illustrates the flow chart of the enrollment within the study. The total database included 55,929 individuals. After the first exclusion for individuals younger than 40 years old (n = 4,086), individuals with incomplete identification (n = 1,944), individuals with HbA1c levels < 6.5% (n = 50,695) and individuals with incomplete or missing biochemical exam results (n = 30), the total number of subjects with DM within the cohort was 2,797. Among these 2,797 subjects, the participants were grouped by dietary habits as vegans (n = 207), lacto-ovo vegetarians (n = 941) and omnivores (n = 1,649).

Demographic Information of the Subjects With Different Eating Habits

Table 1 displays the demographic information between the groups. There were 207 and 941 participants with vegan and lacto-ovo vegetarian diets, respectively, both of which were lower than the number of participants with an omnivore diet (n = 1,649, $p < 0.001$). The age of the participants with an omnivore diet (61.8 ± 10.7 years old) was lower than that of the participants with a vegan or lacto-ovo vegetarian diet

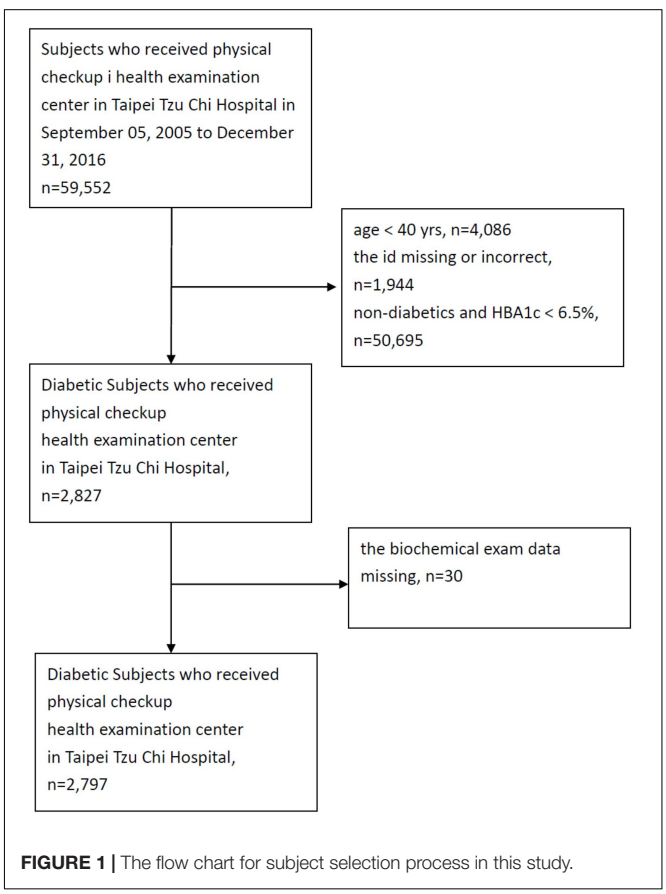

FIGURE 1 | The flow chart for subject selection process in this study.

(67.5 ± 8.9 and 64.3 ± 8.4 years old, respectively, $p < 0.001$). Females were less common in the omnivore group [38.7 vs 60.4% (vegans) and 60.4% (lacto-ovo vegetarian), $p < 0.001$]. Chronic exposure to cigarettes was more prevalent in the omnivore group [12.6 vs 1.9% (vegans) and 1.1% (lacto-ovo vegetarian), $p < 0.001$]. Among the physiological parameters, the omnivore group had a higher BMI than the other groups [25.5 ± 4.1 (kg/m^2) vs 24.4 ± 3.8 kg/m^2 (vegans) and 24.3 ± 3.7 kg/m^2, (lacto-ovo vegetarians) $p < 0.001$]. The percentage of patients with hypertension was higher in the omnivore group [38.6 vs 33.3% (lacto-ovo vegetarian) and 32.9% (vegans), $p = 0.002$]. Among the biochemical parameters, HbA1c level (7.2 ± 1.6%, $p < 0.001$), the ratio of triglyceride/high-density lipoprotein (5.7 ± 1.6, $p < 0.001$) and the percentage of participants with hyperuricemia (14.8%, $p < 0.001$) were all higher in the omnivore group. Regarding the parameters indicating CKD, the omnivore group had a higher incidence of proteinuria [27.7 vs 21.7% (vegans) and 20.5% (lacto-ovo vegetarian), $p < 0.001$]. The proportion of participants with stage 1-2 CKD was also higher in the omnivore group [21.4 vs 14.5% (vegans) and 16.6% (lacto-ovo vegetarian), $p = 0.002$]. The incidence of overall CKD was higher in the omnivore group [36.6 vs 30.4% (vegans) and 28.5% (lacto-ovo vegetarian), $p < 0.001$].

The Odds Ratio for the Occurrence of Chronic Kidney Disease by Demographic Factors and the Different Dietary Habits in DM Individuals in the Logistic Regression Model

Table 2 displays the odds ratio for CKD by risk factors such as physiological and biochemical parameters and dietary habits. In the crude logistic regression model, older age (OR 1.03, 95% CI: 1.02–1.04), male sex (OR 1.41, 95% CI: 1.21–1.67), smoking habit (OR 1.51, 95% CI: 1.15–2.00), BMI > 27 kg/m^2 (OR 1.58, 95% CI: 1.33–1.88), every 10 mmHg increase in systolic pressure (OR 1.18, 95% CI: 1.12–1.24), HbA1c level (OR 1.15, 95% CI: 1.10–1.21), the ratio of triglycerides to high-density lipoprotein (OR 1.05, 95% CI: 1.02–1.07) and the occurrence of hyperuricemia (OR 2.00, 95% CI: 1.60–2.53) all posed hazards for the occurrence of CKD in diabetic patients. When compared with the omnivore diet, the lacto-ovo vegetarian diet had a protective effect against

TABLE 1 | The demographic characteristic of subjects in the community.

	Vegan	Lacto-ovo vegetarians	Omnivore diet	*p*-value
N	207	941	1,649	<0.001
Age group, years-old, *n* (%)				
40–49	5 (2.4)	26 (2.8)	109 (6.6)	<0.001
50–69	14 (6.8)	150 (15.9)	376 (22.8)	<0.001
60–69	77 (37.2)	386 (41.0)	583 (35.4)	0.016
>70	111 (53.6)	375 (39.9)	540 (32.7)	<0.001
Age, years-old, mean(SD)	67.5 (8.9)	64.3 (8.4)	61.8 (10.7)	<0.001
Gender, *n* (%)				
male	82 (39.6)	373 (39.6)	1011 (61.3)	<0.001
female	125 (60.4)	568 (60.4)	638 (38.7)	<0.001
Current smoking, n (%)	4 (1.9)	10 (1.1)	208 (12.6)	<0.001
BMI, Kg/m^{2,} mean(SD)	24.4 (3.8)	24.3 (3.7)	25.5 (4.1)	<0.001
> 27, n (%)	41 (19.8)	196 (20.8)	526 (31.9)	<0.001
Systolic BP, mmHg, mean(SD)	126 (17)	125 (16)	126 (15)	0.42
Hypertension, n (%)	68 (32.9)	313 (33.3)	637 (38.6)	0.013
HbA1c,%, mean(SD)	7.1 (1.9)	6.8 (1.5)	7.2 (1.6)	0.002
TG/HDL ratio, mean(SD)	3.5 (3.2)	3.3 (3.1)	4 (4.2)	<0.001
Uric acid, mg/dL, mean(SD)	5.2 (1.3)	5.3 (1.4)	5.7 (1.6)	<0.001
Hyperuricemia, n (%)	15 (7.2)	77 (8.2)	244 (14.8)	<0.001
Proteinuria, n (%)	45 (21.7)	193 (20.5)	457 (27.7)	<0.001
Creatinine, mg/dL, mean(SD)	0.9 (0.7)	0.9 (0.4)	1.0 (0.4)	<0.001
CKD-EPI eGFR, mL/min/1.73 m^2, mean(SD)	76 (16)	79 (15)	78 (17)	0.99
CKD stage, n (%)				
1–2	30 (14.5)	156 (16.6)	353 (21.4)	0.002
3	31 (15.0)	108 (11.5)	233 (14.1)	0.12
4–5	2 (1.0)	4 (0.4)	17 (1.0)	0.25
CKD, n (%)	63 (30.4)	268 (28.5)	603 (36.6)	<0.001

SD: standard deviation.
BMI: body mass index, Systolic BP: systolic blood pressure, HbA1c: hemoglobin A1c.
TG/HDL ratio: triglyceride to high-density lipoprotein cholesterol ratio.
CKD, chronic kidney disease.

TABLE 2 | Risk in different demographic characteristics and eating habits for occurrence of CKD (n = 2,797).

	Model 1 Odd ratio (95% confidence interval)	Model 2 Odd ratio (95% confidence interval)	Model 3 Odd ratio (95% confidence interval)
Vegan vs. Omnivores	0.76 (0.56–1.04)	0.68 (0.49–0.94)	0.75 (0.54−1.04)
Lacto-ovo vegetarian vs. Omnivores	0.69 (0.58–0.82)	0.68 (0.57–0.82)	0.78 (0.65−0.95)
Age per years old	1.03 (1.02–1.04)	1.03 (1.02–1.04)	1.03 (1.02−1.04)
Male vs. female	1.42 (1.21–1.67)	1.38 (1.17–1.62)	1.24 (1.04−1.47)
Current smoking	1.51 (1.15–2.00)		1.40 (1.03−1.90)
BMI > 27	1.58 (1.33–1.88)		1.32 (1.09−1.59)
Systolic BP, per 10 mmHg	1.18 (1.12–1.24)		1.09 (1.04−1.16)
HbA1c	1.15 (1.10–1.21)		1.12 (1.07−1.18)
TG/HDL ratio	1.05 (1.02–1.07)		1.03 (1.01−1.05)
Hyperuricemia	2.00 (1.60–2.53)		1.80 (1.42−2.29)

MI: body mass index, Systolic BP: systolic blood pressure, HbA1c: hemoglobin A1c.
TG/HDL ratio: triglyceride to high-density lipoprotein cholesterol ratio.
CKD, chronic kidney disease.
Model 1 crude model.
Model 2 adjusted age, gender, vegan, lacto-ovo vegetarians and omnivores.
Model 3 adjusted the variables in model 2 and current smoking, BMI > 27, systolic BP, HbA1c, TG/HDL ratio and hyperuricemia.

the occurrence of CKD (OR 69, 95% CI: 0.58–0.82). The vegan diet also had a lower risk of CKD (vs omnivores, OR 0.76, 95% CI: 0.56–1.04). When adjusting for age and sex, lacto-ovo vegetarian (OR: 0.68, 95% CI: 0.57–0.82) and vegan habits (OR 0.68, 95% CI: 0.49–0.94) may both have a lower risk of CKD. In the full model adjustment, lacto-ovo vegetarians showed a lower risk of CKD occurrence (OR 0.78, 95% CI: 0.65–0.95).

The Adjunctively Lowering Effect of Chronic Kidney Disease Occurrence by Different Dietary Habits in DM Individuals in the Structural Equation Modeling Model

Figure 2 shows the effect of dietary habits on the interactions of the risk factors for CKD in DM patients by using the SEM model. The lacto-ovo diet had the direct effect on lowering the occurrence of CKD. The vegan diet did not lower the occurrence of CKD in SEM model, although it provided the possible protective effect after adjusting age and gender in model 2. However, a vegan diet lowered the risk related to higher BMI (OR: 0.45, $p < 0.001$) and hyperuricemia (OR: 0.53, $p = 0.004$) for the occurrence of CKD. The lacto-ovo diet lowered the risk of CKD directly, as illustrated in **Table 2**. The Lacto-ovo diet also mitigated the risk related to higher BMI (OR: 0.58, $p < 0.001$) and hyperuricemia (OR: 0.55, $p < 0.001$) for the occurrence of CKD. It also meaning that the lacto-ovo-vegetarian diet also indirectly affects occurrence of CKD through effects on hyperuricemia acid and risk related to higher BMI.

DISCUSSION

We found that traditional risk factors, such as hypertension, obesity, hyperuricemia and consumption of cigarettes, were associated with the occurrence of CKD in DM patients and that vegetarian dietary habits were associated with a lower risk of hyperuricemia and BMI > 27 kg/m^2 in the occurrence of CKD in the SEM model. Among the different dietary patterns, vegetarians and lacto-ovo vegetarians had a lower incidence of CKD than the omnivores. While the traditional risk factors pose hazards for the occurrence of CKD, lacto-ovo and vegetarian diets provided protective effects after adjusting for sex and age and other traditional risk factors in a multivariable logistic regression model.

Dietary intervention has been applied to alleviate the complications of metabolic diseases. In controlling hypertension, a dietary approach to stop hypertension encourages reduced ingestion of sodium and high consumption of whole grains and low-fat dairy products. During the past 2 decades, the dietary behavior trend changed in Taiwan. The concept of a healthy diet encouraged adults to increase the ingestion of vegetables, grains, and soy products and avoid excessive ingestion of red meat or animal oils (24). Vegan and lacto-ovo vegetarian diets also provide similar effects for controlling blood pressure. The major components of the vegetarian diet are nuts, wheat and soy-based protein. As described in the previous sections, protein restriction is the cornerstone of dietary intervention in treating CKD, and the proportion of protein might shift from meat to whole grains, legumes or soy-based food (25). In advanced CKD patients, the enhanced consumption of grains might accompany hyperphosphatemia, but the calories from grains could trade off energy and reduce total protein ingestion (25, 26). Whole grain food also improved blood sugar control in DM (26), which might reflect less severe CKD in DM patients (27). A clinical trial by Dobre et al. showed that 12 weeks of supplementation with β-glucan from the grains also lowered the production of trimethylamine N-oxide within the body (28). Soy-based foods are common in Eastern Asian countries, and

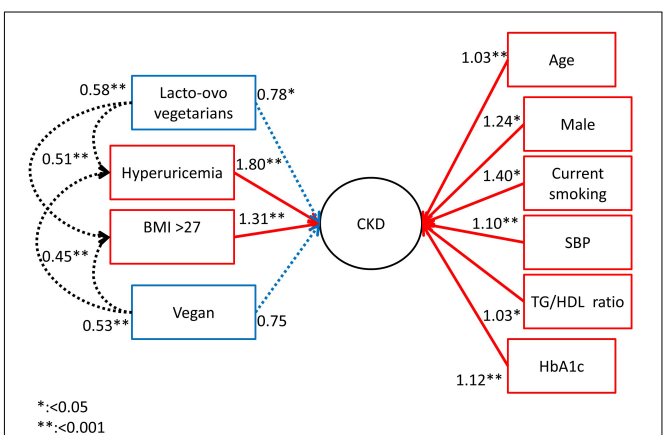

FIGURE 2 | The odds ratio of risk factors on CKD in SEM model.

a soy-based diet habit was associated with a lower incidence of mild cognitive impairment (29). A soy-based diet also improved the survival associated with cognitive impairment (30). The soy-base protein content also has a renoprotective effect. From the *in vivo* study by Chen et al. soy β-conglycinin could directly enhance insulin sensitivity and alleviate the activation of renin-angiotensin-aldosteronism in streptozotocin-treated Wistar rats. The histologic progression of DM nephropathy could be retarded after the administration of soy β-conglycinin (31). The increased expression of nephrin in streptozotocin-treated rats was noted if soy β-conglycinin was given in the diet (32). From previous *in vivo* studies, energy expenditure and energy gain increased in rats receiving a low-protein diet compared with rats receiving a normoproteic diet. At the same time, brown adipose tissue could be lessened by increasing insulin sensitivity even when sympathetic tone increased (33). Sympathetic hyperactivity is common in DM patients, and sympathetic hyperactivity is associated with higher cardiovascular comorbidity. Since a vegan diet provides benefits for insulin sensitivity and metabolic adjustment related to adipose tissue, our result is also consistent with the conclusions from other studies. In the multivariates logistic regression, the vegan diet, in comparison with lacto-ovo vegetarians diet, did not provide the protective effect in CKD in crude model. However, the protective effect was demonstrated after adjusting age and gender. The demographic result illustrated that the age in vegan group was higher than other groups, and the advanced age was a risk factor for development of CKD. Our result might illustrate that both lacto-ovo vegetarians and vegan diet might provide a protective role in decreasing the development of CKD in DM subjects.

The role of a vegan diet in alleviating CKD progression has aroused growing attention. Dietary intervention for CKD prevention or progression includes restriction of daily protein, salt and inorganic phosphorus (15). From the aspect of protein restriction, daily protein ingestion is an important strategy for lowering intraglomerular hypertension and reducing the generation of urea and acid accumulation within the body (13). A protein restriction strategy reduces the decline in glomerular filtration rate in CKD patients and therefore delays entry into dialysis. In the daily diet, processed food and meat have been regarded as sources of exogenous acid in CKD because of excessive catabolism. In addition, animal-based proteins are the major source of purine, which is converted to uric acid. Hyperuricemia is an important risk factor for cardiovascular comorbidities in patients with metabolic syndrome since it serves as an important source of inflammation and oxidative stress. The dietary approach to stop hypertension, which is composed of grains, fish, and milk rather than red meat, might contribute a partial effect in lowering serum uric acid. From the study by Miller et al. the DASH diet lowered the serum uric acid level compared with an omnivore diet (34). When comparing the vegetarian diet with the omnivore diet, the urate-lowering effects differed in different studies. The EPIC-Oxford study enrolled 65,429 subjects in the United Kingdom, and the results demonstrated that subjects fed vegetarian diets had higher serum concentrations of uric acid (35). From a study by Chiu et al. vegans had lower uric acid levels than non-vegetarians in the cohort study initiated in the Buddhist hospital in Taiwan (36). In a study by Chiu et al. a vegetarian diet lowered serum uric acid levels, and the lowering effect was more obvious in patients with hyperlipidemia and diuretic users. The urate-lowering effect was not observed in the DM patients from the cohort study by Chiu et al. but diuretics are commonly used in CKD patients for adequate control of body fluid and blood pressure. However, diuresis accompanies the enhanced reabsorption of urate from the proximal tubules. Therefore, a vegetarian diet might be an important intervention to manage hyperuricemia in patients with DM nephropathy.

The safety of the low-protein diet has been confirmed in multiple studies. Soy is an important component of the vegetarian diet in Taiwanese society to replace the protein source from red meat or fish. Such dietary habits could provide sufficient calories compared with a non-vegetarian diet (36). It has been confirmed that nutritional markers such as serum albumin and BMI are similar when a low-protein diet is applied. From the clinical evidence, the low-protein diet habit provided a protective role in lowering the overall mortality in the younger population from the NHANES III database (37). A recent meta-analysis from Naghshi et al. also provided evidence that all-cause mortality could be lessened by consuming a plant-based diet (38). From the aspect of mortality, the plant-based diet provides a benefit compared with a high-animal protein diet. In specific subgroups of CKD, a low-protein diet also provided clinical benefits. In pregnant CKD patients, the incidence of small for gestational age or extreme preterm babies was lowered when the patients used vegan-based protein restriction (16). From the aspect of homeostasis of calcium and phosphate in CKD, a vegetarian diet also played a conjunctive role in lowering the phosphate burden, while the body mass or fat might not be influenced (21). Beyond the consideration of religious beliefs, a vegetarian diet might be a safe dietary intervention when managing CKD.

There are still several limits in this study. This cohort study did not provide precise gradients of daily intake for all participants. This category was defined by the reply from the participants. We did not define the vegans by using the scales reflecting the daily food frequency, and the vegans or lacto-ovo vegans would not be digitalized. Further validated questionnaire such as 64-item food frequency questionnaire might be helpful to validate the accuracy of the self-report dietary habit. Therefore, the effect of calories and the proportion of protein could not be reflected directly. However, the study from Chiu et al. (21) demonstrated that the caloric content in vegetarian and non-vegetarian was similar for patients in Tzu-Chi medical system (1,705 vs 1,740 kcal, $p = 0.11$). The percentage of protein was $13 \pm 1\%$ and $12 \pm 1\%$, respectively. The more precise measurement on the composition of diet in each individual might be needed in future study. Second, the study was a retrospective study, not a longitudinal study. We used the SEM model to validate the effect of vegetarian diets. The SEM model could express the interactions between different variables to predict the specific disease. From a previous study, the SEM model helped validate the efficacy of biomarkers for predicting CKD, such as Kim-1 (22, 39). In DM patients, the SEM model also played an important role in predicting the risk of CKD. From a study by Lee et al. hyperuricemia also contributed to the

occurrence of DM nephropathy based on the SEM model (10, 40). However, such a model could not demonstrate the longitudinal variation in physiological or biochemical parameters, such as the change in blood pressure and decline in estimated glomerular filtration. Third, the study population was from a single institute with the foundation of the Buddhist religion. Forty-one percent of the participants of the cohort study were vegans or lacto-ovo vegetarians, which might be higher than that in other cohort studies (40, 41). Therefore, further studies with large populations and longitudinal follow-up might be needed. Finally, our database used the result for the self-paid health exams at the health checkup center. As the definition of American Diabetes association, the diagnosis of diabetes mellitus should be confirmed based on the random sugar, the fasting sugar and glycosylated hemoglobin (HbA1c) and Oral Glucose Tolerance Test (42). HbA1c was a convenient measurement for diagnosing DM. Our database measured HbA1c only, and therefore the diagnosis of diabetes mellitus could not be fulfilled according to ADA. However, the HbA1c might not detect DM with advanced CKD. However, the advanced CKD in our database was 1%, and the false-negative effect of HbA1c might not occurred. Besides, the effect of medication such as anti-diabetic or anti-hypertensive medications and the legacy of diabetes were not assessed in the study. The classification of diabetes mellitus, such as insulin deficient diabetes mellitus or mature onset of diabetes of the young could not be differentiated. The connection between the self-paid examination with the medical record in Tzu-Chi Medical system might provide more comprehensive aspects to understanding the different effect of diabetes mellitus.

In summary, the study investigated the role of lacto-ovo vegetarian and vegan diets in DM nephropathy. In our study, vegan and lacto-ovo vegetarian diets decreased CKD in DM patients. The protective effect of a vegan diet might be mediated by alleviating hyperuricemia.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The study was approved by the Institutional Board of Taipei Tzu-Chi Hospital based on the Declaration of Helsinki (06-XD12-033). Further written informed consent were waived in this retrospective study by the Ethical Committee of Taipei Tzu-Chi Hospital.

AUTHOR CONTRIBUTIONS

Y-CH drafted the manuscript. J-SL executed statistical analysis of the dataset. S-YH and H-WL provided the data base. W-HT, H-FH, and K-LK designed the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2014) 37 (Suppl. 1):S81–90.
2. American Diabetes Association. Economic costs of diabetes in the U.S. in 2017. *Diabetes Care.* (2018) 41:917–28. doi: 10.2337/dci18-0007
3. American Diabetes Association. 3. Prevention or delay of type 2 diabetes: standards of medical care in diabetes—2021. *Diabetes Care.* (2020) 44:S34–9. doi: 10.2337/dc21-S003
4. Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L, et al. Effect of weight loss with lifestyle intervention on risk of diabetes. *Diabetes Care.* (2006) 29:2102–7. doi: 10.2337/dc06-0560
5. Esposito K, Chiodini P, Maiorino MI, Bellastella G, Panagiotakos D, Giugliano D. Which diet for prevention of type 2 diabetes? A meta-analysis of prospective studies. *Endocrine.* (2014) 47:107–16. doi: 10.1007/s12020-014-0264-4
6. Chiu THT, Pan WH, Lin MN, Lin CL. Vegetarian diet, change in dietary patterns, and diabetes risk: a prospective study. *Nutr Diabetes.* (2018) 8:12. doi: 10.1038/s41387-018-0022-4
7. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *Am J Kidney Dis.* (2014) 63:713–35. doi: 10.1053/j.ajkd.2014.01.416
8. Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. *J Formos Med Assoc.* (2018) 117:662–75. doi: 10.1016/j.jfma.2018.02.007
9. De Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett.* (2008) 582:97–105.
10. Underwood PC, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. *Curr Hypertens Rep.* (2013) 15:59–70. doi: 10.1007/s11906-012-0323-2
11. Moreira MC, Pinto IS, Mourão AA, Fajemiroye JO, Colombari E, Reis A, et al. Does the sympathetic nervous system contribute to the pathophysiology of metabolic syndrome? *Front Physiol.* (2015) 6:234. doi: 10.3389/fphys.2015.00234
12. Silva Dos Santos D, Polidoro JZ, Borges-Júnior FA, Girardi ACC. Cardioprotection conferred by sodium-glucose cotransporter 2 inhibitors: a renal proximal tubule perspective. *Am J Physiol Cell Physiol.* (2020) 318:C328–36. doi: 10.1152/ajpcell.00275.2019
13. Kalantar-Zadeh K, Fouque D. Nutritional management of chronic kidney disease. *N Engl J Med.* (2017) 377:1765–76.
14. Chauveau P, Aparicio M, Bellizzi V, Campbell K, Hong X, Johansson L, et al. Mediterranean diet as the diet of choice for patients with chronic kidney disease. *Nephrol Dial Transplant.* (2018) 33:725–35. doi: 10.1093/ndt/gfx085
15. Carrero JJ, González-Ortiz A. Plant-based diets to manage the risks and complications of chronic kidney disease. *Nat Rev Nephrol.* (2020) 16:525–42. doi: 10.1038/s41581-020-0297-2
16. Attini R, Leone F, Parisi S, Fassio F, Capizzi I, Loi V, et al. Vegan-vegetarian low-protein supplemented diets in pregnant CKD patients: fifteen years of experience. *BMC Nephrol.* (2016) 17:132. doi: 10.1186/s12882-016-0339-y

17. Garneata L, Stancu A, Dragomir D, Stefan G, Mircescu G. Ketoanalogue-supplemented vegetarian very low-protein diet and CKD progression. *J Am Soc Nephrol.* (2016) 27:2164–76. doi: 10.1681/ASN.2015040369
18. Liu HW, Liu JS, Kuo KL. Vegetarian diet and blood pressure in a hospital-base study. *Ci Ji Yi Xue Za Zhi.* (2018) 30:176–80. doi: 10.4103/tcmj.tcmj_91_17
19. Chang CY, Chang HR, Lin HC, Chang HH. Comparison of renal function and other predictors in lacto-ovo vegetarians and omnivores with chronic kidney disease. *J Am Coll Nutr.* (2018) 37:466–71. doi: 10.1080/07315724.2018.1424588
20. Liu HW, Tsai WH, Liu JS. Association of vegetarian diet with chronic kidney disease. *Nutrients.* (2019) 11:279. doi: 10.3390/nu11020279
21. Moe SM, Zidehsarai MP, Chambers MA, Jackman LA, Radcliffe JS, Trevino LL, et al. Vegetarian compared with meat dietary protein source and phosphorus homeostasis in chronic kidney disease. *Clin J Am Soc Nephrol.* (2011) 6:257–64. doi: 10.2215/CJN.05040610
22. Stevens LA, Claybon MA, Schmid CH, Chen J, Horio M, Imai E, et al. Evaluation of the chronic kidney disease epidemiology collaboration equation for estimating the glomerular filtration rate in multiple ethnicities. *Kidney Int.* (2011) 79:555–62. doi: 10.1038/ki.2010.462
23. Chou YT, Li CH. Association of sleep quality and sleep duration with serum uric acid levels in adults. *PLoS One.* (2020) 15:e0239185. doi: 10.1371/journal.pone.0239185
24. Pan WH, Wu HJ, Yeh CJ, Chuang SY, Chang HY, Yeh NH, et al. Diet and health trends in Taiwan: comparison of two nutrition and health surveys from 1993-1996 and 2005-2008. *Asia Pac J Clin Nutr.* (2011) 20:238–50.
25. Seo YK, Lee H, Kim H, Kim TY, Ryu H, Ju DL, et al. Foods contributing to nutrients intake and assessment of nutritional status in pre-dialysis patients: a cross-sectional study. *BMC Nephrol.* (2020) 21:301. doi: 10.1186/s12882-020-01958-8
26. Marventano S, Vetrani C, Vitale M, Godos J, Riccardi G, Grosso G. Whole grain intake and glycaemic control in healthy subjects: a systematic review and meta-analysis of randomized controlled trials. *Nutrients.* (2017) 9:769. doi: 10.3390/nu9070769
27. Brennan EP, Mohan M, Andrews D, Bose M, Kantharidis P. Specialized pro-resolving mediators in diabetes: novel therapeutic strategies. *Clin Sci (Lond).* (2019) 133:2121–41. doi: 10.1042/CS20190067
28. Hill E, Sapa H, Negrea L, Bame K, Hostetter T, Barkoukis H, et al. Effect of Oat β-glucan supplementation on chronic kidney disease: a feasibility study. *J Ren Nutr.* (2020) 30:208–15. doi: 10.1053/j.jrn.2019.06.012
29. Lin HC, Peng CH, Huang CN, Chiou JY. Soy-based foods are negatively associated with cognitive decline in Taiwan's elderly. *J Nutr Sci Vitaminol (Tokyo).* (2018) 64:335–9. doi: 10.3177/jnsv.64.335
30. Chen RC-Y, Chang Y-H, Lee M-S, Wahlqvist ML. Dietary quality may enhance survival related to cognitive impairment in Taiwanese elderly. *Food Nutr Res.* (2011) 55:doi: 10.3402/fnr.v3455i3400.7387
31. Yeh WJ, Yang HY, Chen JR. Soy β-conglycinin retards progression of diabetic nephropathy via modulating the insulin sensitivity and angiotensin-converting enzyme activity in rats fed with high salt diet. *Food Funct.* (2014) 5:2898–904. doi: 10.1039/c4fo00379a
32. Yang HY, Wu LY, Yeh WJ, Chen JR. Beneficial effects of β-conglycinin on renal function and nephrin expression in early streptozotocin-induced diabetic nephropathy rats. *Br J Nutr.* (2014) 111:78–85. doi: 10.1017/S0007114513001876
33. Aparecida de França S, Dos Santos MP, Garófalo MA, Navegantes LC, Kettelhut Ido C, et al. Low protein diet changes the energetic balance and sympathetic activity in brown adipose tissue of growing rats. *Nutrition.* (2009) 25:1186–92. doi: 10.1016/j.nut.2009.03.011
34. Lei L, Wang JG. Dietary sodium intake and serum uric acid: a mini-review. *Pulse (Basel).* (2018) 6:124–9. doi: 10.1159/000490573
35. Schmidt JA, Crowe FL, Appleby PN, Key TJ, Travis RC. Serum uric acid concentrations in meat eaters, fish eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. *PLoS One.* (2013) 8:e56339. doi: 10.1371/journal.pone.0056339
36. Chiu THT, Liu CH, Chang CC, Lin MN, Lin CL. Vegetarian diet and risk of gout in two separate prospective cohort studies. *Clin Nutr.* (2020) 39:837–44. doi: 10.1016/j.clnu.2019.03.016
37. Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, et al. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab.* (2014) 19:407–17. doi: 10.1016/j.cmet.2014.02.006
38. Naghshi S, Sadeghi O, Willett WC, Esmaillzadeh A. Dietary intake of total, animal, and plant proteins and risk of all cause, cardiovascular, and cancer mortality: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ.* (2020) 370:m2412. doi: 10.1136/bmj.m2412
39. Gardiner L, Akintola A, Chen G, Catania JM, Vaidya V, Burghardt RC, et al. Structural equation modeling highlights the potential of Kim-1 as a biomarker for chronic kidney disease. *Am J Nephrol.* (2012) 35:152–63. doi: 10.1159/000335579
40. Wang CP, Lu YC, Hung WC, Tsai IT, Chang YH, Hu DW, et al. Inter-relationship of risk factors and pathways associated with chronic kidney disease in patients with type 2 diabetes mellitus: a structural equation modelling analysis. *Public Health.* (2021) 190:135–44. doi: 10.1016/j.puhe.2020.02.007
41. Huang CJ, Fan YC, Liu JF, Tsai PS. Characteristics and nutrient intake of Taiwanese elderly vegetarians: evidence from a national survey. *Br J Nutr.* (2011) 106:451–60. doi: 10.1017/s0007114511000195
42. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2021. *Diabetes Care.* (2020) 44:S15–33.

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Association Between Dietary Inflammatory Index and S-Klotho Plasma Levels in Middle-Aged and Elderly People

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Background and Aims: Soluble Klotho (S-Klotho) is a protein that has anti-aging properties. Dietary inflammation index (DII) is closely related to various age-related diseases. However, whether DII is related to S-Klotho plasma levels is still controversial. It was the goal of this study to examine the link between DII and S-Klotho in middle-aged and elderly people.

Methods: Between 2007 and 2016, five NHANES cycles were conducted, with 12,315 middle-aged and elderly (aged 40–79) participants having S-Klotho tests and submitting dietary recall data. The inflammatory potential of a diet was determined using the DII. To determine the plasma levels of S-Klotho, we employed a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA).

Results: There was a negative correlation between DII and S-Klotho plasma levels. In the threshold effect analysis model, the breakpoint was DII=1.3, and the negative correlation was more obvious when DII < 1.3 ($\beta = -10.6$, $p = 0.001$). When DII > 1.3, the correlation disappeared ($p = 0.355$). There may be a threshold saturation effect.

Conclusion: In middle-aged and older individuals, there is a negative connection between the pro-inflammatory dietary pattern as evaluated by DII and the plasma level of S-Klotho. Given the rationale for the findings and the study's limitations, the fundamental mechanisms generating inflammation warrant additional exploration.

Keywords: aging, dietary inflammatory index, Klotho, inflammation, diet

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INTRODUCTION

Klotho, a transmembrane protein, possesses extraordinary anti-aging capabilities (1). Clotho is the name of one of Greek mythology's three fate goddesses, a just but forgiving divinity. As a result, when researchers discovered a protein that "secures the human lifeline," they gave it the name Klotho. Klotho protein can be found in two different forms: membrane and secretory. The hydrolysis of protein on the Klotho membrane produces the exfoliated form (S-Klotho) of Klotho protein. In comparison to membrane Klotho protein, S-Klotho protein is more prevalent in the human body, with S-Klotho found in urine, blood, and cerebrospinal fluid (CSF) (2–4). As a humoral factor, it performs a variety of biological functions in the circulatory system, including inflammation regulation, antioxidation, and senescence prevention (5, 6). By contrast, a shortage of Klotho can result in a variety of age-related diseases, including atherosclerosis, endothelial

dysfunction, decreased bone mineral density, osteoporosis, skin atrophy, and cognitive impairment (7–11). As the population is getting old, the number of the elderly population is growing, age-related diseases are also on the rise, and the disability burden of age-related diseases is expected to increase. Therefore, it is of great significance to understand the factors related to anti-aging.

Dietary patterns largely determine life expectancy (12). There is a great deal of evidence that many foods, nutrients, and non-nutritious food ingredients can regulate inflammation in both acute and chronic ways (13). Considering the anti-inflammatory and pro-inflammatory regulatory potential of nutrients, a lot of interest has been aroused by the inflammatory burden of diet. The pro-inflammatory diet pattern is related to some age-related systemic diseases, including malignant tumors, cardiovascular metabolic diseases, diabetes, and so on (14–16). Furthermore, given the frequency of food intake, the dynamic equilibrium of chronic inflammation is more dependent on diet than on medicine use. Then, by properly managing dietary components associated with inflammation, several disorders caused by inflammatory pathways can be avoided or cured. Rather than a single assessment based on nutrition, the inflammatory load of food is investigated more thoroughly in certain ways. From this point of view, a method of describing and measuring the inflammatory potential of a person's diet can help develop tailored and accurate dietary intervention and health maintenance strategies. Using inflammatory biomarkers, the dietary inflammation index (DII) has been validated in several groups to help determine the inflammogenic potential of certain people's diets (17). Therefore, high DII (pro-inflammatory diet) may be related to a grown risk of chronic disease or all-cause death (18, 19).

Aging is an inevitable process throughout life. However, dietary patterns reflect years to decades of inflammation in the body, which can be inferred chronic inflammation may be closely related to aging. DII has been adopted for studies to better know the relationship between DII and disease. However, as far as we know, the relationship between the Mediterranean diet and S-Klotho has been studied, and there is only one article on DII and S-Klotho in middle-aged people, and the sample size is small ($n = 73$) (2), so it seems to be very important to find out whether the pro-inflammatory diet can regulate the plasma level of S-Klotho in humans. The goal of this study is to explore the connection between DII and S-Klotho in middle-aged and elderly adults using data from the National Health and Nutrition Examination Survey (NHANES 2007–2016).

MATERIALS AND APPROACHES

Participants

Because S-Klotho was evaluated exclusively within that period, the research design comprised data from five National Health and Nutrition Examination Survey cycles (NHANES). The National Center for Health Statistics undertakes a hierarchical, multi-phase assessment of the non-institutionalized civilian population in the United States of America and the District of Columbia (NCHS). The NHANES is used to assess individuals' health and nutritional status and to monitor changes over time. Data collection methods included interviews, physical examinations, and laboratory testing, and all NHANES surveys conducted between 2007 and 2016 were examined and approved by the Centers for Disease Control and Prevention (CDC) and the National Center for Health Statistical Research (NCHS) Ethics Review Committees; All participants provided written informed consent. For this research, data on NHANES2007-2008, 2009-2010, 2011-2012, 2013-2014, and 2015-2016 were searched. The inclusion criteria were as follows: original serum samples of participants aged 40–79 were tested for S-Klotho and answered dietary interviews on total nutrient intake. Excluding people with unreliable dietary records, the total sample size was 12,315. We follow the expansion of enhanced observational research in Nutrition Epidemiology (STROBE-NUT) (**Supplementary Table S1**) (20).

Dietary Inflammatory Index (DII)

The DII is a literature-based technique for continuously classifying individual diets from the most anti-inflammatory to the most pro-inflammatory. The DII's development and validation have been detailed elsewhere. DII is a scoring approach that anticipates the mean and standard deviation (SD) for each coefficient using dietary intake data from regionally relevant world datasets. This study calculated the DII scores for 26 commonly consumed foods: Energy (Kcal), Protein (Gm), Carbohydrates (Gm), Total Sugar (Gm), Dietary Fiber (Gm), Total Fat (Gm), Total Saturated Fatty Acids (Gm), Total Monounsaturated Fatty Acids (Gm), Total Polyunsaturated Fatty Acids (Gm), Cholesterol (Mg), Vitamin E, -tocopherol (Mg), Flavane-3-ol, flavone, flavonol, and green/black tea. First, we calculated every food coefficient and every participant's Z-score. Next, every individual Z-score is transformed into a central percentile. Thirdly, a standardized global inflammatory effect score was adopted to multiply every central percentile. Each participant's DII score is, then, summed up. We determined the highest quartile of DII scores (representing a pro-inflammatory diet) and the lowest quartile of DII scores (showing an anti-inflammatory diet) based on **Table 3** (17).

S-Klotho Plasma Levels

During the five cycles of the NHANES project, blood samples were taken from the original serum samples that were available to participants aged 40–79. The samples were stored on dry ice and each package was examined by personnel in the receiving area of the laboratory. The sample is scanned, the data is compared with the data on the received electronic manifest, and input into the laboratory information system. All samples are stored at -80°C until predetermined batches of samples are provided to technicians for analysis every day. S-Klotho is on basis of a solid-phase sandwich enzyme-linked immunosorbent assay kit (IBL International, Japan). The analysis results are automatically transmitted from the instrument to the laboratory Oracle management system, and the regional supervisor evaluates the results. Samples with repetitive results of more than 10% are marked as repetitive analysis. If the value of the quality control sample is not within the 2SD range of the specified value, the

TABLE 1 | Baseline characteristics of participants (N =12,315).

Characteristic	Klotho levels quartiles, pg/mL				*P*-value
	Q1 <654.7	Q2 ≥654.7 to <802.5	Q3 ≥802.5 to <993.3	Q4 ≥993.3	
No. of participants	3,079	3,077	3,079	3,080	
Age(years)	59.05 (11.11)	58.00 (10.85)	57.33 (10.70)	56.51 (10.60)	<0.001
Sex (%)					<0.001
Men	49.06	51.00	48.07	42.52	
Woman	50.94	49.00	51.93	57.48	
Race/ethnicity (%)					<0.001
Mexican American	6.61	6.22	6.97	6.98	
Other Hispanic	3.96	4.48	4.83	5.53	
Non-Hispanic white	73.83	75.75	73.42	68.12	
Non-Hispanic black	9.32	7.26	7.67	12.77	
Other race/ethnicity	6.27	6.29	7.11	6.60	
Energy intake (kcal/day)	1927.49 (882.96)	1936.99 (898.62)	1935.79 (893.63)	1916.92 (880.57)	0.800
DII	1.04 (1.75)	0.91 (1.80)	0.91 (1.80)	0.92 (1.83)	0.001
Body mass index (kg/m^2)	29.99 (6.36)	29.82 (6.64)	29.72 (6.74)	29.64 (6.92)	0.196
Waist circumference (cm)	103.10 (14.67)	102.29 (15.18)	101.46 (15.21)	100.70 (15.78)	<0.001
Use of medication %	72.26	69.00	68.14	67.20	<0.001
SBP(mmHg)	128.80 (18.36)	128.10 (17.96)	126.83 (18.09)	127.17 (17.95)	<0.001
DBP(mmHg)	71.14 (12.48)	71.88 (12.25)	72.29 (11.63)	72.12 (12.22)	0.001

Mean (±SD) for continuous variables: the P-value was calculated by the weighted linear regression.
Percent for categorical variables: P-value was calculated by weighted chi-square test.
DII, dietary inflammatory index; SBP, Syst olic Blood, Pressure; DBP, Diastolic Blood pressure.

entire analysis run will be rejected and the sample analysis will be repeated.

Assessment of Covariates

The calculation of body mass index (BMI) was made on basis of height and weight (kg/m^2). At the end of a normal exhalation, the waistline at the midpoint between the ilium and the base of the ribs was measured. Following a 5-min sit-down period and establishing the maximum inflation level (MIL).

Blood Pressure

Blood pressure (BP) was manually measured in all eligible patients using a Baumanometer-calibrated mercury real gravity wall sphygmomanometer (W. A. Baum). The circumference of the upper arm is used to calculate the size of the cuff. All examiners have gone through a standardized training program. After sitting silently for 5 min in the mobile testing center, the participants were measured three times in a row. Unless unique participant conditions prevented it, measurements were taken with the right arm, except when the left arm was used. The measurement interval is 30 seconds.

Use of Medication

We used the NHANES variable "RXDUSE" to determine the medication status. During the interview, participants were asked, "have you used or taken any prescription drugs in the past month?" Then, participants who reported the use of prescription drugs were asked to show the container of prescription drugs, so that the interviewer could record the relevant information. The analysis did not include dietary supplements reported for this concern.

Statistical Analysis

Data was collected from nhanesR (http://ckr123.synology.me:3838/nhanesR/) on the NHANES project for five consecutive complete cycles from 2007 to 2016. The statistical packages R (The R Foundation; http://www.r-project.org; version 3.5.3) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc.) were adopted to analyze data. All analyses are calculated using sample weights according to analysis guidelines edited by the National Institutes of Health because NHANES aims at producing data that represent the non-institutionalized civilian population of the United States. The weighted chi-square test was carried out for the classified variables, and the *P*-value of the continuous variables was calculated with the weighted linear regression model. A simple linear regression model was set up to test the correlation between DII and S-Klotho plasma levels: model 1, no elements have been adjusted; model 2, modified for age, sex, and race; and model 3, the adjustment of all covariates in **Table 1** was made for further subgroup analysis. The additive model and smooth curve fitting were generalized to explore the potential non-linear correlation. Furthermore, the inflection point is calculated by using a two-stage linear regression model. $P < 0.05$ was of statistical significance.

RESULTS

Table 1 displays the baseline features of the research participants. A total of 12,315 participants participated in the current study. According to the S-Klotho quartile, the characteristics of the target population are shown in **Table 1**. Overall, there were significant differences in age, sex, race, waist circumference, drug use, and blood pressure distribution among S-Klotho quartiles. Participants in the highest quartile were more likely to be middle-aged, female, had lower DII scores, lower waistline, less drug use, and lower systolic and diastolic blood pressure levels than participants in the lowest quartile of S-Klotho. In terms of energy intake and BMI, no significant difference was observed between the quartiles of S-Klotho.

TABLE 2 | Univariate analysis for S-Klotho.

	S-Klotho, pg/mL	
	β (95%CI)	***P*-value**
DII	−2.64 (−5.52, 0.24)	0.0721
Energy intake	−0.00 (−0.01, 0.00)	0.1335
Sex	−36.32 (−46.73, −25.91)	<0.0001
Body mass index	−1.30 (−2.09, −0.51)	0.0013
Waist circumference	−1.24 (−1.58, −0.90)	<0.0001
Systolic blood pressure	−0.83 (−1.13, −0.52)	<0.0001
Diastolic blood pressure	0.57 (0.12, 1.02)	0.0133

Non–standardized β coefficient [95% confidence interval], p-values are provided. DII, dietary inflammatory index.

Based on this univariate analysis, we found significant differences in sex, waist circumference, use of medication, systolic and diastolic blood pressure as a potential confounders (**Table 2**).

We discovered an inverse correlation between DII and S-Klotho plasma levels: ($\beta = -6.1$, $P = 0.001$; **Table 3**).

In addition, we handled this relationship using weighted generalized weighted models and smoothing curve fitting (**Figure 1**). Based on this relationship, we performed an additional threshold effect analysis and found a better fit relative to the linear model to explain the relationship using a non-linear model (log-likelihood ratio = 0.011, **Table 4**), When the DII score increased by 1 unit, the plasma level of S-Klotho decreased by-10.6 pg/ml (DII <-1.3). This result is consistent with the previous curve fitting plots. Suggesting a possible threshold saturation effect.

DISCUSSION

The current study first establishes a negative connection between DII and S-Klotho plasma levels in middle-aged and elderly subjects in this nationally representative cross-sectional investigation. However, recent research on the DII-S-Klotho connection has been inconclusive. While some feel that eating a higher-quality diet or adopting healthier eating habits will help prevent aging, others remain unsure. One possible explanation for this discrepancy is that these studies used different methods for assessing dietary quality, and dietary quality ratings are dependent on sample data. The majority of research on the link between diet and aging has been on the Mediterranean diet. Studies in the United States (21), Spain (22), and Italy

TABLE 3 | Relationship between Dietary Inflammatory Index (DII) and S-Klotho pg/mL.

Outcome	**Crude Model**		**Model I**		**Model II**	
	β (95%CI)	***P*-value**	**β (95%CI)**	***P*-value**	**β (95%CI)**	***P*-value**
DII	−2.6 (−5.5, 0.2)	0.072	−5.5 (−8.4, −2.6)	<0.001	−6.1 (−9.4, −2.7)	0.001
DII(quartile)						
Q1 < −2.36	Reference		Reference		Reference	
Q2 ≥ −2.36 to <0.23	−35.6 (−63.2, −8.1)	0.011	−39.4 (−66.8, −12.1)	0.004	−37.4 (−64.9, −9.8)	0.007
Q3 ≥ 0.23 to <1.90	−43.1 (−70.6, −15.9)	0.001	−54.4 (−81.6, −27.2)	0.001	−50.1 (−77.9, −22.1)	0.001
Q4 ≥ 1.90	−34.5 (−61.7, −7.3)	0.013	−50.5 (−77.7, −23.3)	0.001	−46.5 (−75.4, −17.6)	0.001
P for trend	0.251		0.003		0.004	
Stratified by gender						
Men	−6.4 (−10.3, −2.5)	0.001	−6.4 (−10.4, −2.5)	0.001	−6.2 (−10.2, −2.3)	0.002
Woman	−3.2 (−7.5, 1.1)	0.147	−4.6 (−8.9, −0.3)	0.035	−2.6 (−6.9, 1.7)	0.240
Stratified by race						
Mexican American	−0.6 (−8.8, 7.5)	0.879	0.2 (−8.2, 8.7)	0.955	0.8 (−7.6, 9.3)	0.845
Other Hispanic	4.7 (−4.5, 13.9)	0.319	2.6 (−6.8, 12.0)	0.591	3.4 (−6.0, 12.9)	0.480
Non-Hispanic white	−4.9 (−8.9, −0.8)	0.019	−6.4 (−10.5, −2.2)	0.002	−5.0 (−9.2, −0.9)	0.018
Non-Hispanic black	2.3 (−6.3, 10.9)	0.600	−3.1 (−11.7, 5.5)	0.479	−1.4 (−10.0, 7.2)	0.750
Other race/ethnicity	−8.2 (−18.0, 1.6)	0.103	−9.6 (−19.4, 0.2)	0.054	−8.7 (−18.5, 1.2)	0.085

Crude Model: There are no covariates were adjusted.
Model I: Age, sex, and race/ethnicity were adjusted.
Model II: Age, sex, and race/ethnicity, energy intake, BMI, waist circumference, use of medication, systolic blood pressure, and diastolic blood pressure were adjusted.

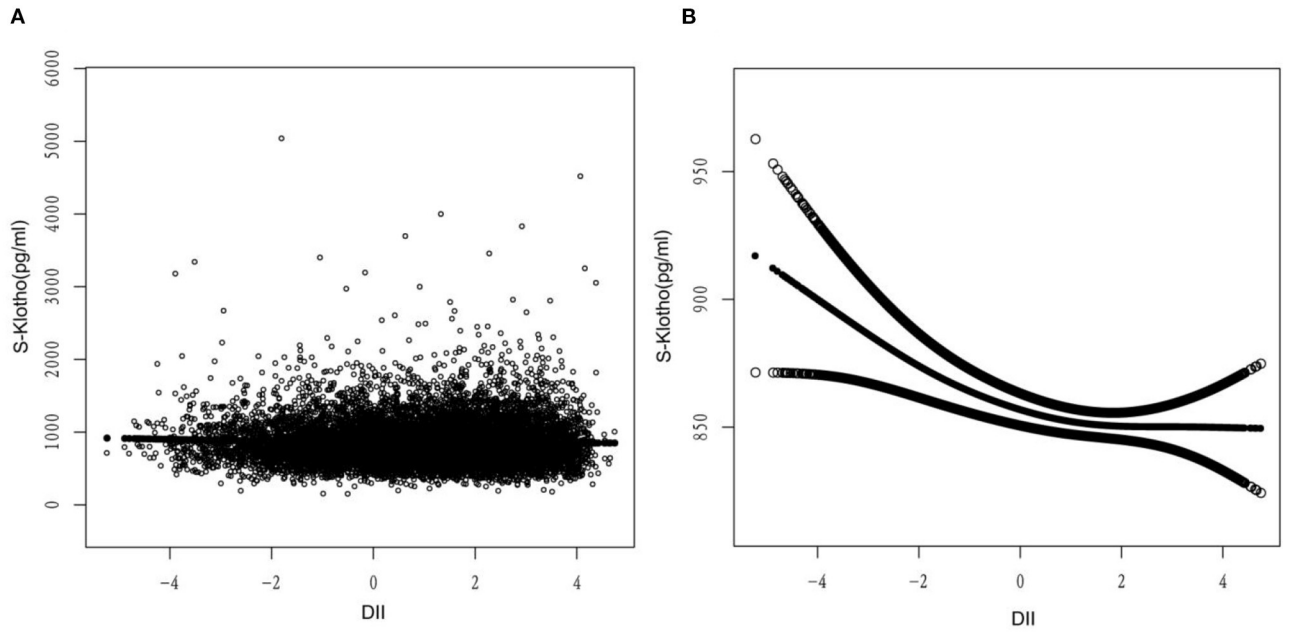

FIGURE 1 | Relationship between DII and S-Klotho. **(A)** Each black dot represents A sample. **(B)** Solid lines represent smooth curve fitting between variables. The black dotted line shows a 95% confidence interval (CI) for the fit. They adjusted for energy intake, age, sex, race, body mass index (BMI), use of medication, and systolic and diastolic blood pressure.

(23) have shown that higher Mediterranean diet scores are associated with anti-aging. In Australia, however, no correlation between the Mediterranean diet and aging was observed (24). Because the Mediterranean diet is not a realistic option for the majority of American adults due to variations in dietary culture and practicality, we employed the DII, an index based on the representative range of dietary intake ingested by humans. This is achieved through the construction of a comprehensive database, including the NHANES database, so it is well-represented nationwide. Only one study in Spain has utilized the DII technique to investigate the association between DII and S-Klotho plasma levels. Interestingly, Lucas et al.'s results (2), based on a survey sample of sedentary middle-aged adults, were just the opposite of ours. Our results support a negative correlation between higher DII scores and higher S-Klotho levels in middle-aged and elderly Americans, which is reasonable and consistent with the hypothetical effect of DII.

Klotho has been linked to aging and is thought to regulate oxidative stress and antioxidant enzymes. Overexpression of S-Klotho can inhibit the expression of retinoic acid-induced gene-I, the activation of NF-κB, and the secretion of proinflammatory cytokines. By contrast, excessive S-Klotho depletion increased the production of pro-inflammatory cytokines, such as tumor necrosis factor-α and IL-1-β, while decreasing the production of anti-inflammatory cytokines, such as IL-10, IL-2, and IL-3 (25). Indeed, DII is a tool that reflects the amounts of six inflammatory markers: IL-1-β, IL-4, IL-6, IL-10, TNF-α, and CRP. The underlying molecular mechanism is that pro-inflammatory substances elevate circulating interleukin (IL) levels (particularly IL-6, IL-1-β, or IL−8) (26–28). As a result, c-reactive protein is extensively generated and released into the circulation by hepatocytes, resulting in more severe systemic inflammation (28). Inflammatory food intake has been shown in the past to have a considerable effect on S-Klotho plasma levels. From a systemic perspective, a pro-inflammatory diet contributes to the elevation of systemic inflammation. Thus, there appear to be grounds to infer that chronic inflammation induced by a pro-inflammatory diet pattern can decrease plasma S-Klotho levels, thereby regulating diet-induced inflammation.

TABLE 4 | Threshold effect analysis of DII on S-Klotho using the two-piecewise linear regression model.

	Adjusted β(95% CI)	*P*-value
Fitting by the standard linear model	−6.1 (−9.4, −2.7)	0.001
Fitting by the two-piecewise linear model		
Inflection point	1.3	
DII <1.3	−10.6 (−15.4, −5.8)	<0.001
DII > 1.3	4.0 (−4.5, 12.4)	0.355
Log-likelihood ratio	0.011	

Age, sex, and race/ethnicity, energy intake, BMI, waist circumference, use of medication, systolic blood pressure, and diastolic blood pressure were adjusted.

We observed that when the DII score increased by 1 unit, the plasma levels of S-Klotho decreased by −10.6 pg/ml (DII < 1.3). These changes in plasma levels of S-Klotho have important clinical significance. In previous studies, when patients with cardiovascular disease were compared with healthy individuals, there were differences in S-Klotho plasma levels in 45 pg/ml (626 and 671 pg/ml, respectively) (29). Furthermore, participants

with a blood sugar level of <575 pg/ml had a higher chance of dying from any cause than those with a blood sugar level of more than 763 pg/ml (30). As a result, our results have clinical implications, because variations in DII can affect not only the risk of cardiovascular disease and all-cause mortality but also the rate of aging (29, 30).

The current research has some limitations. First of all, it has a horizontal design that excludes the construction of causality. Second, we don't know whether these outcomes can be extended to young people. More research is needed to see if these benefits may be seen in different populations. Third, the difficulties of correct dietary evaluation, which may be overstated or misclassified, must be considered. Finally, because the level of Klotho protein in many tissues is unknown and can only be tested by biopsy, the results of Klotho protein cannot be predicted.

CONCLUSION

In middle-aged and older individuals, there is a negative connection between the pro-inflammatory dietary pattern as evaluated by DII and the plasma level of S-Klotho. Given the rationale for the findings and the study's limitations, the fundamental mechanisms generating inflammation warrant additional exploration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Center for Health Statistics Research Ethics Review Board (ERB) for NHANES 2011–2016 (Protocol #2011-17) and NHANES 2007–2010 (Protocol #2005-06) on which data for this analysis was used. Additional details are available at: https://www.cdc.gov/nchs/nhanes/irba98.htm. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was created by T-CM, who also examined the data. FG revised and edited the text for key intellectual content. All authors evaluated and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.853332/full#supplementary-material

REFERENCES

1. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. (1997) 390:45–51. doi: 10.1038/36285
2. Jurado-Fasoli L, Castillo MJ, Amaro-Gahete FJ. Dietary inflammatory index and s-klotho plasma levels in middle-aged adults. *Nutrients*. (2020) 12:281. doi: 10.3390/nu12020281
3. Qian Y, Che L, Yan Y, Lu R, Zhu M, Xue S, et al. Urine klotho is a potential early biomarker for acute kidney injury and associated with poor renal outcome after cardiac surgery. *BMC Nephrol*. (2019) 20:268. doi: 10.1186/s12882-019-1460-5
4. Zimmermann M, Köhler L, Kovarova M, Lerche C, S, Schulte Wurster I, et al. The longevity gene Klotho and its cerebrospinal fluid protein profiles as a modifier for Parkinson's disease. *Eur J Neurol*. (2021) 28:1557–65. doi: 10.1111/ene.14733
5. Kanbay M, Demiray A, Afsar B, Covic A, Tapoi L, Ureche C, et al. Role of Klotho in the development of essential hypertension. *Hypertension*. (2021) 77:740–50. doi: 10.1161/HYPERTENSIONAHA.120.16635
6. Ebert T, Pawelzik SC, Witasp A, Arefin S, Hobson S, Kublickiene K, et al. Inflammation and premature ageing in chronic kidney disease. *Toxins*. (2020) 12:227. doi: 10.3390/toxins12040227
7. da Costa JP, Vitorino R, Silva GM, Vogel C, Duarte AC, Rocha-Santos T. A synopsis on aging-Theories, mechanisms and future prospects. *Ageing Res Rev*. (2016) 29:90–112. doi: 10.1016/j.arr.2016.06.005
8. Martín-Núñez E, Donate-Correa J, López-Castillo Á, Delgado-Molinos A, Ferri C, Rodríguez-Ramos S, et al. Soluble levels and endogenous vascular gene expression of KLOTHO are related to inflammation in human atherosclerotic disease. *Clin Sci*. (2017) 131:2601–9. doi: 10.1042/CS20171242
9. Donato AJ, Machin DR, Lesniewski LA. Mechanisms of dysfunction in the aging vasculature and role in age-related disease. *Circ Res*. (2018) 123:825–48. doi: 10.1161/CIRCRESAHA.118.312563
10. Chalhoub D, Marques E, Meirelles O, Semba RD, Ferrucci L, Satterfield S, et al. Association of serum Klotho with loss of bone mineral density and fracture risk in older adults. *J Am Geriatr Soc*. (2016) 64:e304–8. doi: 10.1111/jgs.14661
11. Abulizi P, Zhou XH, Keyimu K, Luo M, Jin FQ. Correlation between KLOTHO gene and mild cognitive impairment in the Uygur and Han populations of Xinjiang. *Oncotarget*. (2017) 8:75174–85. doi: 10.18632/oncotarget.20655
12. Crous-Bou M, Molinuevo JL, Sala-Vila A. Plant-rich dietary patterns, plant foods and nutrients, telomere length. *Adv Nutr*. (2019) 10:S296–303. doi: 10.1093/advances/nmz026
13. Craddock JC, Neale EP, Peoples GE, Probst YC. Vegetarian-based dietary patterns and their relation with inflammatory and immune biomarkers: a systematic review and meta-analysis. *Adv Nutr*. (2019) 10:433–51. doi: 10.1093/advances/nmy103
14. O'Keefe SJ. Diet, microorganisms and their metabolites, colon cancer. *Nat Rev Gastroenterol Hepatol*. (2016) 13:691–706. doi: 10.1038/nrgastro.2016.165
15. Shah B, Newman JD, Woolf K, Ganguzza L, Guo Y, Allen N, et al. Anti-inflammatory effects of a vegan diet versus the american heart association-recommended diet in coronary artery disease trial. *J Am Heart Assoc*. (2018) 7:e011367. doi: 10.1161/JAHA.118.011367
16. Martín-Peláez S, Fito M, Castaner O. Mediterranean diet effects on type 2 diabetes prevention, disease progression, related mechanisms. A review. *Nutrients*. (2020) 12:2236. doi: 10.3390/nu12082236

17. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr*. (2014) 17:1689–96. doi: 10.1017/S1368980013002115
18. Garcia-Arellano A, Martínez-González MA, Ramallal R, Salas-Salvadó J, Hébert JR, Corella D, et al. Dietary inflammatory index and all-cause mortality in large cohorts: the SUN and PREDIMED studies. *Clin Nutr*. (2019) 38:1221–31. doi: 10.1016/j.clnu.2018.05.003
19. Fowler ME, Akinyemiju TF. Meta-analysis of the association between dietary inflammatory index (DII) and cancer outcomes. *Int J Cancer*. (2017) 141:2215–27. doi: 10.1002/ijc.30922
20. Lachat C, Hawwash D, Ocké MC, Berg C, Forsum E, Hörnell A, et al. Strengthening the reporting of observational studies in epidemiology-nutritional epidemiology (STROBE-nut): an extension of the STROBE statement. *PLoS Med*. (2016) 13:e1002036. doi: 10.1371/journal.pmed.1002036
21. Leung CW, Fung TT, McEvoy CT, Lin J, Epel ES. Diet quality indices and leukocyte telomere length among healthy US adults: data from the national health and nutrition examination survey, 1999–2002. *Am J Epidemiol*. (2018) 187:2192–201. doi: 10.1093/aje/kwy124
22. Jurado-Fasoli L, Amaro-Gahete FJ, Arias-Tellez MJ, Gil A, Labayen I, Ruiz JR. Relationship between dietary factors and S-Klotho plasma levels in young sedentary healthy adults. *Mech Ageing Dev*. (2021) 194:111435. doi: 10.1016/j.mad.2021.111435
23. Vasto S, Buscemi S, Barera A, Di Carlo M, Accardi G, Caruso C. Mediterranean diet and healthy ageing: a Sicilian perspective. *Gerontology*. (2014) 60:508–18. doi: 10.1159/000363060
24. Milte CM, Russell AP, Ball K, Crawford D, Salmon J, McNaughton SA. Diet quality and telomere length in older Australian men and women. *Eur J Nutr*. (2018) 57:363–72. doi: 10.1007/s00394-016-1326-6
25. Fisher FM, Maratos-Flier E. Understanding the physiology of FGF21. *Annu Rev Physiol*. (2016) 78:223–41. doi: 10.1146/annurev-physiol-021115-105339
26. Shivappa N, Hebert JR, Marcos A, Diaz LE, Gomez S, Nova E, et al. Association between dietary inflammatory index and inflammatory markers in the HELENA study. *Mol Nutr Food Res*. (2017) 61:10.1002/mnfr.201600707. doi: 10.1002/mnfr.201600707
27. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol*. (2018) 9:754. doi: 10.3389/fimmu.2018.00754
28. Rhodes B, Fürnrohr BG, Vyse TJ. C-reactive protein in rheumatology: biology and genetics. *Nat Rev Rheumatol*. (2011) 7:282–9. doi: 10.1038/nrrheum.2011.37
29. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, et al. Plasma klotho and cardiovascular disease in adults. *J Am Geriatr Soc*. (2011) 59:1596–601. doi: 10.1111/j.1532-5415.2011.03558.x
30. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, et al. Plasma klotho and mortality risk in older community-dwelling adults. *J Gerontol A Biol Sci Med Sci*. (2011) 66:794–800. doi: 10.1093/gerona/glr058

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Effect of Probiotics on Liver Enzymes in Patients With Non-alcoholic Fatty Liver Disease: An Umbrella of Systematic Review and Meta-Analysis

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Non-alcoholic fatty liver disease (NAFLD) has become prevalent in recent decades, especially in developed countries; yet the approaches for preventing and treating NAFLD are not clear. This study aimed to summarize meta-analyses of randomized controlled trials that examined the effects of probiotics on NAFLD. We systematically searched PubMed, Scopus, Embase, Web of Science, and Cochrane Central Library databases up to August 2021. All Meta-analysis studies assessing the effect of probiotics on liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Gamma-glutamyl transferase (GGT)] were included. Meta-analysis was conducted using a random-effects model. Sensitivity and subgroup analyses were also performed. The umbrella study covered ten eligible studies involving 5,162 individuals. Beneficial effects of probiotics supplementation were revealed on ALT (ES = −10.54 IU/L; 95% CI: −12.70, −8.39; $p < 0.001$; $I^2 = 60.9\%$, $p = 0.006$), AST (ES = −10.19 IU/L, 95%CI: −13.08, −7.29, $p < 0.001$; $I^2 = 79.8\%$, $p < 0.001$), and GGT (ES = −5.88 IU/L, 95% CI: −7.09, −4.67, $p = 0.009$; $I^2 = 0.0\%$, $p = 0.591$) levels. Probiotics have ameliorating effects on ALT, AST, and GGT levels in patients with NAFLD. Overall, Probiotics could be recommended as an adjuvant therapeutic method for the management of NAFLD.

Keywords: non-alcoholic fatty liver disease, probiotics, liver enzyme, umbrella meta-analysis, systematic review

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by lipid deposition in liver cells (1). On the other hand, untreated NAFLD can cause non-alcoholic hepatitis (NASH) as well as other liver-related severe sicknesses like cirrhosis, liver failure, and liver cancer (2). Considering the newest epidemiological studies, the estimated prevalence of NAFLD in Middle Eastern countries

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, Gamma-glutamyl transferase; NASH, Non-alcoholic hepatitis; MTTs, microbiome-targeted treatments; ES, effect sizes; CI, corresponding confidence intervals.

is 31.8%, the highest in comparison with other regions in the world (3). Also, studies display a higher prevalence of NAFLD in patients having obesity or hyperlipidemia (4). NAFLD has a strong relationship with metabolic syndromes like obesity, insulin resistance, type 2 diabetes, dyslipidemia, and hypertension (1, 5–8). Besides liver, this disease can progress and affect many other organs, such as systemic arteries, heart and kidneys, and causes irreversible problems and death (7). It has been suggested that lifestyle changes such as diet and exercise interventions, can help manage NAFLD and lead to a decrease in occurrence and progression of NAFLD (6, 7, 9). Therefore, studies showed that nutritional changes and exercise could lower liver lipid, improve liver enzyme functions and reduce plasma triglyceride (10, 11). However, earlier studies reported that natural compounds can reduce the complications caused by NAFLD (1, 12).

Some studies have shown that there is an association between the gut-liver axis and NAFLD. More than 10,000 microbiomes live in a synergetic relationship with the intestinal tract and have different effects on the host's health and disease condition (13–15). Recently, microbiome-targeted treatments (MTTs) are a proposed way to influence the gut microbiome. In this regard, probiotics are used as a treatment strategy. Probiotics are explained as live microbial organisms used as dietary supplements that are beneficial for the human or animal host in terms of health. Moreover, when provided in an adequate dosage and for a capable duration, probiotics can improve intestinal microbial balance (16) and cause disease occurrence to delay by balancing intestinal flora, permeability, and inflammations (17). Improved production of short-chain fatty acids such as butyrate through microbial pathways impacts the metabolism of energy in the intestine and overall body (18, 19). In addition, probiotics can develop anti-microbial compounds and acidity of the intestinal lumen and cause a reduction in generation of pathogens (20). Finally, a variety of microorganism-based products can impact the immunity of the host positively (21, 22).

Although, many accumulative evidences have shown the beneficial effects of probiotics on liver enzymes by affecting specific biological processes; however, there are some contradictions in this regard (23–27). For more definite results, we aimed to investigate the effects of probiotics supplementation on serum levels of alanine transaminase (ALT), AST, and GGT in an umbrella meta-analysis study.

METHODS

Search Strategy and Study Selection

We followed standardized methods to carry out this umbrella review (a systematic review of multiple meta-analyses) to provide a clear understanding in terms of probiotics supplementation and serum levels of ALT, AST, and GGT. The scientific international databases including, PubMed, Scopus, EMBASE, Web of Science, and Cochrane Central Library databases were searched for relevant studies published up to August 2021. The pattern of search strategy is provided in **Supplementary Table 1**. To enhance the sensitivity of our search strategy, the wild-card term "*" was used. English-language articles were included in this study.

Inclusion and Exclusion Criteria

Meta-analysis studies examining the effects of probiotics supplementation on liver enzymes (ALT, AST, and GGT) which reported the effect sizes (ES) and corresponding confidence intervals (CI), were included in the umbrella meta-analysis. Additionally, we excluded the following studies: *in vitro*, *in vivo*, and *ex-vivo* studies, case reports, observational studies, quasi-experimental studies, and controlled clinical trials.

Quality Evaluation

The methodological quality of meta-analyses was evaluated by two reviewers (VM and SSA) independently using the AMSTAR questionnaire (28). The AMSTAR questionnaire contains 11 items that asks reviewers to answer "Yes," "No," "Can't answer" or "Not applicable." The maximum score is 11. Articles with scores higher than seven are considered as high-quality studies.

Study Selection and Data Extraction

Two independent reviewers (VM and SSA) screened the articles based on mentioned eligibility criteria. After checking and excluding irrelevant studies by the titles and abstracts, the full text of the relevant articles was evaluated to identify the study's eligibility for the umbrella meta-analysis. Any disagreement was resolved through the consensus with the third author (PD).

The first authors' name, year of publication, sample size, study location, dosage, and duration range of supplementation, ESs and CIs for ALT, AST, and GGT were extracted from the selected meta-analyses.

Data Synthesis and Statistical Analysis

ESs and CIs were used to estimate the overall effect sizes. Heterogeneity was determined by I^2 statistics and Cochrane's Q-test. I^2-value $> 50\%$ or $p < 0.1$ for the Q-test was considered as significant between-study heterogeneity. A random-effects model was applied to perform meta-analysis when the between-study heterogeneity was significant; otherwise, the fixed-effects model was employed. To detect probable sources of heterogeneity, subgroup analyses were performed according to the predefined variables, including type of effect size, study duration, mean age, sample size, and study location. Sensitivity analysis was used to identify the dependency of the overall effect size on a special study. The Egger's and Begg's tests was performed to detect a small-study effect. Publication bias was identified by visual inspection of the funnel plot. If there was any evidence of publication bias or small-study effect, trim and fill analysis was carried out accordingly. The meta-analysis was done using Stata, version 16 (Stata Corporation, College Station, TX, US). P-value < 0.05 was considered as significance level.

RESULTS

Study Selection and Study Characteristics

A total number of 85 articles were identified through a systematic search of electronic databases. Putting aside the 31 duplicate articles, 54 articles were screened carefully by titles and abstracts, among which 22 articles were selected for consideration by full-text evaluation. Considering the inclusion criteria, ten articles were included in the umbrella meta-analysis. **Figure 1** presented the flow diagram of the study selection process. According to the studied variables, the distribution of identified articles was ten articles for ALT, nine for AST, and three for GGT. The included studies were conducted between 2013 and 2019, and the mean age of participants was 44 years. The average dose of probiotics in studies was between 2.6×10^9 and 5×10^{11} CFU. Studies were fulfilled in China (23, 25, 26, 29, 30), United States (31–33), India (34), and France (27). The duration of studies ranged between 8 and 20 weeks. Cochrane Risk of Bias Tool (35), adapted from Littell et al. (36), Physiotherapy Evidence Database (PEDro) scale tool (37) and Jadad scores (38) were used for quality assessment. Overall, almost 90% of meta-analyses included high quality RCTs. The quality of the RCTs included in the meta-analyses is summarized in **Table 1**.

Methodological Quality

AMSTAR checklist assessments for the methodological quality of the covered studies are summarized in **Table 2**. All meta-analyses included in the umbrella meta-analysis had a high-quality.

Effect of Probiotics on Alanine Aminotransferase

There was a significant reducing impact of probiotics on ALT (**Figure 2A**). Notable heterogeneity was observed among studies (I^2 = 60.9%, p = 0.006). The high heterogeneity was reduced after subgroup analysis based on the type of effect size, sample size, study location, and duration of intervention. Reductions in ALT levels were more pronounced in the subgroups of sample size (<300) and an intervention duration ≥ 16 weeks when

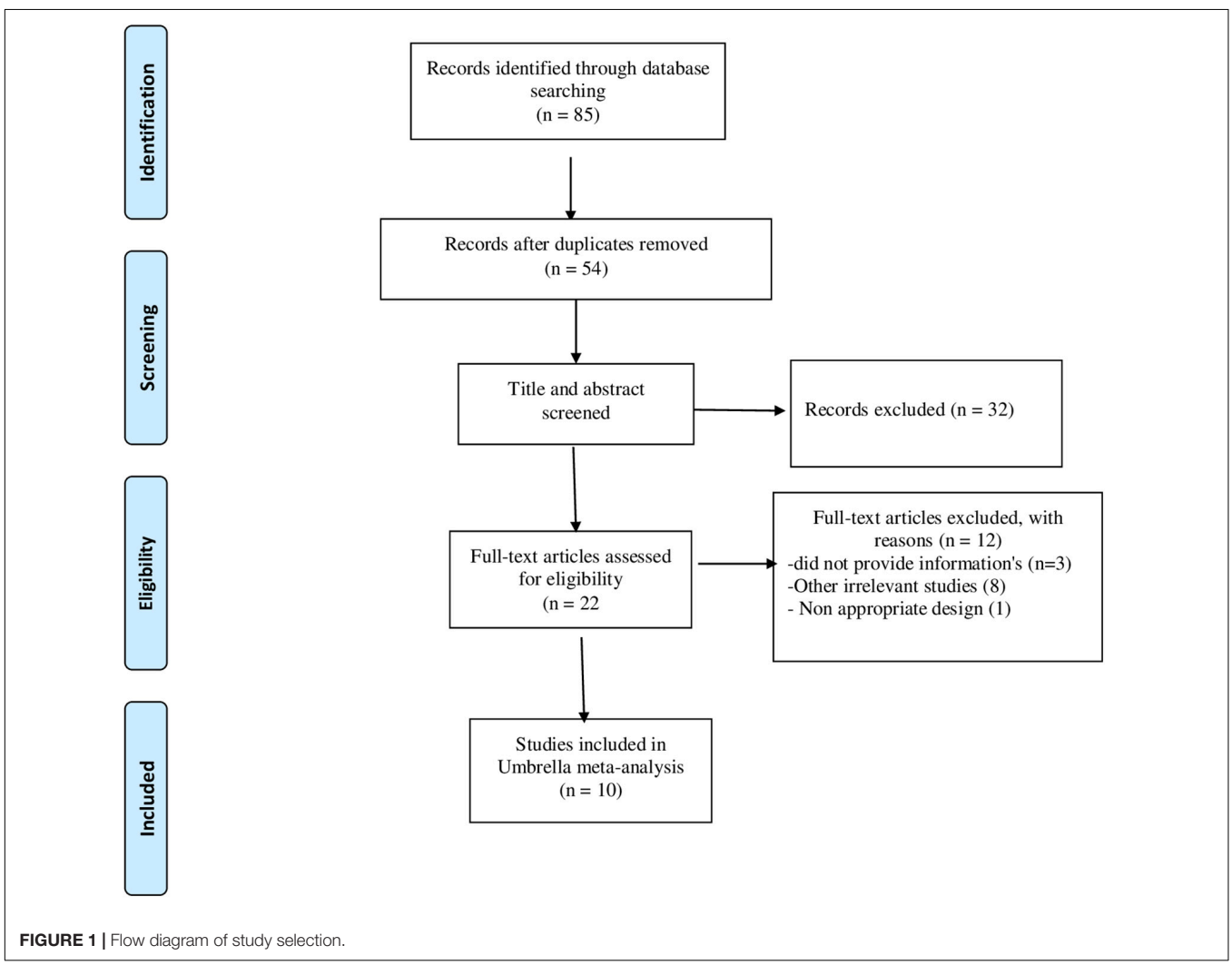

FIGURE 1 | Flow diagram of study selection.

TABLE 1 | Study characteristics of included studies.

References	No. of studies in meta-analysis	Location duration (week)	No. of participants in meta-analysis	Age (year)	Dose (mg/day)	Quality assessment scale and outcome
Gao et al. (23)	9	China 16 week	268	37	*Bifidobacterium*, *Lactobacillus*, *Streptococcus*, VSL#3 NR	Yes (Cochrane) 9/9 high
Khan et al. (32)	12	United States 17 week	292	50	*Lactobacillus*, *Bifidobacterium*, probiotics yogurt $11.4*10^9$	Yes (Cochrane) 9/13 high
Loman et al. (31)	11	United States 8 week	195	34	*Lactobacillus*, *Bifidobacterium*, *Streptococccus* 2.6*109	Yes (adapted from Littell et al.) 7/11 high
Sharpton et al. (33)	16	United States 10 week	322	NR	*Lactobacillus*, *Bifidobacterium*, probiotics yogurt NR	Yes (Cochrane) 11/16 high
Liu et al. (29)	15	China 11 week	673	37	*Acetobacter*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus*	Yes (Cochrane) 13/15 high
Lavekar et al. (34)	7	India 19.5 week	138	NR	*Lactobacillus* NR	Yes (Jodad) 7/7 high
Tang et al. (26)	22	China 13.5 week	879	30	*Lactobacillus*, *Bifidobacterium*, *Streptococccus*, *Bacillus*, *Enterococcus* NR	Yes (Cochrane) 20/22 high
Xiao et al. (25)	28	China 16 week	420	40	*Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Propionibacterium*, *Acetobacter* NR	Yes (Cochrane) 20/28 high
Koutnikova et al. (27)	99	France 8 week	1971	NR	*Bifidobacteria*, *Streprococcus*, *Salivarius*, *Lactobacilli* 5×10^{11}	Yes (PEDro scale tool) 84/99 high
Ma et al. (30)	4	China 17 week	268	44	*Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Lepicol probiotic* NR	Yes (Jodad) 4/4 high

PEDro, Physiotherapy Evidence Database scale tool.

TABLE 2 | Detailed evaluation of the methodological quality with AMSTAR[a].

Study	Q1[b]	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Quality score
Khan et al. (32)	Yes	Yes	Yes	Yes	Yes	NO	Yes	NO	Yes	NO	Yes	8
Lavekar et al. (34)	Yes	CA	NO	Yes	NO	Yes	Yes	NO	Yes	CA	NO	5
Xiao et al. (25)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
Liu et al. (29)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NO	Yes	10
Loman et al. (31)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
Ma et al. (30)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NO	Yes	NO	NO	8
Sharpton et al. (33)	Yes	Yes	Yes	Yes	Yes	CA	Yes	NO	Yes	Yes	NO	8
Tang et al. (26)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
Gao et al. (23)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NO	NO	NO	NO	7

[a]*AMSTAR, assessment of multiple systematic reviews; CA, can't answer; Q, Question.*

[b]*Q1, Was an "a priori" design provided?; Q2, Was there duplicate study selection and data extraction?; Q3, Was a comprehensive literature search (at least two databases) performed?; Q4, Was the status of publication (i.e., gray literature) used as an inclusion criterion?; Q5, Was a list of studies (included and excluded) provided?; Q6, Were the characteristics of the included studies provided?; Q7, Was the scientific quality of the included studies assessed and documented?; Q8, Was the scientific quality of the included studies used appropriately in formulation conclusions?; Q9, Were the methods used to combine the findings of studies appropriate?; Q10, Was the likelihood of publication bias assessed?; Q11, Was the conflict of interest included?*

compared to their counterparts (**Table 3**). The sensitivity analysis revealed that the calculated overall effect sizes for ALT were not significantly changed after omitting each study. No significant small-study effects was found using Egger's and Begg's tests ($p = 0.1$ and $p = 0.074$, respectively). Moreover, visual inspection of the funnel plot indicated an asymmetric distribution of studies (**Figure 2B**). Thus, trim and fill analysis was conducted with ten studies (none imputed studies). The corrected effect

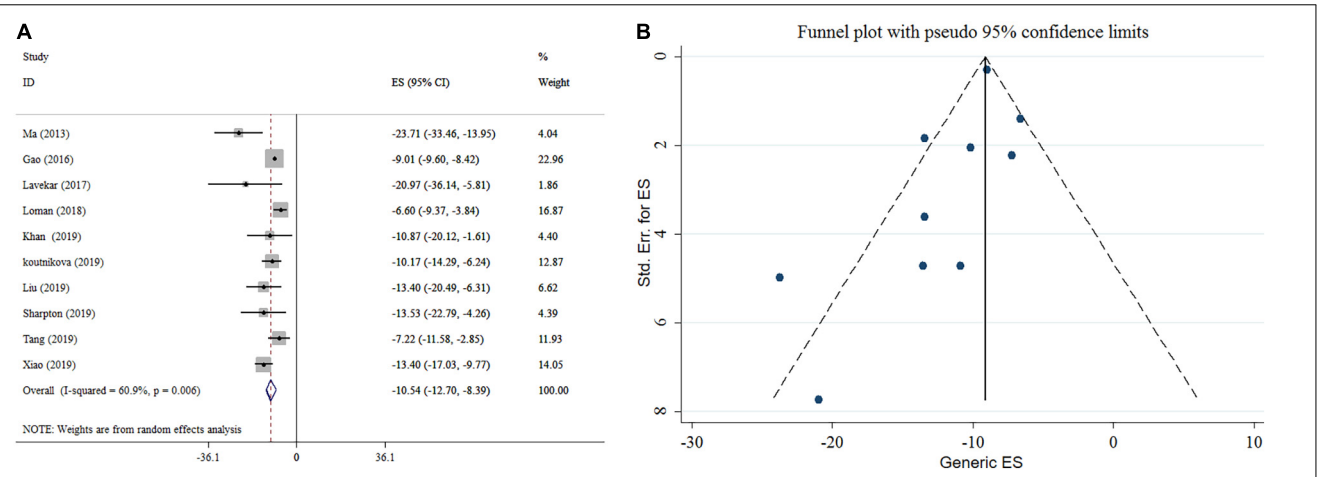

FIGURE 2 | Forest plot **(A)** detailing mean difference and 95% confidence intervals (CIs) and funnel plot **(B)** displaying publication bias in the studies reporting, the effects of probiotics supplementation on ALT levels.

TABLE 3 | Pooled estimates of probiotics effects on liver enzymes within different subgroups.

Variables	No. study	Pooled effect size (95% CI)	*p*-value	I^2 (%)	P heterogeneity
ALT					
Total	10	−10.54 (−12.70, −8.39)	<0.001	60.9	0.006
Type of effect size					
WMD	5	−11.61 (−15.24, −7.97)	<0.001	74.4	0.004
MD	5	−9.91 (−13.35, −6.46)	<0.001	44.0	0.129
Sample size					
<300	5	−13.77 (−20.49, −7.05)	<0.001	74.5	0.004
≥300	5	−9.96 (−11.98, −7.93)	<0.001	45.4	0.119
Age (years)					
≤45	3	−13.89 (−22.66, −5.13)	0.002	79.7	0.007
>45	3	−8.47 (−10.14, −6.80)	<0.001	32.5	0.227
NR	4	−12.33 (−14.88, −9.77)	<0.001	0.0	0.432
Intervention duration (week)					
<16	5	−8.76 (−11.25, −6.28)	<0.001	30.2	0.22
≥16	5	−13.50 (−18.18, −8.81)	<0.001	75.7	0.002
Country					
China	5	−11.66 (−15.21, −8.21)	<0.001	75.3	0.003
Other	5	−9.62 (−13.00, −6.23)	<0.001	38.0	0.168
AST					
Total	9	−10.19 (−13.08, −7.29)	<0.001	79.8	<0.001
Type of effect size					
WMD	4	−10.37 (−13.66, −7.07)	<0.001	59.4	0.06
MD	5	−10.58 (−16.13, −5.02)	<0.001	82.0	<0.001
Age (years)					
≤ 45	3	−11.51 (−17.80, −5.23)	<0.001	52.6	0.121
> 45	3	−7.25 (−11.98, −2.52)	<0.001	91.2	<0.001
NR	4	−12.85 (−17.00, −8.70)	<0.001	44.7	0.164
Intervention duration (week)					
<16	4	−7.79 (−12.10, −3.49)	<0.001	77.0	0.005
≥16	5	−12.89 (−17.13, −8.66)	<0.001	64.7	0.023
Country					
China	5	−10.65 (−13.58, −7.71)	<0.001	53.3	0.073
Other	4	−10.02 (−16.24, −3.79)	0.002	83.4	<0.001

size for publication bias didn't show any change after trim and fill analysis.

Effect of Probiotics on Aspartate Aminotransferase

Probiotics supplementation significantly reduced AST level according to the meta-analysis of nine studies (**Figure 3A**). Heterogeneity was observed between studies (I^2 = 79.8%, $p < 0.001$). For AST; pooled analysis, type of effect size, sample size, study location, and duration of intervention were possible sources of heterogeneity. Subgroup analysis indicated that probiotics supplementation with an intervention duration ≥ 16 weeks and a sample size of < 300 contributes to a more significant effect in lowering AST (**Table 3**). Sensitivity analysis revealed that no single study likely affected the pooled results. No significant small-study effect was shown using Egger's and Begg's tests ($p = 0.318$ and 0.466, respectively). Visual inspection of the funnel plot demonstrated a significant publication bias among included studies (**Figure 3B**). In the following trim and fill analysis, the effect size did not alter.

Effect of Probiotics on Gamma-Glutamyl Transferase

The effect of probiotics on GGT level was reported in three studies (**Figure 4**). Our analysis revealed a significant reduction in GGT levels by probiotics intervention. No remarkable heterogeneity was observed between studies (I^2 = 0.0%, p = 0.591). Subgroup analysis wasn't conducted on studies. Sensitivity analysis provided no evidence of the impact of an individual study on the overall effect size. After performing the Begg's tests, no small-study effects was detected ($p = 0.296$).

DISCUSSION

Exposure to probiotics has been the subject of numerous meta-analyses in the realm of health as well as a diverse range of disorders. We conducted this umbrella review to summarize the available evidence and draw conclusions for the effects of probiotics in NAFLD treatment. We identified 10 meta-analyses of RCTs which had high quality based on the AMSTAR checklist. There seem to be beneficial associations between probiotic consumption and liver function in terms of serum ALT, AST, and GGT levels. All-inclusive, probiotic therapy generally appears to be safe without any evidence of adverse effects.

Even though the findings suggest that probiotics supplementation may be efficacious for controlling NAFLD, it must be stated that, the effects of probiotics on liver enzymes (ALT & AST) were heterogeneous. Differences in treatment dosage, sample size, gender, and duration of intervention, mean age of participants, study location, and population may explain this heterogeneity. Also, the evidence from this study implies that probiotic supplementation in studies with sample size < 300 and supplement duration ≥ 16 weeks can meaningfully decrease ALT. In line with ALT reduction, probiotic supplementation with an intervention duration ≥ 16 weeks and a sample size of < 300 contributes to a more significant effect in lowering AST. Also, probiotic consumption had beneficial effect on reducing GGT level without any significant heterogeneity (39, 40).

NAFLD is a chronic liver disease with a worldwide prevalence of 20–30%. It can be attributed to numerous roots, including environmental parameters, metabolic, genetic, and gut microbial factors (41). In the past decade, considerable evidence has been accumulated regarding the critical role of gut microbiota unbalance and various metabolic disorders, including NAFLD. The causal role of gut microbiota dysbiosis (an imbalance in microbial homeostasis) in NAFLD genesis and development has been reported (42, 43). Host physiology, age, drugs like antibiotics, diet, and environmental factors can influence the gut microbial ecosystem (44).

The gut dysbiosis may influence the development of NAFLD via various signaling pathways including (45, 46):

- Impact on intestinal hormone production affecting glucose control.

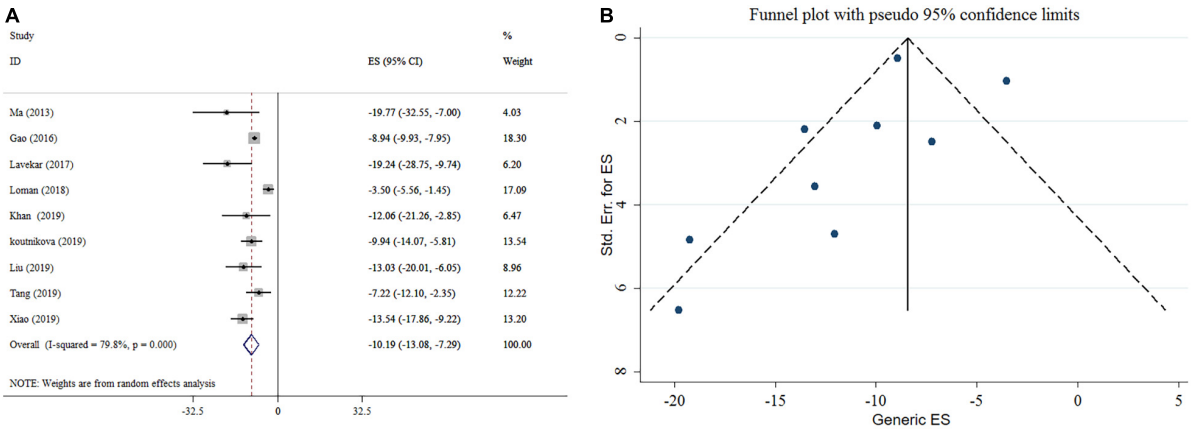

FIGURE 3 | Forest plot **(A)** detailing mean difference and 95% confidence intervals (CIs) and funnel plot **(B)** displaying publication bias in the studies reporting, the effects of probiotics supplementation on AST levels.

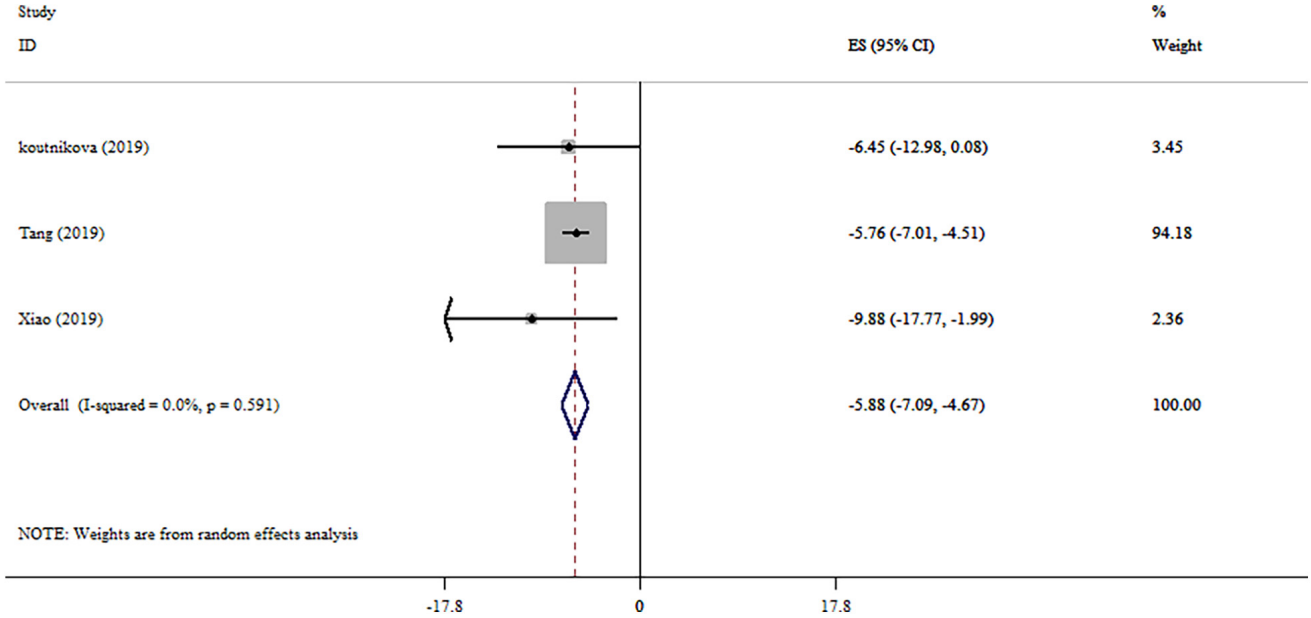

FIGURE 4 | Forest plot detailing mean difference and 95% confidence intervals (CIs), the effects of probiotics supplementation on GGT levels.

- Effect on short-chain production affecting glucose and lipid metabolism.
- Increasing liver toxicity and cardiovascular risk.
- Disturbance of bile acid homeostasis.
- Contribution in metabolic endotoxemia via bacterial lipopolysaccharide (LPS) which subsequently triggers intestinal and hepatic inflammation.

In addition, gut dysbiosis cause the intestinal more permeable which leading to an elevation in fatty acid absorption, migration of the bacteria via the gut epithelial barrier, release of toxic bacterial products and pro- inflammatory cytokines that can initiate an inflammatory cascade. Interestingly, recent studies indicated that gut dysbiosis is related to higher fecal concentrations of some metabolites (2-butanone and 4-methyl-2-pentanone) which lead to hepatocellular toxicity (47).

Probiotics are believed to be important for liver health via specific biological processes as follows: (1) increment in insulin sensitivity; (2) decreasing the absorption of glucose and LDL cholesterol; (3) modification of gut dysbiosis and inducing the production of short-chain fatty acids; (4) decrement in endotoxin concentrations; (5) reducing the oxidative stress status and inflammatory markers; (6) diminishing total cholesterol levels and (7) trapping of bile acids' substances (48–51). Interestingly, probiotics have been introduced as an effective agent in inhibiting pathogens. The fermented products of many probiotic bacteria, containing lactic and acetic acids, are significant causes of probiotics' antimicrobial features. Furthermore, bacteriocins (small antimicrobial proteins secreted by some probiotics) are another cause for this trait (52).

The overall evidence indicates that supplementation with probiotics led to a reduced level of liver enzymes (53). ALT, AST, and GGT are often used to indicate the quality of liver function. The correlation of the entitled enzymes with NAFLD has been shown in some previous studies (54, 55). In recent prospective studies, GGT is considered as a sensitive indicator of liver damage and a novel marker for oxidative stress as well as for inflammation (56, 57). In addition, there is an association between liver aminotransferase enzymes' concentration (AST and ALT) and the quality of liver function (58, 59). Based on previous reports, increased ALT levels in patients with NAFLD may be related to insulin resistance and intrahepatic fat content (60). Lower AST and ALT levels were perceptible in various meta-analyses following probiotics supplementation (25–27, 29–34). Beyond that, microbial therapies reduced circulating GGT according to other evidence-based meta-analyses (25, 27, 31). Several probiotic strains have particular abilities to improve liver function through the modulation of the gastrointestinal tract. These improvements were mostly observed with *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* or multispecies probiotic therapy (23, 27). Studies specifically analyzing the gut microbiome composition revealed that the imbalanced gut–liver axis can be a major factor in NAFLD development and progression (61). In general, the potential therapeutic effects of the gut microbiome manipulation by probiotics and changes in the relative abundance of selective bacteria can be considered as an alternative therapy in NAFLD patients.

STRENGTHS AND LIMITATIONS

This umbrella review used systematic methods with robust search strategies of various databases and independent study selection and extraction methods, which systematically summarized

the current evidence regarding the effects of probiotics supplementation on serum levels of ALT, AST, and GGT. Moreover, the quality of covered systematic reviews and meta-analyses were evaluated using the AMSTAR questioner. However, the use of existing meta-analyses is the main limitation of the umbrella review. The results may depend on what assessments to choose from each preliminary study and how to report them in the meta-analysis. Finally, the most critical limitation of this study is to consider that an impressive body of pioneering studies has supported the concept that in the absence of aminotransferase levels abnormalities, the NAFLD may pretend (62–64). So in fact these criteria may not be reliable for the diagnosis of NAFLD.

CONCLUSION

The beneficial impacts of probiotics as new promising therapeutic agents for patients with NAFLD have been summarized in this studies. Convincing evidence was obtained regarding associations between probiotic intake and liver function improvement reflecting as the serum levels of ALT, AST, and GGT.

However, due to the high heterogeneity of the results and the small number of studies included in each subgroup, the outcomes of the present study should be interpreted with caution.

Even though most studies showed hepatic enzymes as biomarkers for liver function, there is still no comprehensive agreement on counting on the enzyme levels in order to indicate liver function. Adding other non-invasive assessments in line with enzyme levels in RCTs seems to be a necessity. Conclusively, further studies need to be conducted for a definite presumption.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s. All the materials used in this systematic review and meta-analysis have been fully referenced.

AUTHOR CONTRIBUTIONS

VM and SA wrote the original manuscript and contributed to the conception of the article. VM and NR contributed to data collection and analyze and provided advice and consultation. PD contributed to the final revision of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.844242/full#supplementary-material

Supplementary Table 1 | Electronic search strategy.

REFERENCES

1. Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol.* (2012) 56:944–51. doi: 10.1016/j.jhep.2011.08.018
2. Jennings J, Faselis C, Yao MD. NAFLD-NASH: an under-recognized epidemic. *Curr Vasc Pharmacol.* (2018) 16:209–13. doi: 10.2174/1570161115666170622074007
3. Perdomo CM, Frühbeck G, Escalada J. Impact of nutritional changes on nonalcoholic fatty liver disease. *Nutrients.* (2019) 11:677. doi: 10.3390/nu11030677
4. Angulo P. Obesity and nonalcoholic fatty liver disease. *Nutr Rev.* (2007) 65(Suppl. 1):S57–63.
5. Guo X-F, Yang B, Tang J, Li D. Fatty acid and non-alcoholic fatty liver disease: meta-analyses of case-control and randomized controlled trials. *Clin Nutr.* (2018) 37:113–22. doi: 10.1016/j.clnu.2017.01.003
6. Le Yu MY, Wang L. The effect of omega-3 unsaturated fatty acids on non-alcoholic fatty liver disease: a systematic review and meta-analysis of RCTs. *Pak J Med Sci.* (2017) 33:1022. doi: 10.12669/pjms.334.12315
7. Lee C-H, Fu Y, Yang S-J, Chi C-C. Effects of omega-3 polyunsaturated fatty acid supplementation on non-alcoholic fatty liver: a systematic review and meta-analysis. *Nutrients.* (2020) 12:2769. doi: 10.3390/nu12092769
8. Zheng HJ, Guo J, Jia Q, Huang YS, Huang WJ, Zhang W, et al. The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: a systematic review and meta-analysis of randomized controlled trials. *Pharmacol Res.* (2019) 142:303–13. doi: 10.1016/j.phrs.2019.02.016
9. Musa-Veloso K, Venditti C, Lee HY, Darch M, Floyd S, West S, et al. Systematic review and meta-analysis of controlled intervention studies on the effectiveness of long-chain omega-3 fatty acids in patients with nonalcoholic fatty liver disease. *Nutr Rev.* (2018) 76:581–602. doi: 10.1093/nutrit/nuy022
10. Hannah WN, Harrison SA. Lifestyle and dietary interventions in the management of nonalcoholic fatty liver disease. *Digest Dis Sci.* (2016) 61:1365–74. doi: 10.1007/s10620-016-4153-y
11. Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *J Hepatol.* (2012) 56:255–66. doi: 10.1016/j.jhep.2011.06.010
12. Araya J, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli P, et al. Increase in long-chain polyunsaturated fatty acid n− 6/n− 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci.* (2004) 106:635–43. doi: 10.1042/CS20030326
13. Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. *J Hepatol.* (2018) 68:280–95. doi: 10.1016/j.jhep.2017.11.014
14. Zarezadeh M, Musazadeh V, Faghfouri AH, Roshanravan N, Dehghan P. Probiotics act as a potent intervention in improving lipid profile: an umbrella systematic review and meta-analysis. *Crit Rev Food Sci Nutr.* (2021):1–14. doi: 10.1080/10408398.2021.2004578
15. Yu JS, Youn GS, Choi J, Kim CH, Kim BY, Yang SJ, et al. *Lactobacillus* lactis and Pediococcus pentosaceus-driven reprogramming of gut microbiome and metabolome ameliorates the progression of non-alcoholic fatty liver disease. *Clin Transl Med.* (2021) 11:e634. doi: 10.1002/ctm2.634
16. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *N Engl J Med.* (2016) 375:2369–79.
17. Buss C, Valle-Tovo C, Miozzo S, de Mattos AA. Probiotics and synbiotics may improve liver aminotransferases levels in non-alcoholic fatty liver disease

patients. *Ann Hepatol.* (2014) 13:482–8. doi: 10.1016/s1665-2681(19)31246-3
18. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* (2011) 13:517–26. doi: 10.1016/j.cmet.2011.02.018
19. Den Besten G, Van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res.* (2013) 54:2325–40. doi: 10.1194/jlr.R036012
20. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol.* (2013) 13:790–801. doi: 10.1038/nri3535
21. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol.* (2016) 16:341–52. doi: 10.1038/nri.2016.42
22. Musazadeh V, Zarezadeh M, Faghfouri AH, Keramati M, Jamilian P, Jamilian P, et al. Probiotics as an effective therapeutic approach in alleviating depression symptoms: an umbrella meta-analysis. *Crit Rev Food Sci Nutr.* (2022) 62:1–9. doi: 10.1080/10408398.2022.2051164
23. Gao X, Zhu Y, Wen Y, Liu G, Wan C. Efficacy of probiotics in non-alcoholic fatty liver disease in adult and children: a meta-analysis of randomized controlled trials. *Hepatol Res.* (2016) 46:1226–33. doi: 10.1111/hepr.12671
24. Khalesi S, Johnson DW, Campbell K, Williams S, Fenning A, Saluja S, et al. Effect of probiotics and synbiotics consumption on serum concentrations of liver function test enzymes: a systematic review and meta-analysis. *Eur J Nutr.* (2018) 57:2037–53. doi: 10.1007/s00394-017-1568-y
25. Xiao M-W, Lin S-X, Shen Z-H, Luo W-W, Wang X-Y. Systematic review with meta-analysis: the effects of probiotics in nonalcoholic fatty liver disease. *Gastroenterol Res Pract.* (2019) 2019:1484598.
26. Tang Y, Huang J, Zhang WY, Qin S, Yang YX, Ren H, et al. Effects of probiotics on nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Ther Adv Gastroenterol.* (2019) 12:1756284819878046.
27. Koutnikova H, Genser B, Monteiro-Sepulveda M, Faurie J-M, Rizkalla S, Schrezenmeir J, et al. Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: a systematic review and meta-analysis of randomised controlled trials. *BMJ Open.* (2019) 9:e017995. doi: 10.1136/bmjopen-2017-017995
28. Higgins JP, Lane PW, Anagnostelis B, Anzures-Cabrera J, Baker NF, Cappelleri JC, et al. A tool to assess the quality of a meta-analysis. *Res Synthesis Methods.* (2013) 4:351–66. doi: 10.1002/jrsm.1092
29. Liu L, Li P, Liu Y, Zhang Y. Efficacy of probiotics and synbiotics in patients with nonalcoholic fatty liver disease: a meta-analysis. *Digest Dis Sci.* (2019) 64:3402–12. doi: 10.1007/s10620-019-05699-z
30. Ma Y-Y, Li L, Yu C-H, Shen Z, Chen L-H, Li Y-M. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. *World J Gastroenterol WJG.* (2013) 19:6911. doi: 10.3748/wjg.v19.i40.6911
31. Loman BR, Hernández-Saavedra D, An R, Rector RS. Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Nutr Rev.* (2018) 76:822–39. doi: 10.1093/nutrit/nuy031
32. Khan MY, Mihali AB, Rawala MS, Aslam A, Siddiqui WJ. The promising role of probiotic and synbiotic therapy in aminotransferase levels and inflammatory markers in patients with nonalcoholic fatty liver disease – a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol.* (2019) 31:703–15. doi: 10.1097/MEG.0000000000001371
33. Sharpton SR, Maraj B, Harding-Theobald E, Vittinghoff E, Terrault NA. Gut microbiome-targeted therapies in nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression. *Am J Clin Nutr.* (2019) 110:139–49. doi: 10.1093/ajcn/nqz042
34. Lavekar AS, Raje DV, Manohar T, Lavekar AA. Role of probiotics in the treatment of nonalcoholic fatty liver disease: a meta-analysis. *Euroasian J Hepato Gastroenterol.* (2017) 7:130. doi: 10.5005/jp-journals-10018-1233
35. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928
36. Littell JH, Corcoran J, Pillai V. *Systematic Reviews and Meta-Analysis*. Oxford: Oxford University Press (2008).
37. Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. *Phys Ther.* (2003) 83:713–21. doi: 10.1093/ptj/83.8.713
38. Clark HD, Wells GA, Huët C, McAlister FA, Salmi LR, Fergusson D, et al. Assessing the quality of randomized trials: reliability of the Jadad scale. *Control Clin Trials.* (1999) 20:448–52. doi: 10.1016/s0197-2456(99)00026-4
39. Vallianou N, Christodoulatos GS, Karampela I, Tsilingiris D, Magkos F, Stratigou T, et al. Understanding the role of the gut microbiome and microbial metabolites in non-alcoholic fatty liver disease: current evidence and perspectives. *Biomolecules.* (2021) 12:56. doi: 10.3390/biom12010056
40. Jadhav K, Cohen TS. Can you trust your gut? Implicating a disrupted intestinal microbiome in the progression of NAFLD/NASH. *Front Endocrinol.* (2020) 11:592157. doi: 10.3389/fendo.2020.592157
41. Singal AG, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol.* (2014) 109:325–34. doi: 10.1038/ajg.2013.476
42. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut.* (2013) 62:1787–94. doi: 10.1136/gutjnl-2012-303816
43. Tarantino G, Finelli C. Systematic review on intervention with prebiotics/probiotics in patients with obesity-related nonalcoholic fatty liver disease. *Future Microbiol.* (2015) 10:889–902. doi: 10.2217/fmb.15.13
44. Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol.* (2020) 17:279–97. doi: 10.1038/s41575-020-0269-9
45. Scorletti E, Afolabi PR, Miles EA, Smith DE, Almehmadi A, Alshathry A, et al. Design and rationale of the INSYTE study: a randomised, placebo controlled study to test the efficacy of a synbiotic on liver fat, disease biomarkers and intestinal microbiota in non-alcoholic fatty liver disease. *Contemp Clin Trials.* (2018) 71:113–23. doi: 10.1016/j.cct.2018.05.010
46. Loscalzo J. Gut microbiota, the genome, and diet in atherogenesis. *Mass Med Soc.* (2013) 368:1647–9. doi: 10.1056/NEJMe1302154
47. Nair S, Cope K, Terence RH, Diehl AM. Obesity and female gender increase breath ethanol concentration: potential implications for the pathogenesis of nonalcoholic steatohepatitis. *Am J Gastroenterol.* (2001) 96:1200–4. doi: 10.1111/j.1572-0241.2001.03702.x
48. Ghavami A, Roshanravan N, Alipour S, Barati M, Mansoori B, Ghalichi F, et al. Assessing the effect of high performance inulin supplementation via KLF5 mRNA expression in adults with type 2 diabetes: a randomized placebo controlled clinical trail. *Adv Pharm Bull.* (2018) 8:39–47. doi: 10.15171/apb.2018.005
49. Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci.* (2012) 57:545–53. doi: 10.1007/s10620-011-1887-4
50. Manzhalii E, Virchenko O, Falalyeyeva T, Beregova T, Stremmel W. Treatment efficacy of a probiotic preparation for non-alcoholic steatohepatitis: a pilot trial. *J Digest Dis.* (2017) 18:698–703. doi: 10.1111/1751-2980.12561
51. Wong VW, Won GL, Chim AM, Chu WC, Yeung DK, Li KC, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Ann Hepatol.* (2013) 12:256–62. doi: 10.1016/s1665-2681(19)31364-x
52. Shoaf K, Mulvey GL, Armstrong GD, Hutkins RW. Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. *Infect Immun.* (2006) 74:6920–8. doi: 10.1128/IAI.01030-06
53. Huang Z, Mu C, Chen Y, Zhu Z, Chen C, Lan L, et al. Effects of dietary probiotic supplementation on LXRα and CYP7α1 gene expression, liver enzyme activities and fat metabolism in ducks. *Br Poultry Sci.* (2015) 56:218–24. doi: 10.1080/00071668.2014.1000821
54. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* (2016) 64:73–84. doi: 10.1002/hep.28431
55. Oh HJ, Kim TH, Sohn YW, Kim YS, Oh YR, Cho EY, et al. Association of serum alanine aminotransferase and γ-glutamyltransferase levels within the reference range with metabolic syndrome and nonalcoholic fatty liver disease. *Korean J Hepatol.* (2011) 17:27. doi: 10.3350/kjhep.2011.17.1.27
56. Lee DH, Blomhoff R, Jacobs DR Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radic Res.* (2004) 38:535–9. doi: 10.1080/10715760410001694026

57. Lee DH, Jacobs DR Jr. Serum gamma-glutamyltransferase: new insights about an old enzyme. *J Epidemiol Commun Health.* (2009) 63:884–6. doi: 10.1136/jech.2008.083592
58. Askarpour M, Djafarian K, Ghaedi E, Sadeghi O, Sheikhi A, Shab-Bidar S. Effect of L-carnitine supplementation on liver enzymes: a systematic review and meta-analysis of randomized controlled trials. *Arch Med Res.* (2020) 51:82–94. doi: 10.1016/j.arcmed.2019.12.005
59. Sanyal D, Mukherjee P, Raychaudhuri M, Ghosh S, Mukherjee S, Chowdhury S. Profile of liver enzymes in non-alcoholic fatty liver disease in patients with impaired glucose tolerance and newly detected untreated type 2 diabetes. *Indian J Endocrinol Metab.* (2015) 19:597–601. doi: 10.4103/2230-8210.163172
60. Maximos M, Bril F, Portillo Sanchez P, Lomonaco R, Orsak B, Biernacki D, et al. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. *Hepatology.* (2015) 61:153–60. doi: 10.1002/hep.27395
61. Dai X, Hou H, Zhang W, Liu T, Li Y, Wang S, et al. Microbial metabolites: critical regulators in NAFLD. *Front Microbiol.* (2020) 11:567654. doi: 10.3389/fmicb.2020.567654
62. Manco M, Alisi A, Nobili V. Risk of severe liver disease in NAFLD with normal ALT levels: a pediatric report. *Hepatology.* (2008) 48:2087–8. doi: 10.1002/hep.22631
63. Kohli R. There's more under the nonalcoholic fatty liver disease umbrella than an elevated alanine aminotransferase level. *J Pediatr.* (2014) 164:684–6. doi: 10.1016/j.jpeds.2013.12.026
64. Vajro P. Transaminases and pediatric nonalcoholic fatty liver disease diagnosis. *J Pediatr Gastroenterol Nutr.* (2017) 65:e114. doi: 10.1097/MPG.0000000000001668

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Linear Growth Trajectories, Catch-up Growth, and Its Predictors Among North Indian Small-for-Gestational Age Low Birthweight Infants: A Secondary Data Analysis

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Background: Low birthweight small-for-gestational-age (SGA-LBW) (birthweight below the 10th percentile for gestational age; SGA-LBW) infants are at an increased risk of poor postnatal growth outcomes. Linear growth trajectories of SGA-LBW infants are less studied in South Asian settings including India.

Objectives: To describe the linear growth trajectories of the SGA-LBW infants compared with appropriate-for-gestational-age LBW (AGA-LBW) infants during the first 6 months of life. In addition, we estimated catch-up growth (ΔLAZ $>$ 0.67) in SGA-LBW infants and their performance against the WHO linear growth velocity cut-offs. Additionally, we studied factors associated with poor catch-up growth in SGA-LBW infants.

Methods: The data utilized came from an individually randomized controlled trial that included low birthweight (LBW) infants weighing 1,500–2,250 g at birth. A total of 8,360 LBW infants were included. For comparison between SGA-LBW and AGA-LBW infants, we presented unadjusted and adjusted estimates for mean differences (MDs) or risk ratios (*RR*s) for the outcomes of length, linear growth velocity, length for age *z*-score (LAZ) score, and stunting. We estimated the proportion of catch-up growth. Generalized linear models of the Poisson family with log links were used to identify factors associated with poor catch-up growth in SGA-LBW infants.

Results: Low birthweight small-for-gestational-age infants had a higher risk of stunting, lower attained length, and a lower LAZ score throughout the first 6 months of life compared with AGA-LBW infants, with differences being maximum at 28 days and minimum at 6 months of age. The linear growth velocity in SGA-LBW infants compared

with AGA-LBW infants was significantly lower during the birth–28 day period [MD −0.19, 95% confidence interval (*CI*): −0.28 to −0.10] and higher during the 3- to 6-month period (MD 0.17, 95% *CI*: 0.06–0.28). Among the SGA-LBW infants, 55% showed catch-up growth for length at 6 months of age. Lower wealth quintiles, high birth order, home birth, male child, term delivery, non-exclusive breastfeeding, and pneumonia were associated with the higher risk of poor catch-up in linear growth among SGA-LBW infants.

Conclusion: Small for gestational age (SGA) status at birth, independent of gestational age, is a determinant of poor postnatal linear growth. Promotion of institutional deliveries, exclusive breastfeeding, and prevention and early treatment of pneumonia may be helpful to improve linear growth in SGA-LBW infants during early infancy.

Clinical Trial Registration: [https://clinicaltrials.gov/], identifier [NCT02653534].

Keywords: catch-up growth (CUG), linear growth, small for gestational age (SGA), low birthweight (LBW) infant, growth faltering

INTRODUCTION

Small for gestational age (SGA) infants are at higher risk of mortality, poor postnatal growth, morbidities, and long-term neurodevelopmental outcomes compared with appropriate for gestational age (AGA) infants (1–4). Estimates from 2012 suggest that 62.5% of the SGA births globally are from South or Southeast Asia. In India, 36.1% [95% confidence interval (*CI*): 25–52.8%] of all live births are SGA, as per the INTERGROWTH 21st standards (5). Evidence suggests that SGA infants account for approximately 40% of the stunting among under-two children in the Indian population (6). However, the postnatal linear growth patterns of SGA infants have been less studied in South Asian settings, including India.

Previous studies have used different definitions and country-specific standards to define SGA births and catch-up growth, which makes it difficult to compare across studies (7, 8). The Brighton Collaboration Working Group has defined SGA infants as those with birthweight below the 10th percentile for gestational age, and for computing birthweight centiles, the INTERGROWTH-21st standards are globally accepted (5, 7). However, for measurement of catch-up growth, multiple cut-offs have been used including a change in length for age *z*-score (LAZ) of >0.67 between two-time points, or achieving an LAZ of >2 SD or >1.3 SD, or growth above the third percentile for LAZ of the WHO 2006 growth standards at any time during follow-up (8–11). A systematic review by Campisi et al. in 2019 reported that ∼70–80% of the SGA infants showed catch-up growth in the first year after birth and by 2 years of age, >85% had catch-up growth (8). However, the studies included in the review were from developed countries and had used inconsistent definitions for catch-up growth. Moving forward to enable future international comparisons, Campisi et al. (8) have suggested using the criteria of ">0.67 change in LAZ scores as per the WHO 2006 child growth standards" to define catch-up growth in SGA infants over a specified time period as this represents a clinically significant response.

In this analysis, using data from an intervention cohort of 8,402 low birthweight (LBW) infants in North India followed up from birth to 6 months of age, our first objective was to describe the linear growth trajectories of the low birthweight small-for-gestational-age (SGA-LBW) infants compared with appropriate-for-gestational-age LBW (AGA-LBW) infants. Second, to estimate the performance of SGA-LBW infants against different growth indicators, such as catch-up growth in length and the WHO linear growth velocity cut-offs at 3 and 6 months of age. Third, to study the factors associated with poor catch-up growth in SGA-LBW infants at 6 months of age.

Abbreviations: AGA, appropriate for gestational age; CUG, catch-up growth; GLMM, generalized linear mixed-effects model; IQ, intelligence quotient; KMC, kangaroo mother care; LAZ, length for age *z*-score; LMIC, lower- and middle-income countries; SD, standard deviation; SGA, small for gestational age.

MATERIALS AND METHODS

Study Design and Population

The present study was a secondary analysis of data from an individually randomized, controlled trial conducted between 2015 and 2018 to assess the efficacy of promoting community-initiated kangaroo mother care (ciKMC) on post-enrollment neonatal and 6-month mortality in 8,402 LBW weighing between 1,500 and 2,250 g within 3 days of birth. The study was conducted in Faridabad and Palwal districts of Haryana, India. The ciKMC intervention included promotion and support of skin-to-skin contact and exclusive breastfeeding through home visits on days 1–3, 5, 7, 10, 14, 21, and 28 of life (12). All infants in the intervention and control arms received usual care, i.e., home-based postnatal care visits (on days 3, 7, 14, 21, 28, and 42) as implemented through the health system (13). The ciKMC intervention had a substantial effect on infant 6-month mortality but had no substantial effect on linear

growth at 6 months of age (12). The trial was registered with ClinicalTrials.gov, NCT02653534.

Procedures

Pregnant women were identified by a screening and enrollment team and followed-up regularly and with increasing frequency as the expected date of delivery approached. Newborn infants weighing between 1,500 and 2,250 g were enrolled within 72 h of birth if kangaroo mother care (KMC) was not initiated in the facility and written informed consent was obtained from the mothers or primary caregivers (12). We excluded infants who were unable to feed, had difficulty in breathing, had less than normal movements, had gross congenital malformations, KMC was initiated the in hospital, or whose caregivers intended to move away over the next 6 months or refused participation. For the anthropometric assessments and obtaining other clinical information, home visits were conducted at the age of 28, 90, and 180 days.

All anthropometric assessments were taken two times by a pair of workers using the standard techniques (14). Standardization exercises were conducted prior to study initiation and repeated every 6 months (15). An infantometer (model 417; Seca, Chino, CA, United States; sensitivity 0.1 cm) and a digital hanging weighing scale (AWS-SR-20; American Weigh Scales, Cumming, GA, United States; 10 g sensitivity) were used to assess length and weight, respectively (15). Standard weights and length measurement rods were used to calibrate the weighing scales and infantometers at regular intervals, respectively (15). Detailed methods of the primary trial are previously published (12, 16).

Assessment of Gestational Age, Small for Gestation Age, and Appropriate for Gestational Age Status

Gestational age at the time of delivery was estimated from antenatal ultrasound reports in 5,372 (64.3%) women. If the ultrasound was not available, we estimated gestational age based on the last menstrual period as documented in hospital records or as per maternal recall, in the given order of preference. For defining SGA/AGA, we calculated birthweight centiles using "growth standards" package based on INTERGROWTH-21 standards in R software (17). Infants with birthweight below the 10th percentile for their gestational age were classified as SGA, and those with $\geq$10th percentile as AGA.

Study Outcomes

The study outcomes for linear growth in the first 6 months of life were mean length, linear growth velocity, LAZ, and stunting rates. Linear growth velocity was defined as the change in length in centimeters with respect to previous time points, i.e., birth to 28, 28–90, 90–180, and birth to 180 days. LAZ according to the WHO 2006 standards were generated using "zscore06" package in Stata 16.0, TX, United States (18, 19) and scores <-6 were excluded (14). Stunting was defined as LAZ score <-2.

Poor catch-up in linear growth was defined as ΔLAZ $\leq$ 0.67 between two-time points, as per the WHO 2006 child growth standards (8, 20). Catch-up growth was estimated in the SGA infants for the periods of birth to 3 months and birth to 6 months.

Statistical Analysis

Analyses were done using STATA16.0 (Stata Corp., College Station, TX, United States) and statistical software R version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria). To compare linear growth trajectories across the groups of LBW infants, i.e., SGA and AGA, we estimated the mean and standard deviation (SD) of absolute length in centimeters, LAZ scores, and proportion stunted at birth, 28, 90, and 180 days of age. In addition, we estimated the mean (SD) of linear growth velocity for time periods of birth to 28, 28–90, 90–180, and birth to 180 days. To compare the two groups, i.e., SGA-LBW and AGA-LBW infants, we estimated unadjusted mean difference (MD) for continuous variables, e.g., length, LAZ scores, and risk ratio (RR) for binary variables, e.g., stunting. We presented both unadjusted estimates, and estimates adjusted for gestational age, intervention using generalized linear models of the Poisson family with log links. In a sensitivity analysis, we compared the linear growth trajectories between SGA and AGA within the subgroup of infants born preterm $<$37 weeks gestation.

We estimated the proportion of SGA-LBW infants showing catch-up growth $>$0.67 SD during the birth to 3-month and birth to 6-month periods. In addition, using the WHO linear growth velocity standards (21), we estimated the proportion of SGA-LBW infants who were above the median, -1 SD, and -2 SD cut-offs during birth to 3-month, and birth to 6-month periods.

To identify the predictors of poor catch-up growth at 180 days, we conducted univariable and multivariable regression analyses using generalized linear models of the Poisson family with log link. In the regression model, we included covariates that have been previously shown to be associated with growth (22–26). The covariates with $p < 0.1$ in the univariable analysis or those biologically plausible were used in the multivariable model (27). In the multivariable analysis, we adjusted for the intervention, i.e., ciKMC and accounted for clustering within households. We reported both unadjusted and adjusted *RR* and its 95% *CI*s of poor catch-up growth for each predictor variable. We have reported *Akaike's information criteria* (AIC) and *Bayesian information criteria* (BIC) value and have calculated the receiver operating characteristic area under curve (ROC-AUC) as a part of regression model diagnostics.

RESULTS

Of the total 8,402 LBW infants, 42 infants with an LAZ score <-6 were excluded from the analysis. In the 8,360 LBW infants included, 3,918 were AGA and 4,442 were SGA. In the included infants, length measurements were available for almost all at birth, 97% at 28 days, 85% at 90 days, and 79% at 180 days of age (**Figure 1**). The mean (SD) gestational age of the AGA-LBW infants was 34.2 (1.6) weeks and that of SGA-LBW infants was 37.1 (1.2) weeks. Overall, 64.2% (5,369/8,360) were preterm; all AGA-LBW infants were preterm, and among SGA-LBW infants 32.6% (1,451/4,442) were preterm. The proportions of SGA and

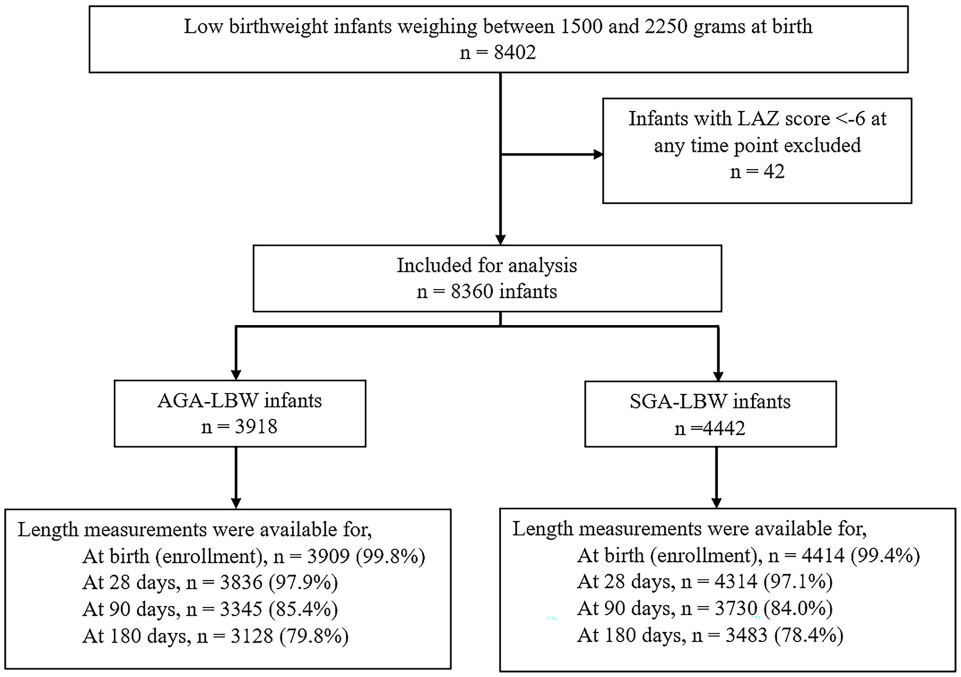

FIGURE 1 | Flowchart showing the number of infants with length measurements included in the analysis.

AGA infants exclusively breastfed at 3 months were 45.8 and 43.0%, respectively.

Growth Trajectory

In the SGA-LBW and AGA-LBW infants, the mean (SD) length at birth was 44.7 (1.6) cm and 44.5 (1.5) cm, and that at 180 days of age was 62.4 (2.4) cm and 62.5 (2.4) cm, respectively. The mean difference (SD) in length between SGA-LBW and AGA-LBW infants adjusted for gestational age was the highest at 28 days (MD −0.98, 95% *CI*: −1.09 to −0.88) and the lowest at 180 days of age (MD −0.73, 95% *CI*: −0.89 to −0.58, **Table 1**).

The 6-month linear growth velocity during the period from birth to 180 days in SGA-LBW and AGA-LBW was 17.8 (2.2) and 17.9 (2.2) cm. Compared with AGA-LBW infants, the linear growth velocity in SGA-LBW infants was substantially lower in the neonatal period (adjusted MD −0.19, 95% *CI*: −0.28 to −0.10), and was significantly higher during the 90- to 180-day period (adjusted MD 0.17, 95% *CI*: 0.06–0.28). The difference in linear growth velocity during other time periods was not statistically significant.

At birth, the LAZ score of SGA-LBW and AGA-LBW infants was −2.7 (0.8) and −2.7 (0.8), respectively. At 180 days of age, the LAZ scores in SGA-LBW and AGA-LBW infants were −1.9 (1.0) and −1.9 (1.1), respectively. Adjusted analysis showed that the LAZ score of SGA-LBW infants was substantially lower than AGA-LBW infants at all time points of measurement (**Table 1**). The adjusted MD in LAZ score between SGA-LBW and AGA-LBW infants was maximum at 28 days and (MD −0.53, 95% *CI*: −0.59 to −0.48) and minimum at 180 days of age (MD −0.38, 95% *CI*: −0.45 to −0.31).

The proportion of stunting in SGA-LBW and AGA-LBW infants at 28 days was 64 and 68%, and that at 180 days was 45 and 44%, respectively. Adjusted analysis showed that the risk of stunting was higher by 24, 37, and 33% in SGA-LBW infants against AGA-LBW infants at 28, 90, and 180 days of age, respectively (**Table 1**).

The findings were similar in the subgroup of preterm infants (**Supplementary Table 1**).

Catch-up Growth in Length Among Low Birthweight Small-for-Gestational-Age Infants

Among the SGA-LBW infants, 47% (1,760/3,724) showed catch-up growth by 3 months, and 55% (1,908/3,477) showed catch-up growth by 6 months. In AGA-LBW infants, 59% (1,890/3,226) were above the cut-off for catch-up growth at 6 months. As per the WHO length velocity standards from birth to 6 months, 65, 88, and 97% of the SGA-LBW infants were above the cut-offs for median, −1 SD and −2 SD, respectively (**Figure 2**). Among the AGA-LBW infants, 68, 88, and 97% were above the median, −1 SD, and −2 SD of the WHO length velocity standards from birth to 6 months, respectively.

Predictors of Poor Catch-up in Linear Growth at 6 Months Among Low Birthweight Small-for-Gestational-Age Infants

Multivariable analysis showed that the adjusted risk of poor catch-up growth was substantially lower in the infants born to

TABLE 1 | Linear growth patterns in small for gestational age (SGA) and appropriate for gestational age (AGA) low birthweight infants at different time points.

Characteristics	Time-point	SGA-LBW	AGA-LBW	Unadjusted mean difference/IRR	Adjusted[b] mean difference/RR
Attained length, cm: mean (SD)	Birth[a]	44.67 (1.57)	44.54 (1.53)	0.12 (0.06 – 0.19)	−0.82 (– 0.91 to – 0.72)
	28 days	49.40 (1.85)	49.22 (1.86)	0.18 (0.10 – 0.26)	−0.98 (– 1.09 to – 0.88)
	90 days	56.20 (2.11)	56.11 (2.12)	0.08 (– 0.02 to 0.18)	−0.89 (– 1.02 to – 0.75)
	180 days	62.38 (2.35)	62.49 (2.35)	−0.11 (– 0.22 to 0.01)	−0.73 (– 0.89 to – 0.58)
Linear growth velocity, cm: mean (SD)	Birth[a]-28 days	4.72 (1.45)	4.66 (1.46)	0.05 (– 0.01 to 0.12)	−0.19 (– 0.28 to – 0.10)
	28-90 days	6.81 (1.45)	6.92 (1.46)	−0.11 (– 0.18 to – 0.04)	0.06 (– 0.04 to 0.15)
	90-180 days	6.23 (1.54)	6.37 (1.61)	−0.14 (– 0.22 to – 0.06)	0.17 (0.06 – 0.28)
	Birth-180 days	17.75 (2.19)	17.95 (2.23)	−0.20 (– 0.31 to – 0.10)	0.07 (– 0.08 to 0.22)
LAZ score: mean (SD)	Birth[a]	−2.66 (0.84)	−2.73 (0.82)	0.06 (0.03 – 0.10)	−0.46 (– 0.51 to – 0.41)
	28 days	−2.40 (0.96)	−2.49 (0.97)	0.09 (0.05 – 0.14)	−0.53 (– 0.59 to – 0.48)
	90 days	−2.06 (1.02)	−2.11 (1.02)	0.04 (– 0.01 to 0.09)	−0.47 (– 0.53 to – 0.40)
	180 days	−1.89 (1.04)	−1.85 (1.05)	−0.05 (– 0.10 to 0.01)	−0.38 (– 0.45 to – 0.31)
Stunting[c]: n/N (%)	28 days	2, 734/4, 314 (63.6)	2, 596/3, 836 (67.7)	0.94 (0.89 – 0.99)	1.24 (1.16 – 1.34)
	90 days	1, 846/3, 730 (49.4)	1, 737/3, 345 (51.7)	0.95 (0.89 – 1.02)	1.37 (1.25 – 1.50)
	180 days	1, 552/3, 483 (44.7)	1, 367/3, 128 (43.9)	1.02 (0.95 – 1.10)	1.33 (1.20 – 1.47)

[a]Birth measurements were within 3 days of birth. [b]Adjusted for gestational age and intervention. [c]For stunting, i.e., length for age z-score (LAZ) < −2 SD, risk ratio (RR) is estimated.

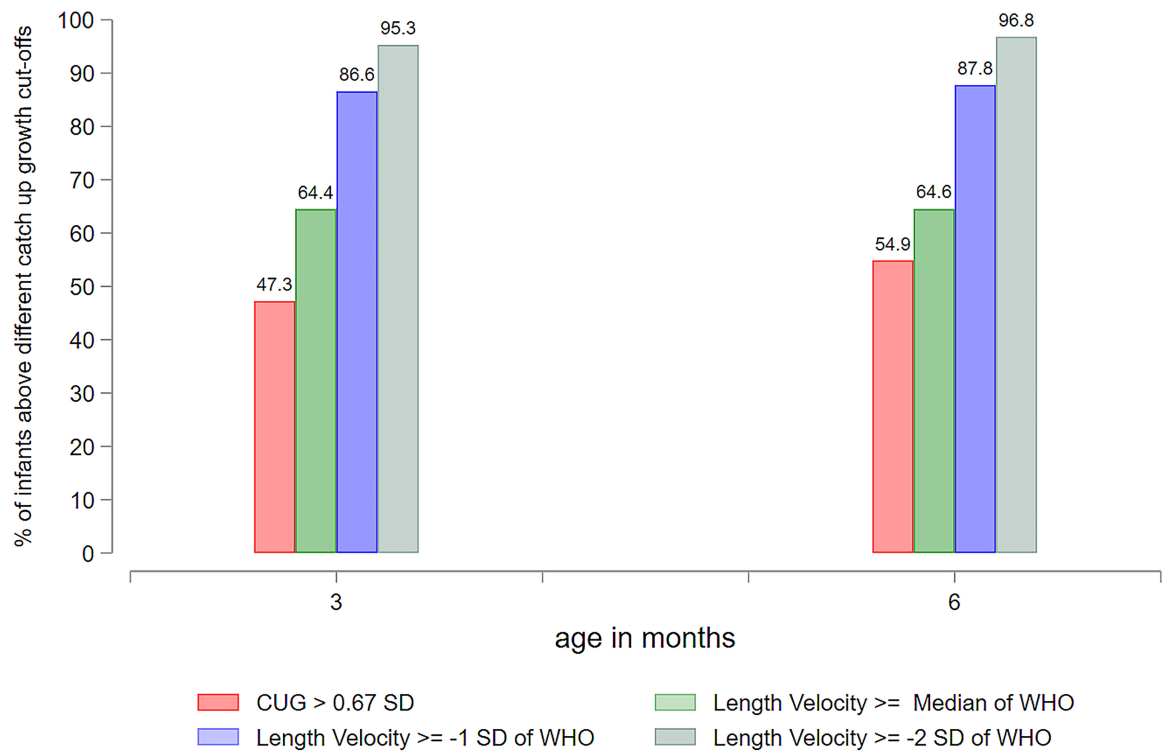

FIGURE 2 | Performance of low birthweight small-for-gestational-age (SGA-LBW) infants with respect to different growth cut-offs at 3 and 6 months of age.

the least poor (*RR* 0.74, 95% *CI*: 0.64–0.86; **Table 2**) or less poor families (*RR* 0.86, 95% *CI*: 0.76–0.97) compared with the poorest families. Infants born at hospitals had a lower risk of poor catch-up growth against those born at home (*RR* 0.88, 95% *CI*: 0.81–0.96). Higher birth order infants (>4) had a greater risk of poor catch-up growth compared with infants born to primiparous mothers (*RR* 1.26, 95% *CI*: 1.09–1.47). The *RR* of poor catch-up growth was 0.89 (95% *CI*: 0.83–0.96) in the girl child vs. boys. Exclusive breastfeeding at 3 months was associated with a lower risk of poor catch-up growth

TABLE 2 | Factors associated with poor catch-up in linear growth status at 6 months among low birthweight small-for-gestational-age (SGA-LBW) infants.

Variables	Unadjusted RR (95% *CI*)	Adjusted[1,4] RR (95% *CI*)	*P*-value (adjusted)
Sociodemographic factors			
Maternal age, year			
≤20	Reference	Reference	
21–29	1.03 (0.95, 1.13)	0.98 (0.88, 1.08)	0.644
≥30	1.31 (1.15, 1.50)	1.04 (0.89, 1.22)	0.609
Maternal education			
Educated	Reference	Reference	
Not educated	1.30 (1.21, 1.39)	1.06 (0.97, 1.15)	0.191
Wealth quintile			
Poorest	Reference	Reference	
Very poor	0.91 (0.82, 0.99)	0.95 (0.86, 1.06)	0.361
Poor	0.84 (0.76, 0.93)	0.94 (0.84, 1.05)	0.254
Less poor	0.75 (0.67, 0.84)	0.86 (0.76, 0.97)	0.018
Least poor	0.60 (0.53, 0.68)	0.74 (0.64, 0.86)	0.000
Social category with reservations[2]			
SC/ST	Reference	Reference	
OBC	1.05 (0.98, 1.14)	1.04 (0.96, 1.12)	0.337
Unreserved	0.71 (0.63, 0.79)	0.82 (0.73, 0.93)	0.002
Birth-related factors			
Birth order			
1	Reference	Reference	
2–4	1.22 (1.13, 1.32)	1.15 (1.05, 1.27)	0.002
>4	1.53 (1.36, 1.72)	1.26 (1.09, 1.47)	0.002
Place of birth			
Home	Reference	Reference	
Hospital	0.78 (0.72, 0.84)	0.88 (0.81, 0.96)	0.006
Infant factors			
Sex			
Male	Reference	Reference	
Female	0.90 (0.84, 0.97)	0.89 (0.83, 0.96)	0.001
Gestational age category			
Term ≥37 weeks	Reference	Reference	
Preterm <37 weeks	0.84 (0.78, 0.92)	0.82 (0.75, 0.89)	0.000
Exclusively breastfed at 3 months			
No	Reference	Reference	
Yes	0.90 (0.84, 0.97)	0.89 (0.83, 0.96)	0.004
Hospitalization at 6 months			
No	Reference	Reference	
Yes	1.12 (0.98, 1.26)	1.06 (0.94, 1.20)	0.329
H/O diarrhea[3]			
No	Reference	Reference	
Yes	1.04 (0.96, 1.13)	1.03 (0.95, 1.11)	0.485
H/O pneumonia[3]			
No	Reference	Reference	
Yes	1.35 (1.16, 1.57)	1.24 (1.07, 1.43)	0.004
Study group			
Control	Reference	Reference	
Intervention	1.00 (0.93, 1.08)	1.01 (0.94, 1.09)	0.684

[1]Adjusted estimates are based on multivariable regression (predictor model). We have also adjusted for the intervention and accounted for any household clustering.
[2]OBC (other backward class)—term used by the GOI to classify socially and educationally disadvantaged category of population, SC/ST (schedule caste/schedule tribe)—historically disadvantaged indigenous people as identified by the Government of India.
[3]Based on history obtained (H/O) at 3 and 6 months visits (for last 14 days).
[4]Akaike's information criteria (AIC) = 1.60, Bayesian information criteria (BIC) = −25705.1.

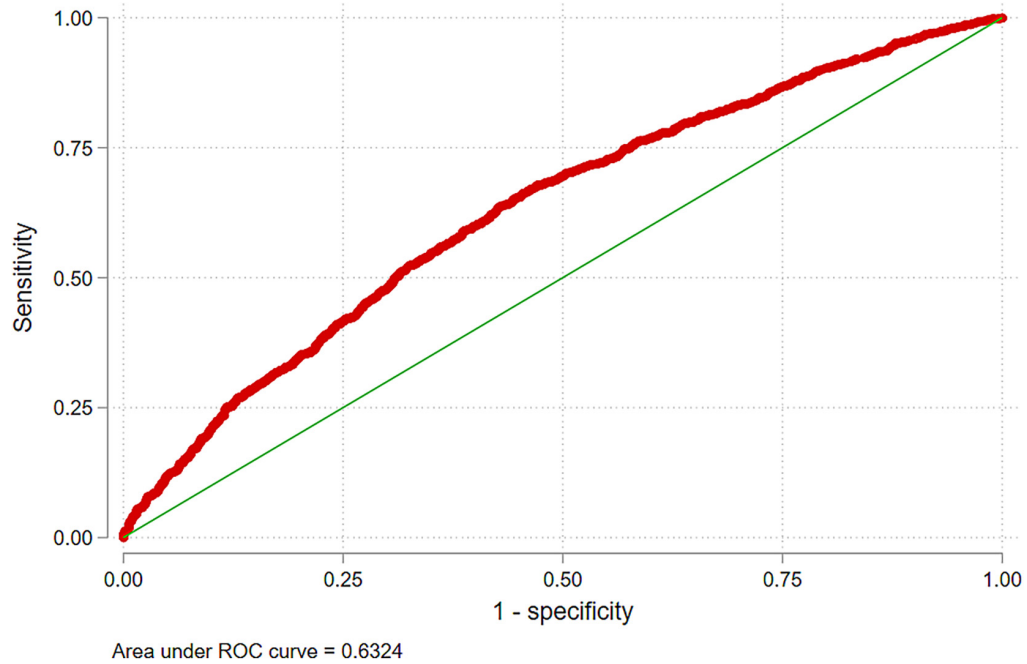

FIGURE 3 | A receiver operating characteristic (ROC) curve of the multivariable model to predict poor catch-up growth in SGA-LBW infants.

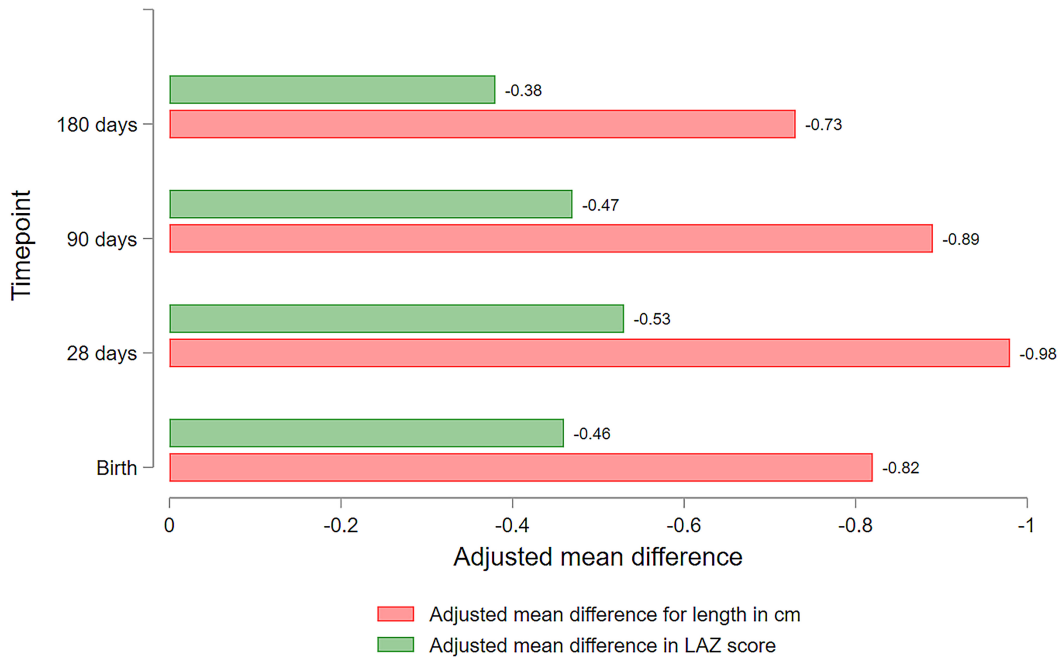

FIGURE 4 | Mean difference (MD) in attained length and length for age *z*-scores (LAZ) between appropriate-for-gestational-age LBW (AGA-LBW) and SGA-LBW infants.

(*RR* 0.89, 95% *CI*: 0.83–0.96). Preterm infants (<37 weeks gestational age) had a lower risk of poor catch-up growth (*RR* 0.82, 95% *CI*: 0.75, 0.89) than term infants. History obtained (H/O) pneumonia was associated with a higher risk (*RR* 1.24, 95% *CI*: 1.07, 1.44) of poor catch-up growth. We did not find any substantial association between diarrhea, hospitalization, or the study intervention, i.e., ciKMC with poor catch-up growth. The ROC-AUC of our multivariable regression model to identify predictors of poor catch-up growth was 63.2% (**Figure 3**).

DISCUSSION

The findings showed that SGA-LBW infants in the study population had a higher risk of stunting, lower attained length, and LAZ score throughout the first 6 months of life compared with AGA-LBW infants, with the differences being maximum at 28 days and minimum at 6 months of age (**Figure 4**). The linear growth velocity in SGA-LBW infants compared with the AGA-LBW infants was lower during the neonatal period but was substantially higher during the 3- to 6-month period. More than half of the SGA-LBW infants met the criteria of catch-up growth for length at 6 months of age. Poor catch-up in linear growth among SGA-LBW infants was associated with lower wealth quintiles, high birth order, home birth, male child, term delivery, non-exclusive breastfeeding, and pneumonia.

Previous studies have observed the linear growth patterns in SGA and/or LBW infants but reports on the longitudinal growth of SGA-LBW infants is limited in Indian settings. In an earlier study using data from the third national health and nutritional examination survey in the United States (1988–1994), it was shown that despite catch-up, the SGA infants remain shorter and lighter compared with AGA or LGA infants from 2 to 47 months of age (28).

A recent study in Australia suggested that catch-up growth (defined by $\geq$0.67 SD change) is more frequent in SGA infants compared with AGA infants with normal intrauterine growth and most of the catch-up growth is observed at approximately 4 months of age (29). A systematic review in 2019 (8) that included 11 studies with full-term SGA infants showed that 87% of the children achieved catch-up growth across all the different definitions used, at latest follow-up (ranging between 1 and 18 years). The review reported that 58% of the SGA infants achieve catch-up growth at 6 months age, while 69–82% infants showed catch-up by 1 year of age. The most common definitions used to classify SGA births were a birthweight of <–2 SD, followed by a birthweight of less than the 10th percentile. To define catch-up growth, the most common definitions used were HAZ of > -2 SD or ΔLAZ > 0.67. A study in Western India that followed-up 247 LBW infants with 73% being SGA, reported that 80% of the infants were above the -2 SD HAZ score at 4 years of age.

The observation from our study corroborates with the previous reports and suggests that more than half of the SGA-LBW infants in our population in North India demonstrate catch-up growth during the first 6 months of life. However, SGA-LBW infants continue to remain relatively shorter than the AGA-LBW infants adjusted for gestational age at 6 months of age. In a longitudinal survey of full-term SGA babies followed-up to 1 year of age, belonging to upper socio-economic strata and representing North-Western India, SGA infants had significantly lower weight and length than AGA infants (30). Our study, along with previous reports, suggests that SGA status at birth, independent of gestational age, is a determinant with postnatal growth trajectory (28). The accelerated linear growth in early infancy in the SGA-LBW infants seems to compensate for intrauterine growth restriction, and the SGA infants not showing catch-up growth may be a high-risk group for short stature in adult life (31–33).

The association of poor catch-up in linear growth with lower wealth quintiles, high birth order, non-exclusive breastfeeding, and pneumonia, found in our study was similar to observations in previous studies (23, 34). We found that SGA-LBW infants born at term are at a higher risk of poor catch-up growth compared with preterm infants. This may be explained by the faster compensatory postnatal growth rate in preterm infants as also reported in previous studies (35). The observed lower risk of poor catch-up growth in girls compared with the boys corroborates to the fact that boys are born shorter than girls relative to the gender-specific international norms and continue to remain below these norms during the first 1,000 days (34, 36). The observed reduced risk of poor catch-up growth in hospital-born infants may be explained by better healthcare seeking. The findings highlight the importance of improving modifiable factors, such as institutional deliveries, exclusive breastfeeding, and prevention or early treatment of pneumonia to promote better linear growth of SGA-LBW infants in the first 6 months of life.

Beyond estimating catch-up growth by the definition of $>$0.67 SD change, we used the different cut-points of the WHO linear growth velocity standards to demonstrate the substantial variability in the proportions when different definitions are used (**Figure 2**). Currently, the definition of catch-up growth lacks clear consensus. The findings underscore the critical need to have standard definitions for catch-up growth in infants to enable comparison across studies and settings (8).

Our study is one of the largest studies on LBW infants reported to date in the Indian population with rigorous longitudinal measurements of anthropometry till 6 months of age. However, there are some limitations. First, ultrasound-based gestational age was not available in 36% of the women. However, we checked the proportions of catch-up growth and its determinants (results not shown) among the infants where ultrasonography (USG)-based gestational age was available, and the results were similar. Second, additional information on the maternal and paternal height, and fetal growth restriction could have been valuable, which has known association with postnatal growth. Lastly, as rapid catch-up growth in early life has implications on future cardio-metabolic risks (37), longer follow-up with measures of body composition could be helpful. In future, it may be worthwhile to plan longitudinal follow-up cohorts where serial measurement of fetal growth as well as postnatal growth is captured up to 2 years of life to be able to study and compare growth patterns of different subgroups of infants, such as LBW, SGA, preterm, as well as normal term-AGA infants parallelly.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of the article can be made available by the authors on request, without undue reservations.

ETHICS STATEMENT

The study involved human participants. It was reviewed and approved by Ethics Review Committee, Centre for Health

Research and Development, Society for Applied Studies, New Delhi; the Regional Committee for Medical and Health Research Ethics in Norway; and World Health Organization, Geneva. Written informed consent to participate in this study was provided by the mothers/primary caregivers of the infants included.

AUTHOR CONTRIBUTIONS

BS: conceptualization, data acquisition, data analysis and interpretation, writing the first draft, manuscript editing, and finalization. TC: conceptualization, data analysis and interpretation, manuscript editing, and finalization. NN: data analysis and writing the first draft of the manuscript. MK: manuscript editing and finalization. SM, ST, and NB: investigators of the original study-obtained funding, data access, and critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.827589/full#supplementary-material

REFERENCES

1. Arcangeli T, Thilaganathan B, Hooper R, Khan KS, Bhide A. Neurodevelopmental delay in small babies at term: a systematic review. *Ultrasound Obstet Gynecol.* (2012) 40:267–75. doi: 10.1002/uog.11112
2. Murray E, Fernandes M, Fazel M, Kennedy SH, Villar J, Stein A. Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review. *BJOG.* (2015) 122:1062–72. doi: 10.1111/1471-0528.13435
3. Prendergast AJ, Humphrey JH. The stunting syndrome in developing countries. *Paediatr Int Child Health.* (2014) 34:250–65. doi: 10.1179/2046905514Y.0000000158
4. Walker SP, Wachs TD, Grantham-McGregor S, Black MM, Nelson CA, Huffman SL, et al. Inequality in early childhood: risk and protective factors for early child development. *Lancet.* (2011) 378:1325–38. doi: 10.1016/S0140-6736(11)60555-2
5. Lee AC, Kozuki N, Cousens S, Stevens GA, Blencowe H, Silveira MF, et al. Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21(st) standard: analysis of CHERG datasets. *BMJ.* (2017) 358:j3677. doi: 10.1136/bmj.j3677
6. Danaei G, Andrews KG, Sudfeld CR, Fink G, McCoy DC, Peet E, et al. Risk factors for childhood stunting in 137 developing countries: a comparative risk assessment analysis at global, regional, and country levels. *PLoS Med.* (2016) 13:e1002164. doi: 10.1371/journal.pmed.1002164
7. Schlaudecker EP, Munoz FM, Bardají A, Boghossian NS, Khalil A, Mousa H, et al. Small for gestational age: case definition & guidelines for data collection, analysis, and presentation of maternal immunisation safety data. *Vaccine.* (2017) 35(48 Pt A):6518–28.
8. Campisi SC, Carbone SE, Zlotkin S. Catch-up growth in full-term small for gestational age infants: a systematic review. *Adv Nutr.* (2019) 10:104–11. doi: 10.1093/advances/nmy091
9. Leroy JL, Frongillo EA, Dewan P, Black MM, Waterland RA. Can children catch up from the consequences of undernourishment? Evidence from child linear growth, developmental epigenetics, and brain and neurocognitive development. *Adv Nutr.* (2020) 11:1032–41. doi: 10.1093/advances/nmaa020
10. Mericq V, Ong KK, Bazaes R, Peña V, Avila A, Salazar T, et al. Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. *Diabetologia.* (2005) 48:2609–14. doi: 10.1007/s00125-005-0036-z
11. Soto N, Bazaes RA, Peña V, Salazar T, Avila A, Iñiguez G, et al. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. *J Clin Endocrinol Metab.* (2003) 88:3645–50. doi: 10.1210/jc.2002-030031
12. Mazumder S, Taneja S, Dube B, Bhatia K, Ghosh R, Shekhar M, et al. Effect of community-initiated kangaroo mother care on survival of infants with low birthweight: a randomised controlled trial. *Lancet.* (2019) 394:1724–36. doi: 10.1016/S0140-6736(19)32223-8
13. Ministry of Health and Family Welfare. *Home Based Newborn Care Operational Guidelines [Internet].* New Delhi: Ministry of Health and Family Welfare (2014).
14. World Health Organization and the United Nations Children's Fund (UNICEF). *Recommendations for Data Collection, Analysis and Reporting on Anthropometric Indicators in Children Under 5 Years Old. Contract No.: Licence: CC BY-NC-SA 3.0 IGO.* Geneva: UNICEF (2019).
15. Taneja S, Sinha B, Upadhyay RP, Mazumder S, Sommerfelt H, Martines J, et al. Community initiated kangaroo mother care and early child development in low birth weight infants in India-a randomized controlled trial. *BMC Pediatr.* (2020) 20:150. doi: 10.1186/s12887-020-02046-4
16. Mazumder S, Taneja S, Dalpath SK, Gupta R, Dube B, Sinha B, et al. Impact of community-initiated kangaroo mother care on survival of low birth weight

infants: study protocol for a randomized controlled trial. *Trials.* (2017) 18:262. doi: 10.1186/s13063-017-1991-7
17. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2020).
18. World Health Organization. *WHO Child Growth Standards and the Identification of Severe Acute Malnutrition in Infants and Children: A Joint Statement by the World Health Organization and the United Nations Children's Fund*. Geneva: World Health Organization (2009).
19. Leroy J. *ZSCORE06: Stata Command for the Calculation of Anthropometric Z-Scores Using the 2006 WHO Child Growth Standards*. Boston, MA: Boston College Department of Economics (2011).
20. Raaijmakers A, Jacobs L, Rayyan M, van Tienoven TP, Ortibus E, Levtchenko E, et al. Catch-up growth in the first two years of life in extremely low birth weight (ELBW) infants is associated with lower body fat in young adolescence. *PLoS One.* (2017) 12:e0173349. doi: 10.1371/journal.pone.0173349
21. WHO. *Growth Velocity Based on Weight, Length and Head Circumference: Methods and Development*. Geneva: World Health Organization (2009).
22. Leger J, Limoni C, Collin D, Czernichow P. Prediction factors in the determination of final height in subjects born small for gestational age. *Pediatr Res.* (1998) 43:808–12. doi: 10.1203/00006450-199806000-00015
23. Pradeilles R, Norris T, Ferguson E, Gazdar H, Mazhar S, Bux Mallah H, et al. Factors associated with catch-up growth in early infancy in rural Pakistan: a longitudinal analysis of the women's work and nutrition study. *Matern Child Nutr.* (2019) 15:e12733. doi: 10.1111/mcn.12733
24. Bavdekar AR, Vaidya UV, Bhave SA, Pandit AN. Catch up growth and its determinants in low birth weight babies: a study using Z scores. *Indian Pediatr.* (1994) 31:1483–90.
25. Nguyen PH, Headey D, Frongillo EA, Tran LM, Rawat R, Ruel MT, et al. Changes in underlying determinants explain rapid increases in child linear growth in alive & thrive study areas between 2010 and 2014 in Bangladesh and Vietnam. *J Nutr.* (2017) 147:462–9. doi: 10.3945/jn.116.243949
26. Itabashi K, Mishina J, Tada H, Sakurai M, Nanri Y, Hirohata Y. Longitudinal follow-up of height up to five years of age in infants born preterm small for gestational age; comparison to full-term small for gestational age infants. *Early Hum Dev.* (2007) 83:327–33. doi: 10.1016/j.earlhumdev.2006.07.002
27. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. *Source Code Biol Med.* (2008) 3:17. doi: 10.1186/1751-0473-3-17
28. Hediger ML, Overpeck MD, Maurer KR, Kuczmarski RJ, McGlynn A, Davis WW. Growth of infants and young children born small or large for gestational age: findings from the third national health and nutrition examination survey. *Arch Pediatr Adolesc Med.* (1998) 152:1225–31. doi: 10.1001/archpedi.152.12.1225
29. McLaughlin EJ, Hiscock RJ, Robinson AJ, Hui L, Tong S, Dane KM, et al. Appropriate-for-gestational-age infants who exhibit reduced antenatal growth velocity display postnatal catch-up growth. *PLoS One.* (2020) 15:e0238700. doi: 10.1371/journal.pone.0238700
30. Kaur H, Bhalla AK, Kumar P. Longitudinal growth dynamics of term symmetric and asymmetric small for gestational age infants. *Anthropol Anz.* (2017) 74:25–37. doi: 10.1127/anthranz/2016/0640
31. Albertsson-Wikland K, Karlberg J. Postnatal growth of children born small for gestational age. *Acta Paediatr Suppl.* (1997) 423:193–5. doi: 10.1111/j.1651-2227.1997.tb18413.x
32. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: short stature and beyond. *Endocr Rev.* (2007) 28:219–51. doi: 10.1210/er.2006-0039
33. Karlberg J, Albertsson-Wikland K. Growth in full-term small-for-gestational-age infants: from birth to final height. *Pediatr Res.* (1995) 38:733–9. doi: 10.1203/00006450-199511000-00017
34. Alderman H, Headey D. The timing of growth faltering has important implications for observational analyses of the underlying determinants of nutrition outcomes. *PLoS One.* (2018) 13:e0195904. doi: 10.1371/journal.pone.0195904
35. Kang L, Wang H, He C, Wang K, Miao L, Li Q, et al. Postnatal growth in preterm infants during the first year of life: a population-based cohort study in China. *PLoS One.* (2019) 14:e0213762. doi: 10.1371/journal.pone.0213762
36. Harding JE, McCowan LM. Perinatal predictors of growth patterns to 18 months in children born small for gestational age. *Early Hum Dev.* (2003) 74:13–26. doi: 10.1016/s0378-3782(03)00080-x
37. Cianfarani S, Germani D, Branca F. Low birthweight and adult insulin resistance: the "catch-up growth" hypothesis. *Arch Dis Child Fetal Neonatal Ed.* (1999) 81:F71–3. doi: 10.1136/fn.81.1.f71

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Nutrition Risk Screening and Related Factors Analysis of Non-hospitalized Cancer Survivors: A Nationwide Online Survey in China

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Purposes: This study investigated the nutritional problems and risks of Chinese non-hospitalized cancer survivors through an online survey.

Methods: The survey included nutritional and clinical questions distributed to non-hospitalized cancer survivors. All data were screened and analyzed with strict quality control. Nutrition Risk Screening-2002 (NRS-2002) was adopted and the related factors were analyzed.

Results: Six thousand six hundred eighty-five questionnaires were included. The prevalence of nutritional risk was 33.9%, which varied according to age, sex, cancer type, TNM staging, oncologic treatment, time interval since last treatment, etc. In the regression analysis, nutritional risk was associated with age, TNM staging, and nutrition support. Patients with leukemia and digestive cancer had the highest NRS-2002 score (3.33 ± 1.45 and 3.25 ± 1.61); the prevalence of nutritional risk (NRS-2002 ≥ 3) was 66.7 and 55.1%, respectively. Patients with a higher TNM stage had higher NRS-2002 scores in non-digestive cancer, which was not seen in digestive cancer. Among digestive, bone, nervous, and respiratory cancer patients, the NRS-2002 score mainly consisted of "impaired nutritional status," which coincided with the "disease severity score" in leukemia patients. Nutrition intervention was achieved in 79.7 and 15.2% of patients with nutritional risk and no risk. Of the patients, 60.3% exhibited confusion about nutritional problems, but only 25.1% had professional counseling.

Conclusions: Regular nutritional risk screening, assessment, and monitoring are needed to cover non-hospitalized cancer survivors to provide nutrition intervention for better clinical outcome and quality of life. By online survey, the nutritional risk of non-hospitalized cancer survivors was found high in China, but the nutrition support or professional consultation were not desirable. The composition of nutritional risk should also be aware of.

Keywords: non-hospitalized cancer survivors, online survey, oncology, nutritional risk, nutrition intervention

INTRODUCTION

It is estimated that about 10 million deaths from cancer in 2020 worldwide (1). Malnutrition is commonly seen among cancer patients and account for 10–20% of cancer death (2). Weight loss and dietary reduction promote the occurrence of malnutrition in cancer patients, thus affecting oncologic treatments, overall survival time, and quality of life (3–5). Standardized nutritional support starts with nutritional risk screening, which is an essential first step in structured process of nutrition care, aims to identify nutritional risks, and to provide the appropriate amount of nutritional support for improving patient outcomes (6–8). European guidelines recommend immediate nutritional screening for all patients after cancer detection (9, 10). The Investigation on Nutrition Status and Its Clinical Outcome of Common Cancer (INSCOC), which was conducted in 22 cities covering more than 80 hospitals in China, found that 26.1, 32.1, and 22.2% of in-patients had severe, moderate, and mild malnutrition (11). However, it is worth noting that a large proportion (∼75%) of cancer survivors are non-hospitalized, who are either in remission or between treatment cycles. To date, large sample surveys investigating the nutritional status of this population as well as their nutrition-related clinical problems remain scarce. The nutritional risk and demand of these non-hospitalized cancer survivors should be investigated, therefore there is an urgent requirement for action to fill this blank. Since it is hard for doctors or dietitians to reach this population and provide nutrition service, with the support of online survey, we can assess the nutritional status on non-hospitalized patients in a wide range.

Malnutrition has adverse effect on quality of life and overall survival (5, 12), thus understanding the nutritional status and identification of the nutritional risk would bring benefit to provide appropriate nutrition support in time, so as to improve the clinical outcome. Among the numerous nutrition screening tools used in clinical practice, the nutritional risk screening 2002 (NRS-2002) is a tool developed by Kondrup (8) and an ESPEN working group in 2002, which is based on the outcome observed in randomized controlled trials and identifies patients who are likely to benefit from nutritional support by an improved clinical outcome. As recommended by ESPEN, NRS-2002 is content valid and could be done by various health providers. Even though NRS-2002 is not designed for cancer patients, the high validity is also confirmed by cancer patients, that participants identified with nutritional risk by NRS-2002 developed a decreased overall survival and worse clinical outcome (13, 14). Moreover, the secondary analysis of EFFORT study showed that for cancer patients at high risk, personalized nutrition intervention resulted in increased survival and better quality of life (15). The study suggests that NRS-2002 is also suitable for out-patients and community-living cancer patients (16). Moreover, key influencing factors for nutritional risk includes clinical diagnosis, oncologic treatment, gender, gastrointestinal symptoms, education, and income, all of which can also be collected by online survey.

During the era of COVID-19, in which face-to-face interviews are limited, this study investigated the nutritional status targeting non-hospitalized cancer survivors who are in remission or between treatment cycles by an online questionnaire, and hypothesized the nutritional status of this population was of concern and required attention by physicians and dietitians. Moreover, risk factors and composition of nutritional risk will be analyzed.

MATERIALS AND METHODS

Subjects

These questionnaires were distributed through a network push by three large social management institutions for cancer patients, which have tens of thousands of registered cancer patients across the country. This ensured that large-scale group surveys could be promoted in a short span of time. The administrators of the organizations emailed or sent WeChat messages containing a study invitation to registered patients; "if you are a cancer patient who is currently in the treatment interval or at the end of treatment, you may consider completing the following questionnaire survey." If the patients opted to participate, they will provide an electronic signature on the consent. Then a link to an online questionnaire will be sent to the patients. The time frame for recruitment was between February 2020 and June 2020. The inclusion criteria were as follows: (1) age $\geq$ 18y, regardless of sex; (2) patients with pathological or clinical diagnosis of cancer (unlimited tumor types); (3) non-hospitalized survivors who were in remission or in between treatment cycles; (4) patients who voluntarily participated in the survey. The exclusion criteria were as follows was: (1) patients who refused to participate.

The protocol was approved by the Human Ethics Committee of Peking Union Medical College Hospital (No. ZS-2601); all participants provided written informed consent. The study was registered at ClinicalTrials.gov (NCT 04778540).

Questionnaire Design and Data Collection

The survey aimed to identify the prevalence of nutritional risks in non-hospitalized cancer survivors and describe their nutritional status and support requirements. The questionnaire was developed according to criteria of NRS-2002, including age, nutritional status (percentage of weight loss, general condition, and recent food intake), and disease severity (diagnosis and stage) (8). Moreover, based on literature review, the general risk factors, such as education, residence, payment methods, recent treatment, the time interval between the survey taken and last oncological treatment, and current nutrition support were also listed in the questionnaire. The design followed the principles of voluntariness, acceptability, objectivity, and non-orientation. Patients were first introduced to the study purpose and confidentiality principles. Objective and closed questions were listed at the beginning, followed by subjective questions, such as appetite, food intake, gastrointestinal symptoms, and access to nutritional support. Factual personal questions, such as education and insurance, were placed at the end. The survey was concise and could be answered within 10-min timeframe. Next, the multi-disciplinary research team of the Nationwide Online Survey on Nutritional Risk and Clinical Outcome of Non-hospitalized Cancer Patients (NOS-NOC), consisting of

clinical dietitians, oncologists, epidemiologists, psychologists, and nurses, discussed the questions summarized and evidence from literature reviews with a focus on implementation and domains for nutritional risk screening. During this process, the questions were modified to be more understandable of patients and added with attitudes and perspectives on nutrition, such as the tendency and frequency of nutrition department visits.

The NRS-2002 score is calculated by adding the nutritional status impaired score (0–3) to the severity of disease score (0–3) plus a score of 1 for patients' age ≥70 years. The total NRS-2002 score ranges from 0 to 7. The nutritional status impaired score is determined by quartiles of decreased oral food intake in the previous week, the presence of weight loss of at least 5% during the previous 1–3 months, and a low body mass index (BMI) combined the impaired general condition (7). In this study, we adapted Chinese BMI criteria (normal range $18.5 \leq \text{BMI} < 24.0$) established by Chinese Obese Working group, which is according to population research (17). Weight loss and reduction of food intake were self-reported. The severity of disease was evaluated based on the patient's choice of whether there is severe condition listed in NRS-2002 criteria, and categorized as none, slight, moderate, or severe, and converted to scores of 0-3. According to the recommendations by ESPEN Screening Guideline, an NRS score ≥ 3 means nutritionally at risk and a NRS score < 3 means no at nutritional risk (7).

Investigators distributed 150 questionnaires to perform a pretest in order to assess reliability and validity and retrieved 121 (response rate 80.7%). Researchers conducted telephone follow-ups to evaluate the participant's nutritional status, compared it with their answers to assess the agreement. Those with a completion rate of more than 90% and agreement of more than 95% were considered validated and reliable. Finally 114 questionnaires were thought to be qualified (94.2%). Moreover, the researchers also asked participants whether they felt the questions were too long or complicated and found good acceptability. Hence, the questionnaire was regarded as reasonable, valid, and reliable (see **Figure 1** Flowsheet).

Quality Control

Our research team recruited eight registered dietitians (RDs) from the aforementioned social management institutions for cancer patients; they underwent a 1-week research training for conducting the purpose, methods, procedure, and quality control requirements of this investigation. Furthermore, they became the investigative assistants of the research team for data review and logical inspection.

First, the questionnaires were scanned and filtered according to the inclusion and exclusion criteria. Next, troubleshooting was conducted, which was divided into an automatic program error check and a manual check. Network engineers set up initial graphic verification to intercept the questions answered by a machine. Then, SMS verification was carried out to ensure the real existence of the phone number. At the same time, each IP address was limited to only one submission. The average answering time of this questionnaire was about 300 seconds, depending on the pretest; only those with more than 180 s of answering time were considered valid. Common sense errors, such as selecting EN and PN at the same time, choosing four kinds of cancer at the same time, obviously wrong height and weight reports, were excluded. Additionally, the RDs participated in the audit with 30 working days and a total of more than 800 h. As performed, 20% of randomly selected questionnaires were checked by phone to confirm the accuracy.

Statistical Analysis

Categorical data were described as relative frequencies; quantitative variables were described as means and standard deviations. According to age, gender, different sites of the primary tumor, and staging, we divided the data into different subgroups; only subgroups with more than 30 participants were included for statistical validity. The differences in nutritional risk scores were compared among the subgroups using *t*-test or analysis of variance (ANOVA). The NRS-2002 score was treated as either a continuous or categorical variable for a nutritional risk classification threshold of 3 (NRS-2002 < 3, NRS-2002 ≥ 3); this was to classify the subjects with or without nutritional risk (7). To investigate the reason for nutritional risk, we further analyzed the composition of the NRS-2002 score. The association between NRS-2002 score and age, gender, site of tumor, cancer stage, therapy, interval of oncological treatment, and symptoms were investigated by univariable and multivariable linear regression analysis. NRS-2002 was alternatively treated as a continuous variable, or as categorical toward a classification threshold of 3 (NRS <3, NRS ≥3). The data were entered into SPSS version 24.0; two-sided $P < 0.05$ was considered significant.

RESULTS

Demographic Characteristics

A total of 10,000 questionnaires were sent during the recruitment period and received respondents of 8,429 (84.3%). 379 and 653 questionnaires were excluded because of the short answering time and incompletion, respectively. According to the inclusion and exclusion criteria, a total of 712 patients were excluded as follows: 29 patients aged < 18 years; 402 patients currently undergoing oncological treatment; 79 non-cancer patients; and 202 questionnaires found with common sense or logical mistakes. Finally, 6,685 questionnaires were entered final analysis (see **Figure 1** for the flowsheet).

The online survey enrolled participants from 31 provinces, autonomous regions, and municipalities of China. The demographic characteristics are listed in **Supplementary Table 1**; more females than males as well as more urban residences than rural areas were recorded. The most frequently reported case was lung cancer (2,492, 37.3%), while others included esophageal (246, 3.7%), gastric (402, 6.0%), liver (158, 2.4%), breast (1,051, 15.7%), ovarian (706, 10.6%), and colorectal cancer (277, 4.2%); 1,219 cases were categorized as digestive system cancer, while 5,466 cases were of non-digestive system cancer. The majority of participants suffered from diarrhea (1,416, 21.2%), abdominal distension (1,501, 22.5%), nausea or vomiting (2,864, 42.8%), acid reflux or heartburn (1,062, 15.9%), and constipation (2,230, 33.3%) for at least 1 week.

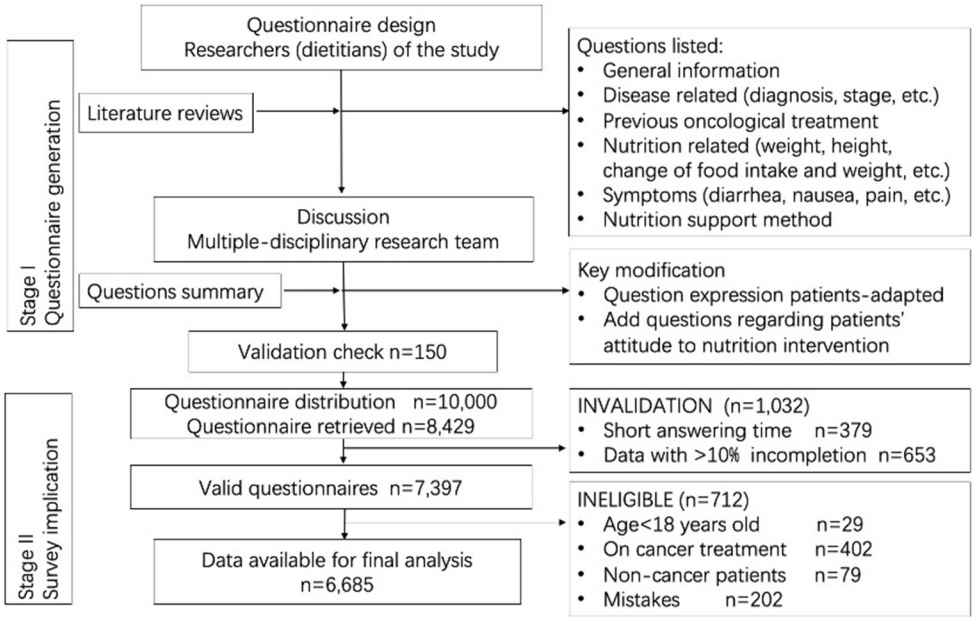

FIGURE 1 | Flowsheet.

Weight Change

The following were revealed: 610 (9.1%) participants being underweight (BMI $<$ 18.5 kg/m^2); 1,693 (25.3%) being overweight; and 575 (8.6%) being obese. A total of 1,089 (16.3%), 417 (6.2%), and 327 (4.9%) patients had $>$ 5% weight loss during the past 3, 2, and 1 months, respectively, while 279 (4.2%) patients reported visible wasting. More than 10% of patients with laryngeal, esophageal, gastric, and bone cancer had $>$ 5% weight loss within 1 month (**Table 1**).

Dietary Intake and Nutrition Support

This part was based on the following parameters: recent dietary change and any nutritional support received recently, such as oral nutritional supplements, tube feeding, or parenteral nutrition. As a result, 337 (5.0%) patients reported more than 75% dietary reduction, while 598 (8.9%) and 741 (11.1%) had a reduction of 50–75 and 25–50%, respectively. In addition, due to the direct impact on food intake, digestion, and absorption, digestive system cancer contributed to 38.6% of all food intake reduction cases (**Table 1**).

In terms of nutritional support, 79.7% of patients with nutritional risk and 15.2% of patients with no risk received nutritional support. A total of 5,953 patients (89.1%) tolerated oral meals. Among them, oral nutritional supplements (ONS) were used in 1,249 cases; nasal enteral nutrition was needed in 365 cases; and parenteral nutrition was needed in 385 cases. Lastly, 672 and 60 cases depended on total nasal feeding and total parenteral nutrition, respectively.

Severity of Disease

This part was based on the questions regarding whether there was any severe pneumonia, dialysis, bone marrow transplantation, stroke, head injury, or organ dysfunction, and evaluated by the experienced oncologist and clinical dietitians in the research team. Patients with hematologic malignancies, severe pneumonia, or stroke were thought to have a score of 2; patients reported to have bone marrow transplantation or head injury were regarded as having a score of 3. If there is nothing severe complications, the score would be rated as 1 score due to cancer. As a result, 19 (0.1%), 72 (1.1%), and 6,604 (98.8%) patients had score of 3, 2, and 1.

Nutrition Risk Screening

Nutritional risk screening was based on the following parameters: age, recent changes in weight, food intake, primary tumor site, diagnosis, and disease severity. A total of 2,268 patients (33.9%) had an NRS-2002 score $\geq$ 3; the prevalence of nutritional risk varied among cancer types. Leukemia patients ranked the highest by both the average score (3.33 $\pm$ 1.45) and the percentage of nutritional risk (66.7%), followed by digestive system cancers with a high incidence of nutritional risk (55.1%) and a high NRS-2002 score. Leukemia and digestive system cancer were the only two diseases with more than half of the patients at nutritional risk. Patients with breast cancer had the lowest NRS-2002 score and percentage (14.3%) with NRS $\geq$ 3 (see **Table 1**, **Supplementary Figure 1**).

Age and Sex

The NRS-2002 scores varied significantly among the different age groups. For $<$ 45, 45–65, 65–85, and $>$ 85-year-old groups, the NRS-2002 scores were 1.72 $\pm$ 1.12, 1.83 $\pm$ 1.43, 2.39 $\pm$ 1.37, and 2.75 $\pm$ 1.72, respectively ($P < 0.05$). Subgroup analysis showed that nutritional risk increased with age in patients with lung and colorectal cancers. For gastric, esophageal, skin, and breast

TABLE 1 | BMI, weight, and dietary reduction, NRS-2002 score of the participants.

Cancer type	N	NRS-2002 score			BMI				Weight change				Dietary reduction		
		Average score	<3 N (%)	≥3 N (%)	<18.5 N (%)	18.5–23.9 N (%)	24–27.9 N (%)	≥28 N (%)	>5% weight loss within 3 months N (%)	>5% weight loss within 2 months N (%)	>5% weight loss within 1 month N (%)	Visibly wasting away N (%)	25–50% dietary reduction in the recent 1 week N (%)	51–75% dietary reduction in the recent 1 week N (%)	76–100% dietary reduction in the recent 1 week N (%)
Lung	2454	1.89 ± 1.32	1709 (69.6)	745 (30.4)	229 (9.3)	1367 (55.7)	640 (26.1)	218 (8.9)	348 (14.2)	134 (5.5%)	99 (4.0)	114 (4.6)	274 (11.2)	209 (8.5)	125 (5.1)
Other respiratory system	50	1.74 ± 1.1	34 (68.0)	16 (32.0)	1 (2.0)	31 (62.0)	12 (24.0)	6 (12.0)	11 (22.0)	7 (14.0)	3 (6.0)	3 (6.0)	10 (20.0)	13 (26.0)	4 (8.0)
Oral	25	2.56 ± 1.29	10 (40.0)	15 (60.0)	1 (4.0)	16 (64.0)	7 (28.0)	1 (4.0)	6 (24.0)	5 (20.0)	1 (4.0)	2 (8.0)	4 (16.0)	5 (20.0)	2 (8.0)
Gastric	402	2.78 ± 1.38	166 (41.3)	236 (58.7)	56 (13.9)	254 (63.2)	70 (17.4)	22 (5.5)	98 (24.4)	47 (11.7)	40 (10.0)	21 (5.2)	58 (14.4)	65 (16.2)	47 (11.7)
Esophageal	245	2.62 ± 1.3	96 (39.2)	149 (60.8)	30 (12.2)	152 (62.0)	43 (17.6)	20 (8.2)	68 (27.8)	24 (10.0)	29 (11.8)	12 (4.9)	48 (19.6)	41 (16.7)	16 (6.5)
Liver	155	2.53 ± 1.21	73 (47.1)	82 (52.9)	16 (10.3)	96 (61.9)	33 (21.3)	10 (6.)	39 (25.2)	14 (9.0)	15 (10.0)	11 (7.1)	25 (16.1)	24 (15.5)	12 (7.7)
Colorectal	271	2.58 ± 1.26	138 (50.9)	133 (49.1)	29 (10.7)	156 (57.6)	67 (24.7)	19 (7.0)	56 (20.7)	18 (6.6)	20 (7.4)	15 (5.5)	38 (14.0)	34 (12.5)	16 (5.9)
Pancreas	45	2.59 ± 1.31	21 (46.7)	24 (53.3)	8 (17.8)	27 (60.0)	7 (15.6)	3 (6.7)	11 (24.4)	4 (8.9)	3 (6.7)	4 (8.9)	5 (11.1)	7 (15.6)	5 (11.1)
Gallbladder	27	2.56 ± 1.25	15 (55.6)	12 (44.4)	4 (14.8)	13 (48.1)	9 (33.3)	1 (3.7)	6 (22.2)	3 (11.1)	0	2 (7.4)	2 (7.4)	4 (14.8)	0
Bile duct	25	2.56 ± 1.29	11 (44.0)	14 (56.0)	3 (12.0)	17 (68.0)	4 (16.0)	1 (4.0)	8 (32.0)	2 (8.0)	1 (4.0)	2 (8.0)	3 (12.0)	3 (12.0)	1 (4.0)
Other digestive system	24	2.38 ± 1.62	17 (71.0)	7 (29.0)	5 (17.0)	8 (33.0)	8 (33.0)	3 (13.0)	5 (21.0)	0	1 (4.0)	0	3 (12.5)	0	2 (8.3)
Kidney	78	2.36 ± 1.1	51 (65.4)	27 (34.6)	3 (3.8)	38 (48.7)	18 (23.1)	19 (24.4)	11 (14.1)	3 (3.8)	3 (3.8)	1 (1.2)	5 (6.4)	6 (7.7)	4 (5.1)
Ureter	60	1.87 ± 1.17	39 (65.0)	21 (35.0)	4 (6.7)	40 (66.7)	12 (20.0)	4 (6.7)	12 (20.0)	9 (15.0)	4 (6.7)	3 (5.0)	5 (8.3)	4 (6.7)	1 (1.7)
Bladder	64	2.42 ± 1.31	36 (56.3)	28 (43.8)	12 (18.)	31 (48.4)	14 (21.9)	7 (10.9)	12 (18.8)	8 (12.5)	4 (6.3)	2 (3.1)	5 (7.8)	4 (6.3)	2 (3.1)
Prostate	67	2.13 ± 1.03	40 (59.7)	27 (40.3)	6 (9.0)	46 (68.7)	12 (17.9)	3 (4.5)	11 (16.4)	5 (7.5)	6 (9.0)	2 (3.0)	12 (17.9)	2 (3.0)	2 (3.0)
Other urinary system	11	2.0 ± 1.34	8 (72.7)	3 (27.3)	0	5 (45.4)	4 (36.4)	2 (18.2)	4 (36.4)	0	0	1 (9.1)	1 (9.1)	1 (9.1)	1 (9.1)

(Continued)

TABLE 1 | Continued

Cancer type	N	NRS-2002 score			BMI				Weight change				Dietary reduction		
		Average score	<3 N (%)	≥3 N (%)	<18.5 N (%)	18.5–23.9 N (%)	24–27.9 N (%)	≥28 N (%)	>5% weight loss within 3 months N (%)	>5% weight loss within 2 months N (%)	>5% weight loss within 1 month N (%)	Visibly wasting away N (%)	25–50% dietary reduction in the recent 1 week N (%)	51–75% dietary reduction in the recent 1 week N (%)	76–100% dietary reduction in the recent 1 week N (%)
Leukemia	33	3.33 ± 1.45	11 (33.3)	22 (67.7)	9 (27.3)	11 (33.3)	11 (33.3)	2 (6.1)	3 (9.1)	9 (27.3)	3 (9.1)	0	1 (3.0)	3 (9.1)	3 (9.1)
Lymphoma	53	2.68 ± 1.14	26 (49.1)	27 (50.9)	6 (11.3)	21 (39.6)	20 (37.8)	6 (11.3)	14 (26.4)	5 (9.4)	2 (3.8)	2 (3.8)	9 (17.0)	3 (5.7%)	2 (3.8)
Other blood system	20	2.55 ± 1.1	11 (55.0)	9 (45.0)	1 (5.0)	7 (35.0)	6 (3.0%)	6 (3.0)	4 (20.0)	2 (10.0)	1 (5.0)	3 (15.0)	1 (5.0)	1 (5.0)	1 (5.0)
Bone	120	2.34 ± 1.49	64 (53.3)	56 (46.7)	18 (15.0)	58 (48.3)	34 (28.3)	10 (8.3)	25 (20.8)	10 (8.3)	14 (11.7)	11(9.2)	21 (7.5)	16 (13.3)	10 (8.3)
Skin	150	1.82 ± 1.18	103 (68.7)	47(31.3)	16 (10.7)	81 (54.0)	38 (25.3)	15 (10.0)	29 (19.3)	14 (9.3)	7 (4.7)	5 (3.3)	19 (12.7)	9 (6.0)	7 (4.7)
Cerebral	79	2.2 ± 1.52	44 (55.7)	35 (44.3)	10 (12.7)	46 (58.2)	12 (15.2)	11 (16.5)	21 (26.6)	11 (13.9)	7 (8.9)	5 (6.3)	7 (8.9)	12 (15.2)	4 (5.1)
Uterus	246	2.45 ± 1.24	135 (54.9)	111 (45.1)	27 (11.0)	146 (59.3)	58 (23.6)	15 (6.1)	64 (26.0)	16 (6.5)	17 (6.9)	9 (3.7)	31 (12.6)	27 (11.0)	14 (5.7)
Ovary	698	2.29 ± 1.05	491 (70.3)	207 (29.7)	42 (6.0)	403 (57.7)	193 (27.7)	60 (8.6)	88 (12.6)	26 (3.7)	14 (2.0)	22 (3.2)	74 (10.6)	42 (6.0)	26 (3.7)
Other gynecologic	124	2.12 ± 1.17	89 (71.8)	35 (28.2)	13 (10.5)	71 (57.3)	34 (27.4)	6 (4.8)	15 (12.1)	6 (4.8)	2 (1.6)	3 (2.4)	8 (6.5)	6 (4.8)	5 (4.0)
Breast	1,033	1.42 ± 0.94	885 (85.7)	148 (14.3)	56 (5.4)	586 (56.7)	297 (28.8)	94 (9.1)	104 (10.1)	27 (2.6)	24 (2.3)	19 (1.8)	61 (5.9)	41 (4.0)	21 (2.0)
Nasopharynx	112	1.66 ± 1.14	86 (76.8)	26 (23.2)	5 (4.5)	70 (62.5)	26 (23.2)	11 (9.8)	15 (13.4)	7 (6.3)	4 (3.6)	4 (3.6)	10 (8.9)	13 (11.6)	4 (3.6)
Larynx	14	2.07 ± 0.96	8 (57.1)	6 (42.9)	0	10 (71.4)	4 (28.6)	0	1 (7.1)	1 (7.1)	3 (21.4)	1 (7.1)	1 (7.1)	3 (21.4)	0
F		24.19													
P		<0.01													

cancer, the NRS-2002 score was like shaped like a "U," that is, high at < 45 years old, decreased at 45–65 years old, and then increased at > 65 years old. Gastric and lung cancer patients in the > 85 years old group had the highest NRS-2002 score (4.02 ± 1.01 and 4.10 ± 1.41, respectively), while breast cancer patients aged 45–65 years old had the lowest score (1.46 ± 0.94). The NRS-2002 scores of males were significantly higher than females, which were 1.96 ± 2.09 and 1.25 ± 1.30, respectively ($P = 0.001$) (**Table 2**).

TNM Staging and Oncological Treatment

The NRS-2002 scores for stages I to IV were 1.80 ± 1.06, 2.03 ± 1.25, 2.03 ± 1.24, and 2.08 ± 1.40, respectively ($P = 0.001$), which increased along with the disease stage. A positive relationship between the NRS-2002 score and the stage of lung, skin, uterine, ovarian, and breast cancer was observed. However, the NRS-2002 scores for digestive system and nasopharyngeal carcinoma, which were closely associated with food intake, did not show significant differences between the different stages (**Table 2**). In addition, there was a significant difference in nutritional risk among the different oncological treatments and the time interval since last oncological treatment. The NRS-2002 score was higher in patients who had bone marrow transplantation (4.32 ± 1.27) than in those had surgery plus radio/chemotherapy, surgery, and radio/chemotherapy, with scores of 2.14 ± 1.27, 2.10 ± 1.20, and 1.94 ± 1.31, respectively ($P = 0.002$). Moreover, the combined treatment was more likely to be at nutritional risk than monotherapy. According to the time interval since last oncological treatment, we divided the participants into <1, 1–6, 6–12, and >12 months, and found the NRS-2002 score were 3.04 ± 1.32, 1.50 ± 1.04, 1.44 ± 0.92, and 1.01 ± 0.71, respectively ($P = 0.032$).

Medical Insurance and Education

Overall, the patients with commercial insurance had the lowest NRS-2002 score (2.25 ± 1.22), followed by self-paid and urban resident insurance (2.48 ± 1.45 and 2.66 ± 1.31, respectively); those with rural cooperative medical insurance had the highest score (2.86 ± 1.34, $P = 0.002$). In terms of education level, the NRS-2002 score of patients with a bachelor's degree or above was 1.91 ± 1.22, whereas those with a high school diploma was 2.32 ± 1.28; on the other hand, those with a primary school diploma and below was the highest (2.65 ± 1.45, $P < 0.001$).

Composition of NRS-2002 Score

We further analyzed the composition of the NRS-2002 score as a source of nutritional risk. Subgroup analysis showed that groups with nutritional impairment, which accounted for ≥ 50% of the total NRS-2002 score, were digestive system (71.4%), bone (67.6%), nervous system (64.2%), and respiratory system cancers (51.2%). In those with hematological cancer, only 34.4% of patients had a proportion of nutritional impairment exceeding 50% of the total score, thereby indicating that the nutritional risk of these patients was mainly contributed by disease severity. **Figures 2**, **3** shows the percentage of different degrees of weight loss and different degrees of dietary reduction. Compared to the average level, the percentage of severe weight loss (incidence of > 5% weight loss within 1 month and obvious weight loss) in respiratory system, digestive system, hematological, bone, brain, and nasopharyngeal cancers was significantly higher. Moreover, the percentage of more than 50% food reduction in digestive system, bone, brain, and nasopharyngeal cancers exceeded the average level. Moreover, in order to further evaluate the relationship between nutritional impairment and cancer advancement, we conducted the correlation between TNM staging and nutritional impairment indices, and found a significant correlation (r = 0.232, $P = 0.030$).

Nutrition Counseling

A total of 4,032 participants were confused about nutrition or needing nutrition guidance; only 1,678 had visited the nutrition department for counseling; and only 1,450 patients at nutritional risk had ever consulted the clinical dietitians. When asked about their willingness to obtain nutrition knowledge, most participants (3,897) wanted to be guided by clinicians; only 585 wanted to be guided by dietitians. Furthermore, 2,099 (31.4%), 2,045 (30.59%), 3,089 (46.21%), and 908 (13.58%) patients achieved nutrition knowledge from TV health programs, nutrition books, WeChat official accounts, and nutrition lectures, respectively. Compared with face-to-face offline consultations, these multimedia platforms were more convenient to access.

The Association Between Influencing Factors and Nutritional Risk

Based on previous analysis, there were significant differences in age, sex, TNM staging, medical insurance, and education between with and without nutritional risk groups. The regression analysis showed that the association between nutritional risk and several factors, including age (OR 1.114, 95%CI 1.106, 1.325), TNM staging III (OR 1.891 95%CI 1.171, 3.916) and IV (OR 2.136, 95%CI 1.054, 4,222), bone marrow transplant (OR 1.427, 95%CI 1.191, 2.901), interval of oncological therapy <1 month (OR 1.472, 95%CI 0.312), and nutritional support (OR 0.497, 95%CI 0.287, 0.812) (**Table 3**).

DISCUSSION

To date, this study is the broadest online survey with the largest sample size to investigate dietary intake and nutritional status of non-hospitalized cancer survivors in China. Being regarded as an early manifestation and an important cause of malnutrition in cancer patients, anorexia was exhibited by 52.7% of patients, thereby presenting with a high prevalence. Anorexia was the main cause for reduced food intake and an important predictor for mortality (18, 19). However, the presence/absence of anorexia was not assessed by nutrition risk screening tools. In fact, patients may present with anorexia but without experiencing significant weight loss due to the administration of artificial nutrition. Therefore, considering anorexia as an early event during cancer progression, its evaluation would be useful in the screening process to early discover of nutritional derangements. The study showed that 31.6% of patients suffered from significant weight loss in the past 3 months and 25.0% had reduced food intake in the past week. In cancer patients weight loss is regarded as

TABLE 2 | NRS-2002 score of subjects according to different age and TNM staging.

Cancer type	Age						TNM staging					
	<45	45–65	65–85	>85	F	*P*	I	II	III	IV	F	*P*
Lung	1.8 ± 1.25	1.88 ± 1.27	2.69 ± 1.51	4 ± 1.0	84.29	<0.01	1.57 ± 1.01	2.05 ± 1.32	1.88 ± 1.29	2.16 ± 1.41	10.58	<0.01
Gastric	3.13 ± 1.33	2.96 ± 1.31	3.73 ± 1.51	4 ± 1.41	6.47	0.002	2.73 ± 1.28	3.15 ± 1.36	3.31 ± 1.29	3.19 ± 1.49	2.19	0.069
Esophageal	3.1 ± 1.28	2.96 ± 1.35	3.02 ± 1.39	0	0.19	0.823	2.48 ± 1.24	3.18 ± 1.24	3.31 ± 1.39	2.52 ± 1.33	2.28	0.105
Liver	–	–	–	–	–	–	2.53 ± 1.12	2.93 ± 1.24	2.95 ± 1.15	3.25 ± 1.29	0	0.945
Colorectal	2.37 ± 1.11	2.79 ± 1.25	3.35 ± 1.49	0	7.63	0.001	2.55 ± 1.24	2.76 ± 1.04	2.89 ± 1.46	3.14 ± 1.59	1.68	0.172
Bone	–											
Skin	2.19 ± 1.38	1.68 ± 1.05	4 ± 0	0	4.39	0.039	1.53 ± 1.03	2.31 ± 1.32	1.71 ± 1.11	1 ± 0	8.71	0.004
Uterus	2.7 ± 1.36	2.72 ± 1.24	3.25 ± 1.82	0	0.01	0.904	2.32 ± 1.21	3.01 ± 1.31	3 ± 1.3	3.19 ± 1.05	7.81	0.001
Ovary	2.35 ± 1.12	2.4 ± 1.11	2.69 ± 0.87	0	1.29	0.275	2.2 ± 0.88	2.62 ± 1.22	2.37 ± 1.06	2.66 ± 1.27	4.63	0.003
Other gynecologic	2.33 ± 1.28	2.2 ± 1.2	2.11 ± 0.78	0	0.32	0.574	–	–	–	–	–	–
Breast	1.54 ± 1.01	1.46 ± 0.94	2.43 ± 1.43	0	23.06	<0.01	1.4 ± 0.83	1.48 ± 0.96	1.58 ± 1.02	1.74 ± 1.22	4.15	0.002
Nasopharynx	1.61 ± 1.05	1.74 ± 1.14	3.2 ± 1.55	0	0.34	0.559	1.72 ± 1.02	2.08 ± 1.27	1.87 ± 1.41	1.31 ± 0.63	0.42	0.519

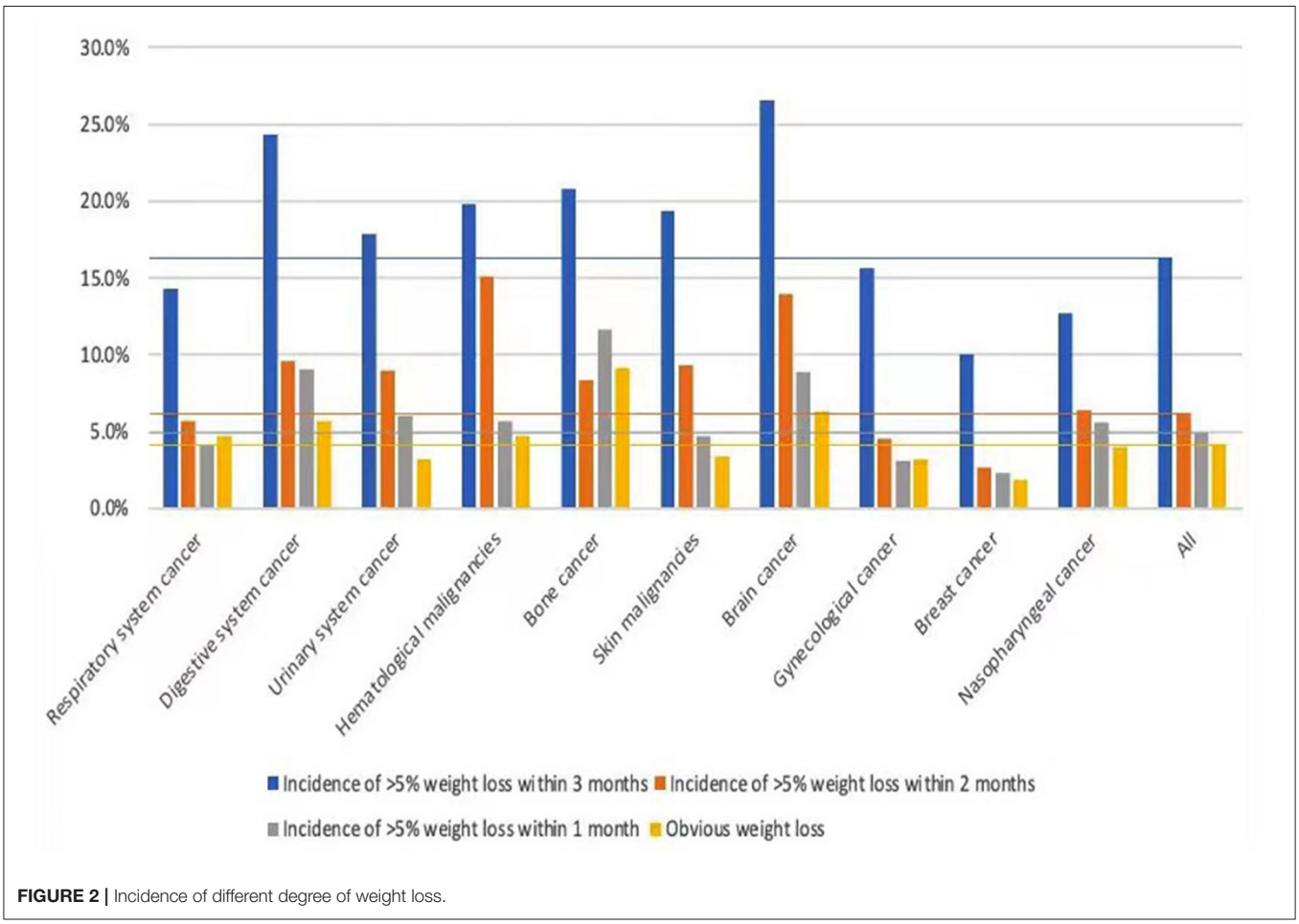

FIGURE 2 | Incidence of different degree of weight loss.

an early warning signal of wasting process involving, and an involuntary body weight loss>5% calls for urgently performing a systematic nutritional assessment in cancer (19).

Discover and diagnosis of nutritional risk and malnutrition are crucial in cancer patients (1, 15). NRS-2002 and Malnutrition Universal Screening Tool (MUST) are two of the most popular tools used in clinical practice (20, 21), but there is no consensus on which screening method is more efficient and appropriate in an oncology population (22). NRS-2002 is widely used as a valid nutrition screening tool in clinics for not only general

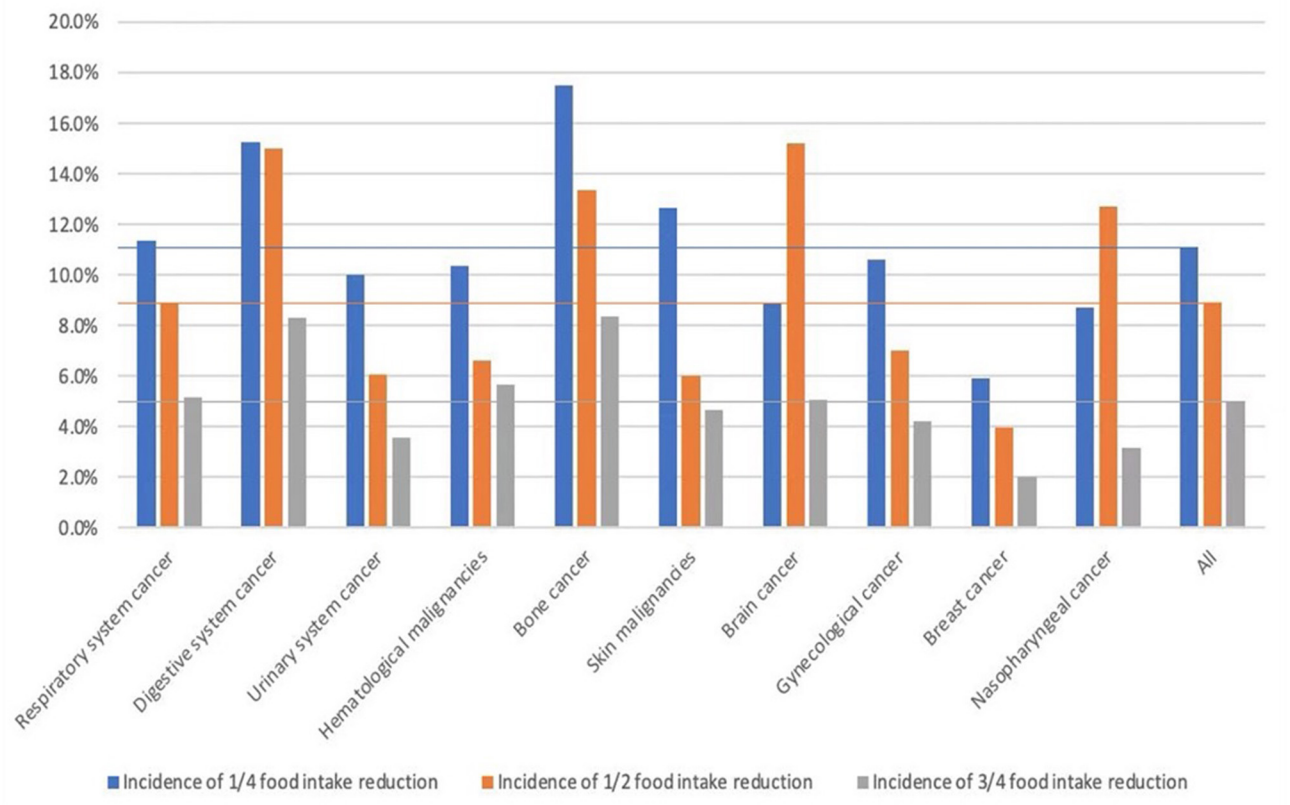

FIGURE 3 | Incidence of different degree of food intake reduction.

population, but also cancer patients. The Patient-Generated Subjective Global Assessment (PG-SGA), a broadly used for nutrition assessment for cancer patients (6, 23), consists of the patient-generated and the professional component (24). The first part, also regarded as PG-SGA Short Form, is perceived comprehensible and easy (25), and can be completed by the patients or their carers quickly (6), while the professional part, especially the physical examination (25), making it not suitable for online survey. The mini-Nutritional Assessment-Short Form (MNA-SF), in addition to the evaluation of current BMI, weight loss, food intake reduction, burden of disease, investigated the presence of neuropsychological problems and low mobility, however it was specifically designed to evaluate the malnutrition in elders (26). The Global Leadership Initiative on Malnutrition (GLIM) has engaged several global clinical nutrition societies to reach a consensus on the diagnostic criteria of malnutrition in clinical settings (27). A study conducted in cancer patients to compare different nutrition risk screening tools found NRS-2002 was better correlated with the GLIM criteria than MUST and PG-SGA, and could serve as a good candidate for first-step malnutrition risk screening according to the GLIM diagnostic scheme. Although the PG-SGA is a sensitive tool to detect compromised nutritional status, the assessment had a low specificity in the diagnosis of malnutrition according to the GLIM criteria (28).

In this study, the overall prevalence of nutritional risk was 33.9%, as screened by NRS-2002; this was lower than the 40.2 and 50% reported among the Chinese population (28, 29). This may be explained by the fact that the participants mentioned above were hospitalized cancer patients, whose disease might be of high severity or who were currently under treatment. However, in this study, the participants were either in remission or between treatment cycles, thereby rendering less treatment effects or disease severity. Even so, the nutritional risk of this population was still high and should not be ignored; even though tumor burden is not severe, appropriate and early nutritional intervention can help achieve a clinical benefit.

Nutritional risk varied according to personal characteristics. The NRS-2002 scores tended to increase with age, which is similar to previous study (30). Elderly patients with cancer are more prone to nutritional problems due to organ dysfunction and reduced treatment tolerance. Nevertheless, for some types of cancer in our study, such as gastric, esophageal, skin, and breast cancers, young and middle-aged patients showed higher NRS-2002 scores. This may be related to the higher malignancy of tumors that occur in young and middle-aged patients. Meanwhile, the treatment plan for this population may also be more aggressive, thus leading to a greater impact on gastrointestinal symptoms and food intake. Besides, our study showed the nutritional risk of this population is gradually

TABLE 3 | The regression analysis of influencing factors and nutritional risk.

Variables	OR (95% CI)	*P*
Age		
≤65 years old	1	0.036
>65 years old	1.114 (1.106, 1.325)	
Sex		
Male	1	0.391
Female	0.924 (0.843, 1.412)	
Payment methods		
Urban resident medical insurance	1	0.417
Commercial insurance	0.965 (0.834, 1.379)	0.312
Rural cooperative medical insurance	1.413 (0.812, 2.341)	0.712
Self-paid	1.109 (0.918, 1.293)	0.059
Education		
Primary school or under	1	0.124
Middle school	0.642 (0.420, 1.012)	0.237
Bachelor's or above	0.142 (0.024, 1.410)	0.062
TNM staging		
I	1	0.118
II	1.452 (0.762, 3.462)	0.247
III	1.891 (1.171, 3.916)	0.030
IV	2.136 (1.054, 4.222)	0.037
Permanent residential		
Capital city	1	
Prefecture level cities	0.462 (0.302, 1.364)	0.248
Country-level city	0.681 (0.325, 1.572)	0.102
Rural areas	0.642 (0.423, 1.416)	0.174
Oncological therapy		
Surgery	1	0.062
Chemotherapy	0.912 (0.791, 1.421)	0.263
Radiotherapy	0.879 (0.364, 1.880)	0.685
Bone marrow transplant	1.427 (1.191, 2.901)	0.037
Interval of oncological therapy		
>12 months	1	0.074
6–12 months	1.082 (0.581, 2.012)	0.166
1–6 months	1.266 (0.481, 2.791)	0.104
<1 month	1.472 (1.112, 2.521)	0.030
Nutritional support		
No	1	0.014
Yes	0.497 (0.287, 0.812)	

deteriorating with malignancy of tumor, especially more than stage III. Moreover, we found that the NRS-2002 scores significantly differed according to education level and medical insurance type. Cancer patients with higher education levels or commercial insurance probably intend to perform early detection and interventions, suggesting an imbalance of medical resources at present.

Interestingly, for most non-digestive cancer survivors, a higher NRS-2002 score seemed to be associated with a higher TNM stage, which was comparable to the result of the largest investigation of hospitalized cancer patient (11). However in subgroup analysis of our study, no significant difference was found between TNM stages in digestive system cancers. This indicates that digestive system cancers could influence food intake and body weight even at an early stage, thus leading to increased nutritional risk. Therefore, nutritional risk screening should be performed as early as possible. The NRS-2002 score was higher in patients who had combined surgery plus radio/chemotherapy, followed by those received surgery or radio/chemotherapy alone, thus suggesting that extra attention should be paid to this population.

In the regression analysis, age, TNM staging, oncological therapy, and time interval of treatment were associated with NRS-2002, and also nutritional impairment score was close correlated with TNM staging, indicating the intimate relationship between nutritional risk and disease. However, the association between nutritional risk and the demographic characteristics was not found, it might be the complicated relationship among these factors.

Patients spend the most time at home or in community sanatoriums, especially in the era of COVID-19, patients had fewer chances to visit a hospital for nutrition counseling. However, the nutritional problems of these non-hospitalized patients were distressingly undertreated. A large number of the participants had nutrition-related queries, but only a minority received professional guidance or intervention from dietitians. A French study found that only 35.8% of cancer patients received regular nutrition counseling; of which, 56.3% were provided by nutritionists or dietitians, 31.9% by doctors, and 11.8% by other medical staff (31). A Chinese study included 1,138 cancer patients and found merely 14% of them had cancer counseling (32). This shows that there are still gaps in the standardized treatment as well as in clinical practice. In addition, the importance of dietitians or nutritional support is not yet fully recognized by clinicians or patients (33–35). This may be due to the insufficient participation of dietitians during oncologic treatments or lack of collaboration between oncologists and clinical nutritionists (36), where nutritional intervention is not routinely included in clinical practice (37).

Nutritional support benefits patients with nutritional risk as to improving clinical outcomes (38); conversely, it may not help but increase the costs for those with no risk. A Chinese study focusing on hospitalized gastric cancer patients found that 59.1% of patients with malnutrition did not receive nutritional support, while 25.5% at no risk were given needless intervention (39). Only 30–60% of cancer patients with nutritional risk were provided with nutritional support (32, 40). In this study, a higher proportion of patients with and without nutritional risk received support, indicating the inappropriate use of nutritional intervention. Besides, nutrition support, such as ONS, nasal enteral nutrition, and parenteral nutrition were used by some non-hospitalized patients, but the appropriateness of the application was not fully evaluated, and whether the nutrition requirement was met remained unclear.

Few studies have focused on the composition of nutritional risk screening to identify the main contributors to NRS-2002. The proportion of nutritional impairment score ranged from 50–70% in respiratory system, bone, and nervous system cancers; however, they were mainly contributed by weight

loss, not dietary reduction. The incidence of weight loss and dietary reduction was high in digestive system, nervous system, and bone cancers, thus indicating that the nutritional status of these patients was seriously impaired. For those with nutritional impairment, proper nutritional intervention can improve the nutritional status. However, for those with more severe diseases, aggressive therapies for primary cancer may bring more benefits. Therefore, in addition to paying attention to the existence of nutritional risk, the composition of NRS-2002 is also important for dietitians in choosing the appropriate intervention. We further found the close correlation between TNM and nutrition impairment, indicating that disease and nutrition impairment have influence on each other and should be paid attention.

Given the patients' intention to acquire nutritional knowledge and modern communication technology, results of this study suggest that online survey is a convenient and quick method to delivery nutritional risk screening for cancer survivors. Several web-based lifestyle or psychological interventions for cancer patients were conducted and shown acceptable and feasible by patients (41, 42). More important, online survey can reach non-hospitalized patients, so that nutrition support can be integrated into patients' daily life more deeply and permanently. Compared to the largest survey of Chinese hospitalized cancer patients conducted between 2013 to 2020 and enrolled 47,448 patients from 22 provinces (10), our study enrolled 6,648 valid surveys covering 31 provinces, autonomous regions, and municipalities within 6 months, suggesting it is efficient. Strict quality control was performed during the entire process. The "impaired nutritional status" part of the NRS-2002 included age, recent food intake, and weight change; these can be easily reported by the patients through an online questionnaire. The "severity of disease" might be difficult to assess; however, it can be evaluated by the site and staging of the tumor as well as the recent therapy methods reported by the subjects. Therefore, NRS-2002 can be conducted online; this study provides evidence for an online-based NRS-2002 evaluation.

This study had several limitations. First, the NRS-2002 score is qualitative and simple; therefore, it may not be possible to comprehensively evaluate complex nutritional problems. Second, self-report measures may result in biased estimates; however, these are presumably equally distributed among all participants. Thirdly, the online survey may lose sight of people who are incapable to use cellphone, which is a common problem of this method. Lastly, since the limitation of online survey, whether the nutrition requirement was meet by nutritional support cannot be known because physical examination and medical status evaluation cannot be done, which is important in nutrition care practice. Therefore, further studies are warranted to assess the nutritional status of non-hospitalized survivors based on more objective and abundant data measures and to determine their long-term clinical outcomes.

CONCLUSIONS

This study found a large proportion of these population presenting dietary intake reduction, weight loss, and high nutritional risk, and regular monitoring and assessment follow-up system should be established to evaluate the nutritional status of non-hospitalized cancer survivors. Moreover, based on our practice, online survey may be a convenient and suitable method for nutrition status investigation. Medical staff must be aware of the nutritional risks, the contributors of the NRS-2002 score, and provide nutrition intervention for non-hospitalized cancer survivors to improve nutritional status and clinical outcomes. Lastly, due to the low percentage of nutrition consultation achieved, we recommend professional nutrition consultation and education should be carried out in clinical practice.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Ethics Committee of Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FW and KY were responsible for the design of the study, revised the paper, and provided feedback. FW, KY, QD, R-rL, and J-yG conducted the research and analyzed the data. FW, QD, and C-wL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.920714/full#supplementary-material

REFERENCES

1. World Health Organization. Cancer fact sheets. Available online at: https://gco.iarc.fr/today/fact-sheets-cancers (accessed January 26, 2022).
2. Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia: understanding the molecular basis. *Nat Rev Cancer*. (2014) 14:754–62. doi: 10.1038/nrc3829
3. Steenhagen E, van Vulpen JK, van Hillegersberg R, May AM, Siersema PD. Nutrition in peri-operative esophageal cancer management. *Expert Rev Gastroenterol Hepatol*. (2017) 11:663–72. doi: 10.1080/17474124.2017.1325320

4. Campillo M, Fernández JM, Salas MA. A randomized controlled trial of preoperative oral immunonutrition in patients undergoing surgery for colorectal cancer: hospital stay and health care costs. *Cir Cir.* (2017) 85:393–400. doi: 10.1016/j.circen.2017.11.008
5. Baracos VE, Martin L, Korc M, Guttridge DC, Fearon KCH. Cancer-associated cachexia. *Nat Rev Dis Primers.* (2018) 4:17105. doi: 10.1038/nrdp.2017.105
6. Cederholm T, Barazzoni R, Austin P, Ballmer P, Biolo G, Bischoff SC, et al. ESPEN guidelines on definitions and terminology of clinical nutrition. *Clin Nutr.* (2017) 36:49–64. doi: 10.1016/j.clnu.2016.09.004
7. Reber E, Schönenberger KA, Vasiloglou MF, Stanga Z. Nutritional risk screening in cancer patients: the first step toward better clinical outcome. *Front Nutr.* (2021) 8:603936. doi: 10.3389/fnut.2021.603936
8. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M; Educational and Clinical Practice Committee, et al. ESPEN Guidelines for Nutrition Screening 2002. *Clin Nutr.* (2003) 22:415-421. doi: 10.1016/S0261-5614(03)00098-0
9. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H. Bozzetti F, et al. ESPEN guidelines on nutrition in cancer patients. *Clin Nutr.* (2017) 36:11–48. doi: 10.1016/j.clnu.2016.07.015
10. Dupuis M, Kuczewski E, Villeneuve L, Bin-Dorel S, Haine M, Falandry C, et al. Age Nutrition Chirugie (ANC) study: impact of a geriatric intervention on the screening and management of undernutrition in elderly patients operated on for colon cancer, a stepped wedge controlled trial. *BMC Geriatr.* (2017) 17:10. doi: 10.1186/s12877-016-0402-3
11. Song CH, Wang KH, Guo ZQ, Fu ZM, Wang C, Weng M, et al. Investigation of nutritional status in Chinese patients with common cancer (in Chinese). *Sci Sin Vitae.* (2020) 50:1437–52. doi: 10.1360/SSV-2020-0297
12. Gupta D, Lis CG, Granick J, Grutsch JF, Vashi PG, Lammersfeld CA. Malnutrition was associated with poor quality of life in colorectal cancer: a retrospective analysis. *J Clin Epidemiol.* (2006) 59:704–9. doi: 10.1016/j.jclinepi.2005.08.020
13. Zhang X, Tang M, Zhang Q, Zhang KP, Guo ZQ, Xu HX, et al. The GLIM criteria as an effective tool for nutrition assessment and survival prediction in older adult cancer patients. *Clin Nutr.* (2021) 40:1224–32. doi: 10.1016/j.clnu.2020.08.004
14. Hsueh SW, Lai CC, Hung CY, Lin YC, Lu CH, Yeh KY, et al. A comparison of the MNA-SF, MUST, and NRS-2002 nutritional tools in predicting treatment incompletion of concurrent chemoradiotherapy in patients with head and neck cancer. *Support Care Cancer*. (2021) 29:5455–62. doi: 10.21203/rs.3.rs-168112/v1
15. Bargetzi L, Brack C, Herrmann J, Bargetzi A, Hersberger L, Bargetzi M, et al. Nutritional support during the hospital stay reduces mortality in patients with different types of cancers: secondary analysis of a prospective randomized trial. *Ann Oncol.* (2021) 32:1025–33. doi: 10.1016/j.annonc.2021.05.793
16. Bozzetti F, Mariani L, Lo Vullo S. SCRINIO Working Group, Amerio ML, Biffi R, et al. The nutritional risk in oncology: a study of 1,453 cancer outpatients. *Support Care Cancer.* (2012) 20:1919–28. doi: 10.1007/s00520-012-1387-x
17. Chen CM. Obesity Working Group, International Life Science Association of China (WGOC). Recommendation of Chinese adults' body mass index reference. *Chin J Prev Med.* (2003) 35:349–50. doi: 10.3760/j:issn:0253-9624.2001.05.019
18. Abraham M, Kordatou Z, Barriuso J, Lamarca A, Weaver JMJ, Cipriano C, et al. Early recognition of anorexia through patient-generated assessment predicts survival in patients with oesophagogastric cancer. *PLoS ONE.* (2019) 14:e0224540. doi: 10.1371/journal.pone.0224540
19. Molfino A, de van der Schueren MAE, Sánchez-Lara K, Milke P, Amabile MI, Imbimbo G, et al. Cancer-associated anorexia: validity and performance overtime of different appetite tools among patients at their first cancer diagnosis. *Clin Nutr.* (2021) 40:4037–42. doi: 10.1016/j.clnu.2021.02.016
20. Poulia KA, Klek S, Doundoulakis I, Bouras E, Karayiannis D, Baschali A, et al. The two most popular malnutrition screening tools in the light of the new ESPEN consensus definition of the diagnostic criteria for malnutrition. *Clin Nutr.* (2017) 36:1130–5. doi: 10.1016/j.clnu.2016.07.014
21. Ye XJ, Ji YB, Ma BW, Huang DD, Chen WZ, Pan ZY, et al. Comparison of three common nutritional screening tools with the new European Society for Clinical Nutrition and Metabolism (ESPEN) criteria for malnutrition among patients with geriatric gastrointestinal cancer: a prospective study in China. *BMJ Open.* (2018) 8:e019750. doi: 10.1136/bmjopen-2017-019750
22. Thompson KL, Elliott L, Fuchs-Tarlovsky V, Levin RM, Voss AC, Piemonte T. Oncology evidence-based nutrition practice guideline for adults. *J Acad Nutr Diet.* (2017) 117:297–310.e47. doi: 10.1016/j.jand.2016.05.010
23. Jager-Wittenaar H, Ottery FD. Assessing nutritional status in cancer: role of the patient-generated subjective global assessment. *Curr Opin Clin Nutr Metab Care.* (2017) 20:322–9. doi: 10.1097/MCO.0000000000000389
24. Ottery FD. Definition of standardized nutritional assessment and interventional pathways in oncology. *Nutrition*. (1996) (1 Suppl):S15–S19. doi: 10.1016/0899-9007(95)00067-4
25. Sealy MJ, Haß U, Ottery FD, van der Schans CP, Roodenburg JLN, Jager-Wittenaar H. Translation and cultural adaptation of the scored Patient-Generated Subjective Global Assessment (PG-SGA): an interdisciplinary nutritional instrument appropriate for Dutch cancer patients. *Cancer Nurs.* (2018) 41:450–62. doi: 10.1097/NCC.0000000000000505
26. Wildiers H, Heeren P, Puts M, Topinkova E, Janssen-Heijnen ML, Extermann M, et al. International society of geriatric oncology consensus on geriatric assessment in older patients with cancer. *J Clin Oncol.* (2014) 32:2595–603. doi: 10.1200/JCO.2013.54.8347
27. Cederholm T, Jensen GL, Correia MITD, Gonzalez MC, Fukushima R, Higashiguchi T, et al. GLIM criteria for the diagnosis of malnutrition - a consensus report from the global clinical nutrition community. *J Cachexia Sarcopenia Muscle.* (2019) 10:207–17. doi: 10.1016/j.clnu.2018.08.002
28. Zhang Z, Wan Z, Zhu Y, Zhang L, Zhang L, Wan H. Prevalence of malnutrition comparing NRS2002, MUST, and PG-SGA with the GLIM criteria in adults with cancer: a multi-center study. *Nutrition.* (2021) 83:111072. doi: 10.1016/j.nut.2020.111072
29. Cao J, Xu H, Li W, Guo Z, Lin Y, Shi Y, et al. Investigation on Nutrition Status and Clinical Outcome of Common Cancers (INSCOC) Group, Chinese Society of Nutritional Oncology. Nutritional assessment and risk factors associated to malnutrition in patients with esophageal cancer. *Curr Probl Cancer.* (2021) 45:100638. doi: 10.1016/j.currproblcancer.2020.100638
30. Song C, Cao J, Zhang F, Wang C, Guo Z, Lin Y, et al. Nutritional risk assessment by scored patient-generated subjective global assessment associated with demographic characteristics in 23,904 common malignant tumors patients. *Nutr Cancer.* (2019) 71:50–60. doi: 10.1080/01635581.2019.1566478
31. Hébuterne X, Lemarié E, Michallet M, de Montreuil CB, Schneider SM, Goldwasser F. Prevalence of malnutrition and current use of nutrition support in patients with cancer. *JPEN J Parenter Enteral Nutr.* (2014) 38:196–204. doi: 10.1177/0148607113502674
32. Li Z, Chen W, Li H, Zhao B. Chinese Oncology Nutrition Survey Group. Nutrition support in hospitalized cancer patients with malnutrition in China. *Asia Pac J Clin Nutr.* (2018) 27:1216–24. doi: 10.6133/apjcn.201811_27(6).0007
33. Gyan E, Raynard B, Durand JP, Lacau Saint Guily J, Gouy S, Movschin ML, et al. Malnutrition in patients with cancer: comparison of perceptions by patients, relatives, and physicians-results of the NutriCancer2012 Study. *JPEN J Parenter Enteral Nutr.* (2018) 42:255–60. doi: 10.1177/0148607116688881
34. Dolatkhah N, Aghamohammadi D, Farshbaf-Khalili A, Hajifaraji M, Hashemian M, Esmaeili S. Nutrition knowledge and attitude in medical students of Tabriz University of Medical Sciences in 2017-2018. *BMC Res Notes.* (2019) 12:757. doi: 10.1186/s13104-019-4788-9
35. Kirbiyik F, Ozkan E. Knowledge and practices of medical oncologists concerning nutrition therapy: a survey study. *Clin Nutr ESPEN.* (2018) 27:32–7. doi: 10.1016/j.clnesp.2018.07.004
36. Caccialanza R, Cereda E, Pinto C, Cotogni P, Farina G, Gavazzi C, et al. Awareness and consideration of malnutrition among oncologists: Insights from an exploratory survey. *Nutrition.* (2016) 32:1028–32. doi: 10.1016/j.nut.2016.02.005
37. Leuenberger M, Kurmann S, Stanga Z. Nutritional screening tools in daily clinical practice: the focus on cancer. *Support Care Cancer.* (2010) 18:S17–27. doi: 10.1007/s00520-009-0805-1
38. Wang R, Cai H, Li Y, Chen C, Cui Y. Impact exerted by nutritional risk screening on clinical outcome of patients with esophageal cancer. *Biomed Res Int.* (2018) 27:7894084. doi: 10.1155/2018/7894084
39. Guo ZQ, Yu JM, Li W, Fu ZM, Lin Y, Shi YY, et al. Investigation on the Nutrition Status and Clinical Outcome of Common Cancers

(INSCOC) Group. Survey and analysis of the nutritional status in hospitalized patients with malignant gastric tumors and its influence on the quality of life. *Support Care Cancer*. (2020) 28:373–80. doi: 10.1007/s00520-019-04803-3

40. Planas M, Álvarez-Hernández J, León-Sanz M, Celaya-Pérez S, Araujo K, García de Lorenzo A, et al. Prevalence of hospital malnutrition in cancer patients: a sub-analysis of the PREDyCES study. *Support Care Cancer*. (2016) 24:429–35. doi: 10.1007/s00520-015-2813-7
41. Galiano-Castillo N, Cantarero-Villanueva I, Fernández-Lao C, Ariza-García A, Díaz-Rodríguez L, Del-Moral-Ávila R, et al. Telehealth system: a randomized controlled trial evaluating the impact of an internet-based exercise intervention on quality of life, pain, muscle strength, and fatigue in breast cancer survivors. *Cancer*. (2016) 122:3166–74. doi: 10.1002/cncr.30172
42. Williams VA, Brown NI, Johnson R, Ainsworth MC, Farrell D, Barnes M, et al. A Web-based Lifestyle Intervention for Cancer Survivors: Feasibility and Acceptability of SurvivorSHINE. *J Cancer Educ*. (2021). doi: 10.1007/s13187-021-02026-x. [Epub ahead of print].

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Body Mass Index Combined With Possible Sarcopenia Status Is Better Than BMI or Possible Sarcopenia Status Alone for Predicting All-Cause Mortality Among Asian Community-Dwelling Older Adults

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Background: Body mass index (BMI) and sarcopenia are common indicators of nutritional status. Possible sarcopenia, defined as low muscle strength or performance, was recently introduced by the Asian Working Group for Sarcopenia (AWGS) in 2019. We investigated for association between all-cause mortality and BMI combined with possible sarcopenia severity in Asian older adults.

Methods: This study included a subpopulation (8,195 participants aged ≥60 years; male gender: 49.4%; mean age: 69.2 ± 6.8 years) from the Fourth Thai National Health Examination Survey (NHES-IV). BMI was classified using Asia-Pacific cut-offs. Possible sarcopenia was defined using quadriceps strength based on AWGS 2019 criteria, and possible sarcopenia severity was determined using study population quartile cut-offs. All-cause mortality data was derived from the national vital registry in 2020.

Results: The prevalence of underweight status and possible sarcopenia was 11.8 and 38.9%, respectively. Multivariate analysis showed underweight individuals with severe possible sarcopenia to be at highest risk for increased mortality [adjusted hazard ratio (aHR): 3.98, 95% confidence interval (CI): 2.89–5.48], and higher risk was found in men compared to women (aHR: 5.35, 95% CI: 1.19–8.97). Obese status without possible sarcopenia was an independent protective factor (aHR: 0.61, 95% CI: 0.38–0.97).

Conclusion: BMI combined with possible sarcopenia severity is a better predictor of mortality risk than either parameter alone.

Keywords: body mass index, BMI, possible sarcopenia status, all-cause mortality, Asian community-dwelling older adults

INTRODUCTION

Malnutrition is a common and important public health problem among older adults due to its strong association with morbidity and mortality (1). Body mass index (BMI) is an anthropometric parameter that is routinely used to assess nutritional status (2). Although BMI is strongly correlated with health outcomes, direct association between BMI and mortality among older adults is still being investigated and debated. Many studies have reported a reverse J curve or U curve association between BMI and mortality; however, other studies that examined these associations did not find a similar relationship between BMI and mortality (3–5). Furthermore, the accuracy of BMI measurement in older adults remains problematic. BMI is calculated from body weight and height. A decrease in height due to aging can increase the BMI value without any change in body weight (6). As a result, no consensus has yet been reached regarding the optimal cut-off point for optimal BMI in older adults.

Apart from BMI—sarcopenia, which is defined as loss of muscle mass plus low muscle strength and/or low physical performance, is also strongly associated with malnutrition and negative health consequences (7). Although sarcopenia is a good predictor of nutritional status, diagnosis requires physical performance measurement, and muscle mass measurement. Dual energy X-ray absorptiometry (DEXA) and bioelectrical impedance analysis (BIA) are the commonly used tools for measuring muscle mass in a research setting; however, these tools are not widely available in community setting, especially in developing countries.

Recently, the Asian Working Group for Sarcopenia (AWGS) introduced the term "possible sarcopenia," which is defined as low muscle strength or reduced physical performance (8). Possible sarcopenia allows for an easier and earlier diagnosis of sarcopenia, particularly in community setting. Limited evidence currently exists specific to the association between possible sarcopenia and mortality in Asian older adults. Therefore, the aim of this study was to determine the optimal BMI in Asian older adults that associates with the lowest all-cause mortality, and to investigate for association between all-cause mortality and BMI combined with various degrees of possible sarcopenia in Asian community dwelling older adults.

MATERIALS AND METHODS

Study Design

This retrospective cohort study used data from the Fourth Thai National Health Examination Survey (NHES-IV). The NHES-IV was a national health examination survey of Thai population that was conducted during August 2008 to March 2009. In the survey, multistage cluster sampling was employed. The sampling technique was described elsewhere (9). A total of 20,450 individuals were enrolled in the NHES-IV. The response rate was 85.5% for men, and 95.4% for women. The inclusion criteria for the present study were age ≥60 years, having BMI data and handgrip strength data. A total of 1,015 participants aged ≥60 years were excluded due to a lack of BMI and/or handgrip strength data. A total sample size of 8,195 was included for the final analyses (**Figure 1**).

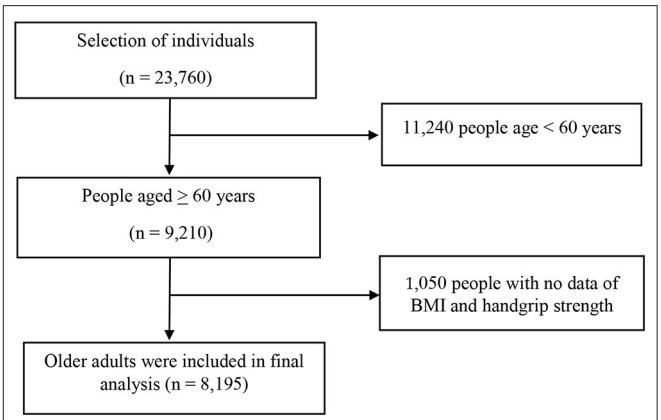

FIGURE 1 | The flowchart of participants' selection process.

Data Collection and Measurement

Data were obtained for the NHES-IV study *via* a semi-structured face-to-face interview between the respondent and trained personnel. Body weight and height were both measured by standardized procedures, and BMI was calculated as weight in kilograms divided by height in meters squared. BMI was classified using the following Asia-Pacific cut-off values: BMI < 18.5 kg/m^2 as underweight, 18.5–22.9 kg/m^2 as normal weight, 23.0–24.9 kg/m^2 as overweight, and ≥25.0 kg/m^2 as obese (10). In the present study, to examine the optimal BMI for achieving the lowest all-cause mortality, the BMI range in our study population (14–36 kg/m^2) was classified into subgroups for every 2 kg/m^2 (e.g., 14–15.9, 16–17.9 kg/m^2, etc.). Hand grip strength was measured using a hydraulic hand dynamometer (Takei Scientific Instrument; Product No. T.K.K.5401). The interface reports grip strength in kilograms to one decimal point. All participants were seated with the elbow flexed at 90°, the, forearm in neutral position and the wrist between 0 and 30°. Each participant was asked to have two assessments per arm at 1-min intervals. The maximum handgrip strength was used for analysis. Possible sarcopenia was defined by low muscle strength using handgrip strength <28 kg for men, and <18 kg for women according to the AWGS 2019 guideline (8). We also set forth to examine the association between severity of possible sarcopenia and all-cause mortality. Therefore, handgrip strength was categorized using quartile cut-offs. The lowest quartile represented the study subjects with the lowest handgrip strength. Handgrip strength were categorized into quartile. The cut-off values of handgrip strength in each quartile among male older people were <24.6, 24.7–29.2, 29.3–33.8, and >33.9 kg. The cut-off values of handgrip strength in each quartile for female older people were <16.6, 16.7–19.7, 19.8–22.8, >22.9 kg. The analysis was performed using the highest quartile as the reference group (defined as no possible sarcopenia), and 1–3 quartiles were sequentially defined as severe, moderate, and mild possible sarcopenia. All-cause mortality data were retrieved in May 2020

from the National Civil Registration and Vital Statistics System, Ministry of Interior, Thailand.

Sociodemographic and clinical data, including age, gender, residential region, years of education, smoking status, and underlying diseases, were collected. Hypertension (HTN) was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg or self-reported diagnosis of hypertension or use of any antihypertensive medications. Diabetes was defined as a fasting plasma glucose level of ≥7.0 mmol/L or self-reported diagnosis of diabetes or use of antidiabetic medications. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) <60 mL/minute calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (11). Depression was diagnosed using the criteria published in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV) (12). Cerebrovascular disease (CVD) was defined by the self-reported diagnosis of CVD. Cardiovascular disease was also defined by the self-reported diagnosis of coronary heart diseases. Assessment of an individual's ability to perform activities of daily living (ADL) was measured using the Barthel Index (BI) (13). Impaired ADL was defined as the need for partial or total assistance in carrying out any basic ADL.

Statistical Analysis

All the statistical analyses were performed using STATA software 16.1 (StataCorp LP, College Station, TX, USA). Study data were sample weighted against the total national registered population of Thailand in 2009, and methods for complex survey design analysis were applied (14). The baseline characteristics of study subjects were compared between groups using descriptive statistics. Parametric and non-parametric tests were applied depending on the distribution of data. Kruskal-Wallis test and chi-square test were used for continuous and categorical variables, respectively. A $p < 0.05$ was considered statistically significant for all tests. The association between groups and all-cause mortality was explored using univariate and multivariate Cox proportional hazards models. The proportional hazards assumption was checked on the basis of Schoenfeld residuals. There was no evidence that the proportional-hazards assumption was violated. Multivariate models were adjusted for potentially confounding factors, including age, residential area, diabetes, chronic kidney disease, cardiovascular disease, cerebrovascular disease, and history of smoking. The results of multivariate analysis are shown as hazard ratio (HR) and 95% confidence interval (CI) for univariate analysis, and as adjusted hazard ratio (aHR) and 95% CI for multivariate analysis.

Ethical Considerations

Reporting of this study was in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline. The protocol for this study was approved by the Institutional Review Boards of both the Faculty of Medicine Ramathibodi Hospital, Mahidol University, and the Faculty of Medicine Siriraj Hospital, Mahidol University. Written informed consent was not obtained for this study because our data was retrospectively collected, but written informed consent was obtained when participants were originally invited to participate in the NHES-IV survey.

RESULTS

A total 8,195 participants were analyzed in this study. The mean age was 69.2 ± 6.8 years, and nearly half of participants were men. Almost 46% of total participants required some assistance in performing ADL. The prevalence of underweight, normal weight, overweight, and obesity was 14.8, 38.6, 16.7, and 29.9%, respectively. Female participants had a mean BMI higher than that of male participants (23.9 vs. 22.5 kg/m^2, respectively). The prevalence of possible sarcopenia was 38.9%. Regarding the BMI and possible sarcopenia categories, 61.3% of underweight participants; 42.9% of normal weight participants; 35.2% of overweight participants and 26.8% of obese participants had possible sarcopenia. Among male participants, 64.3% of underweight participants, 46.2% of normal weight participants, 35.6% of overweight participants, and 28.3% of obese participants had possible sarcopenia. For female participants, the possible sarcopenia was identified at 57.6% for underweight participants; 38.7% for normal weight participants; 34.9% for overweight participants; and 25.8% for obese participants.

There were 1,771 deaths among our study population within 10 years of follow-up. Underweight and normal weight participants had a higher death rate compared to overweight and obese subjects. The all-cause mortality rate by BMI category (underweight, normal weight, overweight, and obesity) was 53.6 [95% confidence interval (CI): 47.2–60.8], 31.2 (95% CI: 28.4–34.2), 25.6 (95% CI: 21.8–30.0), and 25.0 (95%CI: 21.8–28.8) per 1,000 person-years, respectively, among older adult men, and 45.1 (95% CI: 38.8–52.4), 22.5 (95% CI: 20.0–25.5), 18.1(95% CI: 15.0–21.9), and 16.1 (95%CI: 14.1–18.4) per 1,000 person-years, respectively, among older adult women. When comparing according to BMI and possible sarcopenia status, participants with underweight with possible sarcopenia status had the highest mortality, while participants with obesity without possible sarcopenia status had the lowest mortality with death rates per 1,000 person-years of 65.7 (95% CI: 58.8–73.4) and 14.4 (95% CI: 12.7–16.4), respectively. The overall mortality according to BMI categories and possible sarcopenia status among included participants is shown in **Figure 2**. Sociodemographic, functional, lifestyle, clinical, mental health, and mortality characteristics of study participants for different BMI ranges compared between Thai older adults with and without possible sarcopenia are shown in **Table 1**.

Association between the ranges of BMI and all-cause mortality after adjustment for potentially confounding factors is shown in **Figure 3**. BMI 26.0–27.9 kg/m^2, the range with the lowest mortality, was defined as the reference group. Compared with the reference group, every range of BMI < 26.0 kg/m^2 was significantly associated with increased all-cause mortality, whereas the ranges of BMI ≥ 28.0 kg/m^2 were not significantly associated with increased mortality. Older adults with a BMI < 14.0 kg/m^2 had the highest risk for all-cause mortality with an adjusted hazard ratio (aHR) of 6.61 (95% CI: 3.98–10.99).

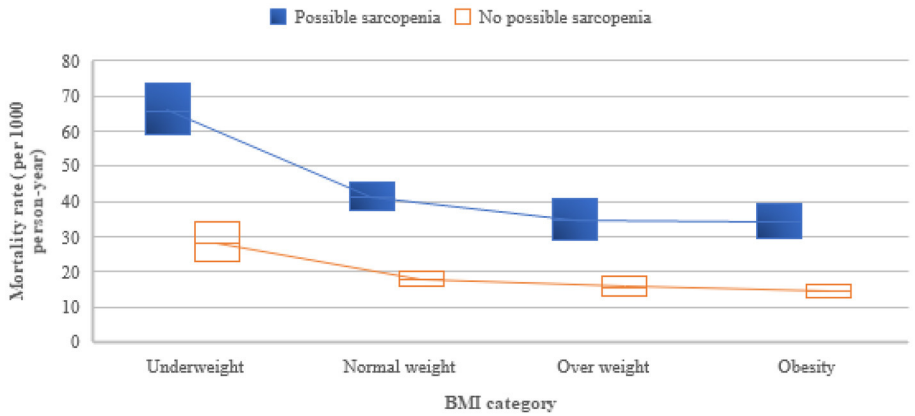

FIGURE 2 | Mortality rate among older people with difference BMI and possible sarcopenia categories.

TABLE 1 | Sociodemographic, functional, lifestyle, clinical, mental health, and mortality characteristics of study participants for different body mass index (BMI) ranges compared between Thai older adults with and without possible sarcopenia ($N = 8,195$).

	Total	BMI < 18.5 kg/m^2 ($n = 1,090$)		BMI 18.5–22.9 kg/m^2 ($n = 3,082$)		BMI 23.0–24.9 kg/m^2 ($n = 1,439$)		BMI > 25.0 kg/m^2 ($n = 2,584$)	
	($n = 8,195$)	With possible sarcopenia ($n = 668$, 61.3%)	Without possible sarcopenia ($n = 422$, 38.7%)	With possible sarcopenia ($n = 1,321$, 42.9%)	Without possible sarcopenia ($n = 1,761$, 57.1%)	With possible sarcopenia ($n = 507$, 35.2%)	Without possible sarcopenia ($n = 932$, 64.8%)	With possible sarcopenia ($n = 692$, 26.8%)	Without possible sarcopenia ($n = 1,892$, 73.2%)
Age (year), mean (SD)	69.2 (6.8)	73.8 (7.2)	68.5 (6.3)	72.6 (7.1)	67.9 (6.2)	71.9 (7.2)	67.0 (5.5)	70.4 (6.8)	66-5 (5.4)
Male, *n* (%)	4,048 (49.4)	387 (57.9)	215 (51.0)	798 (60.4)	931 (52.9)	263 (51.9)	476 (51.1)	277 (40.0)	701 (37.1)
Education (year), mean (SD)	5.1 (3.1)	4.1 (1.8)	4.6 (2.5)	4.7 (2.6)	5.3 (3.3)	4.7 (2.9)	5.7 (3.6)	4.9 (2.9)	5.5 (3.5)
Impaired ADL, *n* (%)	3,736 (45.6)	353 (52.8)	174 (41.2)	646 (48.9)	683 (38.8)	262 (51.7)	346 (37.1)	389 (56.2)	883 (46.7)
Urban, *n* (%)	4,369 (53.3)	285 (38.6)	167 (39.6)	594 (45.0)	885 (50.3)	281 (55.4)	545 (58.5)	428 (61.9)	1,211 (64.0)
Smoking, *n* (%)	1,583 (19.3)	228 (34.1)	140 (33.2)	327 (24.8)	424 (24.1)	59 (11.6)	147 (15.8)	72 (10.4)	186 (9.8)
Hypertension, *n* (%)	4,098 (50.0)	224 (33.6)	120 (28.4)	625 (47.3)	721 (41.0)	292 (57.8)	485 (52.1)	458 (66.2)	1,173 (62.1)
Diabetes, *n* (%)	1,335 (16.3)	36 (5.4)	14 (3.3)	174 (13.2)	182 (10.3)	101 (19.9)	163 (17.5)	202 (29.2)	463 (24.5)
Chronic kidney disease, *n* (%)	2,368 (28.9)	184 (27.6)	82 (19.4)	459 (34.8)	403 (22.9)	184 (36.3)	255 (27.4)	239 (34.5)	562 (29.7)
Depression, *n* (%)	1,583 (19.3)	47 (7.0)	23 (5.5)	63 (4.8)	62 (3.5)	30 (3.9)	35 (3.8)	40 (5.8)	72 (3.8)
Cardiovascular disease, *n* (%)	347 (4.2)	21 (3.1)	10 (2.4)	56 (4.2)	43 (2.4)	25 (4.9)	25 (2.7)	37 (3.4)	57 (3.0)
Cerebrovascular disease, *n* (%)	251 (3.1)	31 (4.6)	6 (1.4)	39 (3.0)	31 (1.8)	29 (5.7)	33 (3.5)	54 (7.8)	101 (5.3)
Death per 1,000 person year (95% CI)	28.2 (27.0–29.4)	65.7 (58.8–73.4)	27.9 (22.8–34.1)	41.2 (37.5–45.4)	17.8 (15.8–20.1)	34.4 (29.2–40.7)	15.5 (13.0–18.5)	34.1 (29.6–39.3)	14.4 (12.7–16.4)

Association between 10-year all-cause mortality and BMI, possible sarcopenia status according to AWGS 2019, and possible sarcopenia severity according to quartile of handgrip strength among Thai older adults who participated in the NHES-IV is shown in **Table 2**. Concerning the BMI category, underweight status associated with a significant risk of mortality (aHR: 1.81, 95% CI: 1.58–2.07), whereas obesity was found to be a protective factor against death (aHR: 0.74, 95% CI: 0.63–0.87) when compared with the normal BMI group. Older adults in the overweight category appear to have similar mortality risk as those in the normal weight group. The results showed similar trends between genders.

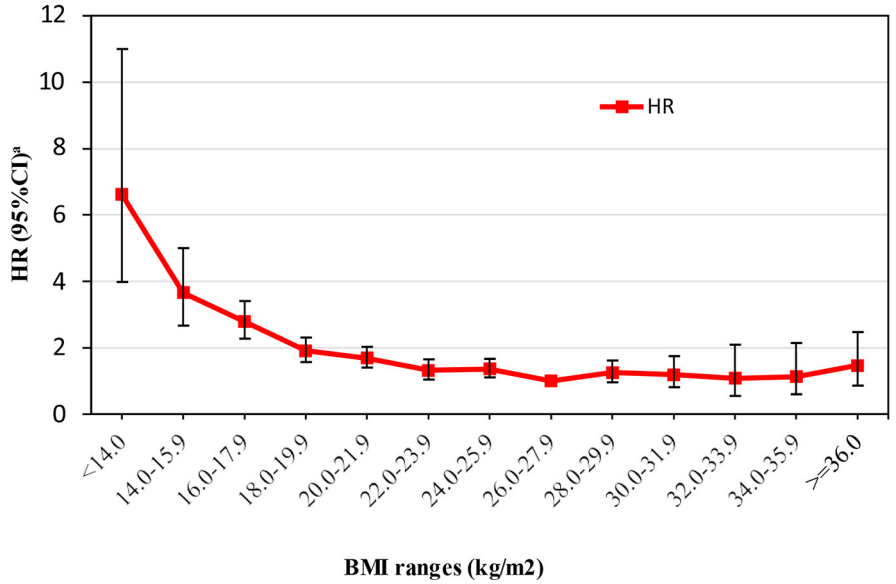

FIGURE 3 | Association between the ranges of body mass index (BMI) and all-cause mortality after adjustment for potentially confounding factors. [a]HR, hazard ratio; CI, confidence interval.

Concerning possible sarcopenia status and defining older adults using the AWGS 2019 cut-off point, participants with possible sarcopenia had significantly higher mortality risk compared to those without possible sarcopenia (aHR: 1.89, 95% CI: 1.70–2.09). When classifying older adults by severity of possible sarcopenia according to quartile group of handgrip strength, the mortality risk was increased in a dose-response manner according to the severity of possible sarcopenia. Older adults with severe possible sarcopenia had the highest risk for mortality (aHR: 2.46, 95% CI: 1.01–3.01) compared to those with moderate or mild possible sarcopenia (aHR: 1.92, 95% CI: 1.65–2.23, and aHR: 1.39, 95% CI: 1.20–1.61, respectively). Similar results were observed between genders.

Association between 10-year all-cause mortality and BMI category combined with possible sarcopenia status for all study participants, and compared between male and female Thai older adults who participated in the NHES-IV is shown in **Table 3**. Multivariate analysis showed that older adults who were underweight with possible sarcopenia were at highest risk for mortality (aHR: 2.81, 95% CI: 2.32–3.40), whereas those who were obese without possible sarcopenia were at lowest risk for mortality (aHR: 0.67, 95% CI: 0.55–0.81) when using normal weight with no possible sarcopenia as the reference group. Our results showed improved classification for defining the risk of death among older adults when BMI and possible sarcopenia status were combined. Among normal weight and overweight older adults, having possible sarcopenia significantly increased mortality risk. In the underweight group, although possible sarcopenia and no possible sarcopenia both exerted increased risk of death, the possible sarcopenia group had substantially higher risk. In the obese group, having possible sarcopenia increased the risk of death with an aHR of 1.53 (95% CI: 1.25–1.88), whereas not having possible sarcopenia was a protective factor against death with an aHR of 0.67 (95% CI: 0.55–0.81). These associations were more pronounced in males compared to females.

Association between 10-year all-cause mortality and BMI category combined with severity of possible sarcopenia for all study participants, and compared between male and female Thai older adults who participated in the 4th Thai NHES-IV is shown in **Table 4**. Multivariate analysis revealed older adults with underweight and severe possible sarcopenia status to be at the highest risk for increased mortality (aHR: 3.98, 95% CI: 2.89–5.48) when using normal weight without possible sarcopenia status as the reference group. In the overweight group, individuals with moderate-to-severe possible sarcopenia showed higher mortality compared to the reference group. Among those in the obesity group, having moderate-to-severe possible sarcopenia posed a higher risk of death, while the opposite was found among those who were obese without possible sarcopenia. Multivariate analysis showed the strongest risk of mortality among older adult males with underweight and severe possible sarcopenia status (aHR: 5.35, 95% CI: 1.19–8.97). In stark contrast, obesity without possible sarcopenia status was a protective factor against mortality (aHR: 0.61, 95% CI: 0.38–0.97).

DISCUSSION

The results of this study affirm the finding that being slightly obese leads to better chance of survival among older adults. It has been shown that higher BMI associated with lower risk of death among older people (15–17). However, the proposed optimal

TABLE 2 | Association between 10-year all-cause mortality and body mass index (BMI), possible sarcopenia status according to AWGS 2019, and possible sarcopenia severity according to quartile of handgrip among Thai older adults who participated in the 4th Thai National Health Examination Survey (NHES-IV) (*N* = 8,195).

	Total		Male		Female	
	Unadjusted	**Adjusted***	**Unadjusted**	**Adjusted***	**Unadjusted**	**Adjusted***
BMI categories						
Underweight (BMI < 18.5)	1.93 (1.70–2.19)	1.81 (1.58–2.07)	1.99 (1.63–2.43)	1.87 (1.53–2.29)	1.92 (1.50–2.45)	1.85 (1.48–2.31)
Normal weight (BMI 18.5–22.9)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Overweight (BMI 23.0–24.9)	0.86 (0.74–1.00)	0.91 (0.78–1.05)	1.03 (0.87–1.22)	0.99 (0.82–1.18)	0.71 (0.56–0.89)	0.81 (0.64–1.03)
Obesity (BMI ≥ 25.0)	0.70 (0.60–0.82)	0.74 (0.63–0.87)	0.83 (0.68–1.02)	0.71 (0.57–0.89)	0.68 (0.54–0.87)	0.83 (0.66–1.04)
Possible sarcopenia categories						
Possible sarcopenia	2.70 (2.45–2.98)	1.89 (1.70–2.09)	2.66 (2.27–3.12)	1.97 (1.65–2.35)	2.57 (2.20–3.00)	1.78 (1.51–2.11)
No possible sarcopenia	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Possible sarcopenia severity						
Severe possible sarcopenia (6.5–24.6 kg in male, 6–16.6 kg in female)	4.11 (3.45–4.89)	2.46 (1.01–3.01)	4.26 (3.48–5.21)	2.92 (2.25–2.78)	3.95 (3.11–4.99)	2.21 (1.72–2.85)
Moderate possible sarcopenia (24.7–29.2 kg in male, 16.7–19.7 kg in female)	2.60 (2.25–3.00)	1.92 (1.65–2.23)	2.77 (2.25–3.41)	2.19 (1.75–2.74)	2.42 (1.90–3.08)	1.72 (1.35–2.20)
Mild possible sarcopenia (29.3–33.8 kg in male, 19.8–22.8 kg in female)	1.59 (1.37–1.84)	1.39 (1.20–1.61)	1.61 (1.24–2.08)	1.48 (1.14–1.91)	1.58 (1.27–1.96)	1.32 (1.05–1.67)
No possible sarcopenia (33.9–54.5 kg in male, 22.9–49.0 kg in female)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

**Adjusted by age, residential area, diabetes, chronic kidney disease, cardiovascular disease, cerebrovascular disease, history of smoke.*

TABLE 3 | Association between 10-year all-cause mortality and body mass index (BMI) category stratified by possible sarcopenia status for all study participants, and compared between male and female Thai older adults who participated in the 4th Thai National Health Examination Survey (NHES-IV) (*N* = 8,195).

BMI categories	**Possible sarcopenia categories**	**Total participants**		**Male**		**Female**	
		Unadjusted	**Adjusted***	**Unadjusted**	**Adjusted***	**Unadjusted**	**Adjusted***
Underweight (BMI < 18.5)	With	4.00 (3.35–4.79)	2.81 (2.32–3.40)	4.07 (3.00–5.52)	3.05 (2.21–4.22)	3.84 (2.89–5.11)	2.66 (2.00–3.53)
	Without	1.53 (1.12–2.09)	1.58 (1.56–2.16)	1.46 (0.98–2.17)	1.54 (1.04–2.29)	1.62 (1.06–2.50)	1.65 (1.07–2.55)
Normal weight (BMI 18.5–22.9)	With	2.26 (1.87–2.74)	1.60 (1.31–1.95)	2.25 (1.77–2.84)	1.67 (1.30–2.15)	2.18 (1.62–2.94)	1.47 (1.07–2.01)
	Without	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Overweight (BMI 23.0–24.9)	With	1.88 (1.53–2.32)	1.37 (1.10–1.72)	2.05 (1.61–2.60)	1.36 (1.03–1.79)	1.73 (1.25–2.39)	1.42 (1.04–1.95)
	Without	1.01 (0.74–1.40)	1.00 (0.71–1.40)	1.40 (0.95–2.06)	1.28 (0.85–1.92)	0.67 (0.47–0.94)	0.70 (0.48–1.03)
Obesity (BMI ≥ 25.0)	With	2.16 (1.78–2.61)	1.53 (1.25–1.88)	3.10 (2.26–4.25)	1.77 (1.32–2.39)	1.70 (1.62–2.47)	1.43 (0.97–2.09)
	Without	0.70 (0.58–0.84)	0.67 (0.55–0.81)	0.68 (0.51–0.91)	0.54 (0.39–0.76)	0.73 (0.57–0.94)	0.81 (0.62–1.04)

**Adjusted by age, residential area, diabetes, chronic kidney disease, cardiovascular disease, cerebrovascular disease, history of smoke.*

TABLE 4 | Association between 10-year all-cause mortality and body mass index (BMI) category stratified by possible sarcopenia severity for all study participants, and compared between male and female Thai older adults who participated in the 4th Thai National Health Examination Survey (NHES-IV) ($N = 8,195$).

BMI categories	Possible sarcopenia severity	Total participants		Male		Female	
		Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted*
Underweight (BMI < 18.5)	Severe	6.82 (4.92–9.47)	3.98 (2.89–5.48)	8.05 (5.00–12.98)	5.35 (1.19–8.97)	5.64 (3.76–8.48)	3.12 (2.06–4.72)
	Moderate	4.09 (2.94–5.67)	2.97 (2.13–4.14)	4.36 (2.95–6.47)	3.35 (2.27–4.95)	3.83 (2.34–6.26)	2.81 (1.75–4.53)
	Mild	2.83 (1.83–4.38)	2.72 (1.75–4.23)	3.03 (1.70–5.38)	3.08 (1.69–5.64)	2.70 (1.54–4.73)	2.56 (1.46–4.48)
	None	0.76 (0.40–1.47)	0.78 (0.41–1.51)	0.63 (0.26–1.54)	0.65 (0.27–1.58)	0.85 (0.39–1.89)	0.92 (0.43–2.01)
Normal weight (BMI 18.5–22.9)	Severe	3.73 (2.71–5.13)	2.18 (1.59–3.00)	4.23 (2.95–6.05)	2.74 (1.86–4.04)	3.22 (1.99–5.19)	1.79 (1.11–2.86)
	Moderate	2.79 (1.92–4.05)	1.99 (1.40–2.82)	3.20 (1.97–5.21)	2.37 (1.46–3.85)	2.31 (1.42–3.76)	1.64 (1.02–2.64)
	Mild	1.85 (1.33–2.57)	1.50 (1.10–2.03)	2.12 (1.46–3.04)	1.77 (1.21–2.59)	1.58 (0.91–2.73)	1.28 (0.75–2.18)
	None	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Overweight (BMI 23.0–24.9)	Severe	3.45 (2.34–5.09)	2.05 (1.39–3.02)	4.36 (2.83–6.72)	2.45 (1.49–4.05)	2.85 (1.73–4.70)	1.89 (1.15–3.10)
	Moderate	2.21 (1.64–2.97)	1.58 (1.19–2.10)	3.13 (2.22–4.45)	2.13 (1.49–3.06)	1.44 (0.86–2.40)	1.13 (0.67–1.89)
	Mild	1.38 (0.91–2.12)	1.17 (0.77–1.78)	1.58 (0.82–3.02)	1.26 (0.66–2.40)	1.20 (0.67–2.15)	1.06 (0.59–1.89)
	None	1.63 (0.81–3.29)	1.48 (0.74–2.96)	2.85 (1.30–6.25)	2.44 (1.09–5.44)	0.67 (0.31–1.42)	0.65 (0.30–1.39)
Obesity (BMI ≥ 25.0)	Severe	3.45 (2.45–4.85)	1.98 (1.41–2.79)	5.60 (3.67–8.54)	2.68 (1.70–4.24)	2.64 (1.65–4.25)	1.76 (1.10–2.81)
	Moderate	2.20 (1.56–3.11)	1.53 (1.10–2.79)	3.80 (2.20–6.58)	2.20 (1.35–3.58)	1.61 (1.08–2.42)	1.26 (0.83–1.90)
	Mild	1.23 (0.87–1.74)	1.01 (0.71–1.42)	1.85 (1.16–2.96)	1.26 (0.79–1.99)	0.99 (0.64–1.53)	0.91 (0.58–1.41)
	None	0.87 (0.59–1.29)	0.77 (0.52–1.14)	0.82 (0.53–1.26)	0.61 (0.38–0.97)	1.01 (0.51–1.62)	0.98 (0.55–1.73)

**Adjusted by age, residential area, diabetes, chronic kidney disease, cardiovascular disease, cerebrovascular disease, history of smoke.*

BMI range varies widely (18, 19). A previous meta-analysis that reported that older adults with a BMI of 27.0–27.9 kg/m^2 had the lowest mortality did not include Asian population in the analysis (16). The present study proposes a BMI of 26.0–27.9 kg/m^2 as the optimal BMI for Asian older adults since it was found to be associated with the lowest mortality after adjusting for potential confounders.

The present study showed the prevalence of underweight status among older adults to be substantially high in Thailand at approximately 15%. This finding is similar to those reported from several studies conducted in Thailand (20, 21), and in other countries in Asia (15, 17). However, these figures are in stark contrast to the 3% rates reported from the US (22) and Europe (23). Moreover, our study emphasizes the higher mortality risk associated with underweight status (when compared to obese status) among community-dwelling Thai older adults. This association is similar to those reported from other Asian countries (15, 17), but is different from the results of studies conducted in non-Asian older adult populations that found that the mortality risk associated with underweight and overweight status increased at similar magnitudes (16, 23, 24). A similar trend toward association between underweight status and increased mortality was found in a previous study of a large volume of pooled data from younger Asian population (25); however, the magnitude of increased mortality risk appears to be stronger among older adults. The possible explanations for synergistic mortality risk among older people with underweight status might stem from the accumulation of several poor prognostic factors, such as risk of infection (26), risk of falls (27), severe functional impairment, and other geriatric syndromes (28). Importantly, some of these factors are potentially modifiable. Since underweight status is one of those potentially modifiable factors, underweight status should be addressed at the healthcare policy level so that appropriate nutritional strategies can be developed and implemented, especially among Asian older adults.

In this study, we found the mortality rate of Thai older people was higher than the European countries (29). Thailand, a

middle-income country, has been undergoing an epidemiological transition with predominant of non-communicable diseases. Cardiovascular diseases accounted for almost one-third of all deaths in Thai population (30). Compared to populations in America and Europe with different stages of economic development and earlier stage of cardiovascular epidemic; the mortality rate in Thai population as with other low-and middle-income countries are relatively higher than in high-income countries where cardiovascular and NCD death rates are lower (31). This might be due to the differences in socioeconomic, health system resources, and system performance for rate of awareness, coverage and treatment and control of the condition.

In contrast to BMI alone, this study showed that the association between BMI and all-cause mortality in older adults was modified by possible sarcopenic status, and was dependent upon the severity of possible sarcopenia. Among underweight older adults, the subgroup without possible sarcopenia was not at elevated risk for mortality; however, the risk of mortality increased in dose-response manner as the severity of possible sarcopenia increased. Furthermore, the classification of possible sarcopenia severity according to quartile of handgrip strength was also able to differentiate mortality risk among older adults in other weight groups. Among obese older adults, severity of possible sarcopenia improved the ability to assess and differentiate mortality risk. Obese people with moderate-to-severe possible sarcopenia were at increased risk, while those without possible sarcopenia realized protective effect relative to mortality risk.

Sarcopenia in older adults increases their mortality risk, as shown in a previous meta-analysis (32). The association between severity of sarcopenia using handgrip strength and all-cause mortality has also been addressed in previous studies (33, 34). The combination of BMI and sarcopenia, however, has been focused mostly on sarcopenia and obesity, namely sarcopenic obesity, as a condition of increased mortality risk (35). This finding has not been widely addressed in the literature. The mechanisms of sarcopenic obesity are based on changes in metabolism and body composition due to aging combined with poor physical activity from both physical and mental illnesses (36). The present study demonstrated similar association with mortality in the sarcopenic obesity group. However, the magnitude of risk in the present study was more pronounced in the possible sarcopenic-undernutrition group. A recent review by the UK group (37) included studies that investigated association between body composition and mortality, and they found no studies that used the combination of BMI and sarcopenia in other form apart from sarcopenic obesity. A previous study from Taiwan (38) reported synergistic mortality risk between the combination of low physical activity with underweight status and low physical activity with sarcopenia. An explanation for this finding was not proposed.

The obesity paradox, which is defined as the finding that people with a higher BMI are associated with a reduced risk of mortality, has been repeatedly established in studies conducted among older adults. Several attempts have been made to explore the underneath mechanism for protective effect of obesity paradox by combining additional measurements. A previous systematic review (39) explored the effect of physical fitness on this phenomenon in older adults, but they failed to arrive at a robust conclusion, and they commented on the limited validity of physical activity measurement in the included studies. A recent study (40) attempted to stratify risk among middle-aged adults according to BMI using cardiorespiratory fitness. They reported that fitness modified mortality risk in obese men, but not in obese women. The present study proposes the explanation for obesity paradox by further stratified BMI using possible sarcopenia status. We discovered a similar trend to previous study using sarcopenic status as a fitness measurement for older adults. The combination of BMI and possible sarcopenic status could better classify obese older adults into different risk groups, which is in accordance with the "fat, but fit" phenomenon (39).

Possible sarcopenic status in combination with BMI appears to be a good body composition measurement for classifying mortality risk among older adults. Possible sarcopenia enhances mortality risk after classified by BMI in all categories. Previous studies attempted to classify risk groups for mortality using several body composition parameters that ranged from a single parameter to a combination of parameters; however, their results were inconclusive (37). One explanation could be that the relationships between these parameters and mortality are not linear and the method of combining matters.

The main strengths of this study include the use of data from a nationwide survey from a representative group of population with a long-term follow-up period. The mortality outcome was assessed using mortality data from a national registry that has been affirmed as a reliable database for mortality data in Thailand. Moreover, the BMI categories used ethnicity-specific cut-off points that were developed for Asia-Pacific population. This study also used a recent definition of possible sarcopenia that is specific for Asian population. The classification of possible sarcopenia severity using quartile of handgrip strength demonstrated robustness by showing a similar trend in all subgroups, and the relationship with mortality risk was in a dose-response manner. The important limitation is that all information was collected only once during 2008–2009, it is unknown whether those anthropometric measures changed during last years of life. Additionally, many confounding factors derived from self-report information, such as history of stroke or cardiovascular disease, might be vulnerable to underreporting bias. Furthermore, similar to other cohort study, residual confounding factors could also be an issue in the present study.

Implications for Policy and Practice

Optimal nutritional status is considered to be a key component of healthy aging. This study demonstrates that a combination of anthropometric measurements may be a better tool, compared to BMI alone, for guiding optimal nutritional status, and for reducing mortality risk. Moreover, parameters need to be age group and ethnicity specific to improve the accuracy and reliability of risk assessment. Among older adults, an attempt should be made to maintain a slightly higher BMI, and to avoid sarcopenic status. In addition to a proper diet, resistance exercise is approved for improving muscle strength, muscle mass, and physical function (41, 42). Therefore, an exercise program should be included in the health promoting

package in all settings (43). Among older adults who are underweight, recommendations should be made to encourage increased both body weight and muscle strength. This would include adequate total caloric intake and increased protein intake in combination with resistance exercise. Among those with overweight/obesity status, possible sarcopenic status should be measured. Appropriate recommendations should then be given. Optimal resistance exercise should be considered in all older people with possible sarcopenia.

In conclusion, the results of this study revealed that the combination of BMI and possible sarcopenia could be a better predictor of all-cause mortality among Asian older adults compared to either parameter alone. In addition to BMI measurement, handgrip strength should be included as a nutritional assessment tool. Multivariate analysis showed underweight individuals with severe possible sarcopenia to be at highest risk for increased mortality, and higher risk was found in men. Obese status without possible sarcopenia was found to be an independent protective factor against mortality. Among older adults with a high BMI, proper diet and resistance exercise should be encouraged to prevent sarcopenia as opposed to encouraging weight reduction alone. "Fat, but fit" might be the most favorable outlook for older people.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research protection Unit, Faculty of Medicine Siriraj Hospital, Mahidol University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CC, VS, and WA: conceptualization, formal analysis, and review and editing of the manuscript. WA: methodology, resources, data curation, and project administration. CC: software. CC and VS: writing—original draft of the manuscript. VS: supervision. All authors have read and are in agreement with the submitted version of the manuscript.

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REFERENCES

1. Hsu YH, Chou MY, Chu CS, Liao MC, Wang YC, Lin YT, et al. Predictive effect of malnutrition on long-term clinical outcomes among older men: a prospectively observational cohort study. *J Nutr Health Aging.* (2019) 23:876–82. doi: 10.1007/s12603-019-1246-2
2. Garn SM. Anthropometry in clinical appraisal of nutritional status. *Am J Clin Nutr.* (1962) 11:418–32. doi: 10.1093/ajcn/11.5.418
3. Veronese N, Cereda E, Solmi M, Fowler SA, Manzato E, Maggi S, et al. Inverse relationship between body mass index and mortality in older nursing home residents: a meta-analysis of 19,538 elderly subjects. *Obes Rev.* (2015) 16:1001–15. doi: 10.1016/S1878-7649(15)30028-0
4. Chen Y, Copeland WK, Vedanthan R, Grant E, Lee JE, Gu D, et al. Association between body mass index and cardiovascular disease mortality in east Asians and south Asians: pooled analysis of prospective data from the Asia cohort consortium. *BMJ.* (2013) 347:f5446. doi: 10.1136/bmj.f5446
5. Greenlee H, Unger JM, LeBlanc M, Ramsey S, Hershman DL. Association between body mass index and cancer survival in a pooled analysis of 22 clinical trials. *Cancer Epidemiol Biomarkers Prev.* (2017) 26:21–9. doi: 10.1158/1055-9965.EPI-15-1336
6. Sorkin JD, Muller DC, Andres R. Longitudinal change in the heights of men and women: consequential effects on body mass index. *Epidemiol Rev.* (1999) 21:247–60. doi: 10.1093/oxfordjournals.epirev.a018000
7. Beaudart C, Sanchez-Rodriguez D, Locquet M, Reginster J-Y, Lengelé L, Bruyère O. Malnutrition as a strong predictor of the onset of sarcopenia. *Nutrients.* (2019) 11:2883. doi: 10.3390/nu11122883
8. Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, et al. Asian working group for sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *J Am Med Dir Assoc.* (2020) 21:300–7.e2. doi: 10.1016/j.jamda.2019.12.012
9. Aekplakorn W, Kessomboon P, Sangthong R, Chariyalertsak S, Putwatana P, Inthawong R, et al. Urban and rural variation in clustering of metabolic syndrome components in the Thai population: results from the fourth national health examination survey 2009. *BMC Public Health.* (2011) 11:854. doi: 10.1186/1471-2458-11-854
10. Pan WH, Yeh WT. How to define obesity? Evidence-based multiple action points for public awareness, screening, and treatment: an extension of Asian-Pacific recommendations. *Asia Pac J Clin Nutr.* (2008) 17:370–4.
11. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF III, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
12. Bell CC. DSM-IV: diagnostic and statistical manual of mental disorders. *JAMA.* (1994) 272:828–9. doi: 10.1001/jama.1994.03520100096046
13. Mahoney FI BD. Functional evaluation: the Barthel index. *Maryland State Med J.* (1965) 14:56–61. doi: 10.1037/t02366-000
14. Saylor J, Friedmann E, Lee HJ. Navigating complex sample analysis using national survey data. *Nurs Res.* (2012) 61:231–7. doi: 10.1097/NNR.0b013e3182533403
15. Wang YF, Tang Z, Guo J, Tao LX, Liu L, Li HB, et al. BMI and BMI changes to all-cause mortality among the elderly in Beijing: a 20-year cohort study. *Biomed Environ Sci.* (2017) 30:79–87. doi: 10.3967/bes2017.011
16. Winter JE, MacInnis RJ, Wattanapenpaiboon N, Nowson CA. BMI and all-cause mortality in older adults: a meta-analysis. *Am J Clin Nutr.* (2014) 99:875–90. doi: 10.3945/ajcn.113.068122
17. Tamakoshi A, Yatsuya H, Lin Y, Tamakoshi K, Kondo T, Suzuki S, et al. BMI and all-cause mortality among Japanese older adults: findings from the Japan collaborative cohort study. *Obesity.* (2010) 18:362–9. doi: 10.1038/oby.2009.190

18. Yi SW, Ohrr H, Shin SA, Yi JJ. Sex-age-specific association of body mass index with all-cause mortality among 12.8 million Korean adults: a prospective cohort study. *Int J Epidemiol.* (2015) 44:1696–705. doi: 10.1093/ije/dyv138
19. Peter RS, Mayer B, Concin H, Nagel G. The effect of age on the shape of the BMI-mortality relation and BMI associated with minimum all-cause mortality in a large Austrian cohort. *Int J Obes.* (2015) 39:530–4. doi: 10.1038/ijo.2014.168
20. Churak P, Praditsorn P, Meenongwah J, Wimonpeerapattana W. Factors associated with nutritional status of elderly in Ubon Ratchathani, Thailand. *Asia Pac J Sci Technol.* (2019) 24. doi: 10.14456/apst.2019.5
21. Vapattanawong P, Aekplakorn W, Rakchanyaban U, Prasartkul P, Porapakkham Y. Obesity and mortality among older Thais: a four year follow up study. *BMC Public Health.* (2010) 10:604. doi: 10.1186/1471-2458-10-604
22. Centers of Disease Control and Prevention. *Prevalence of Underweight Among Adults Aged 20 and Over: United States, 1960–1962 Through 2015–2016.* Centers of Disease Control and Prevention (2018). Available online at: https://www.cdc.gov/nchs/data/hestat/underweight_adult_15_16/underweight_adult_15_16.htm#table1 (accessed July 2, 2021).
23. Song X, Pitkäniemi J, Gao W, Heine RJ, Pyörälä K, Söderberg S, et al. Relationship between body mass index and mortality among Europeans. *Euro J Clin Nutr.* (2012) 66:156–65. doi: 10.1038/ejcn.2011.145
24. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity: An evaluation of potential bias. *Vital Health Stat 3.* (2018) 1–21.
25. Zheng W, McLerran DF, Rolland B, Zhang X, Inoue M, Matsuo K, et al. Association between body-mass index and risk of death in more than 1 million Asians. *N Engl J Med.* (2011) 364:719–29. doi: 10.1056/NEJMoa1010679
26. Lin Y-K, Wang C-C, Yen Y-F, Chen L-J, Ku P-W, Chen C-C, et al. Association of body mass index with all-cause mortality in the elderly population of Taiwan: a prospective cohort study. *Nutr Metab Cardiovasc Dis.* (2021) 31:110–8. doi: 10.1016/j.numecd.2020.08.014
27. Zhang X, Huang P, Dou Q, Wang C, Zhang W, Yang Y, et al. Falls among older adults with sarcopenia dwelling in nursing home or community: a meta-analysis. *Clin Nutr.* (2020) 39:33–9. doi: 10.1016/j.clnu.2019.01.002
28. Huang C-C, Lee J-D, Yang D-C, Shih H-I, Sun C-Y, Chang C-M. Associations between geriatric syndromes and mortality in community-dwelling elderly: results of a national longitudinal study in Taiwan. *J Am Med Direct Assoc.* (2017) 18:246–51. doi: 10.1016/j.jamda.2016.09.017
29. Statistical Office of the European Communities. *Eurostat: Deaths by Age and Sex.* (2021). Available online at: https://ec.europa.eu/eurostat/databrowser/view/DEMO_MAGEC/default/table?lang=en&category=demo.demo_mor (accessed April 14, 2022).
30. World Health Organization ROfS-EA. *WHO Country Cooperation Strategy, Thailand, 2017–2021.* New Delhi: World Health Organization (2017)
31. Tyrovolas S, Koyanagi A, Garin N, Olaya B, Ayuso-Mateos JL, Miret M, et al. Diabetes mellitus and its association with central obesity and disability among older adults: a global perspective. *Exp Gerontol.* (2015) 64:70–7. doi: 10.1016/j.exger.2015.02.010
32. Kelley GA, Kelley KS. Is sarcopenia associated with an increased risk of all-cause mortality and functional disability? *Exp Gerontol.* (2017) 96:100–3. doi: 10.1016/j.exger.2017.06.008
33. Bae EJ, Park NJ, Sohn HS, Kim YH. Handgrip strength and all-cause mortality in middle-aged and older Koreans. *Int J Environ Res Public Health.* (2019) 16:740. doi: 10.3390/ijerph16050740
34. Cai Y, Liu L, Wang J, Gao Y, Guo Z, Ping Z. Linear association between grip strength and all-cause mortality among the elderly: results from the SHARE study. *Aging Clin Exp Res.* (2021) 33:933–41. doi: 10.1007/s40520-020-01614-z
35. Zhang X, Xie X, Dou Q, Liu C, Zhang W, Yang Y, et al. Association of sarcopenic obesity with the risk of all-cause mortality among adults over a broad range of different settings: a updated meta-analysis. *BMC Geriatr.* (2019) 19:183. doi: 10.1186/s12877-019-1195-y
36. Batsis JA, Villareal DT. Sarcopenic obesity in older adults: aetiology, epidemiology and treatment strategies. *Nat Rev Endocrinol.* (2018) 14:513–37. doi: 10.1038/s41574-018-0062-9
37. Knowles R, Carter J, Jebb SA, Bennett D, Lewington S, Piernas C. Associations of Skeletal muscle mass and fat mass with incident cardiovascular disease and All-cause mortality: a prospective cohort study of UK Biobank participants. *J Am Heart Assoc.* (2021) 10:e019337. doi: 10.1161/JAHA.120.019337
38. Lee MS, Chen RC, Chang YH, Huang YC, Wahlqvist ML. Physical function mitigates the adverse effects of being thin on mortality in a free-living older Taiwanese cohort. *J Nutr Health Aging.* (2012) 16:776–83. doi: 10.1007/s12603-012-0379-3
39. Yerrakalva D, Mullis R, Mant J. The associations of "fatness," "fitness," and physical activity with all-cause mortality in older adults: a systematic review. *Obesity.* (2015) 23:1944–56. doi: 10.1002/oby.21181
40. Tarp J, Grøntved A, Sanchez-Lastra MA, Dalene KE, Ding D, Ekelund U. Fitness, fatness, and mortality in men and women from the UK biobank: prospective cohort study. *J Am Heart Assoc.* (2021). 10:e019605. doi: 10.1161/JAHA.120.019605
41. Bao W, Sun Y, Zhang T, Zou L, Wu X, Wang D, et al. Exercise programs for muscle mass, muscle strength and physical performance in older adults with sarcopenia: a systematic review and meta-analysis. *Aging Dis.* (2020) 11:863–73. doi: 10.14336/AD.2019.1012
42. Naseeb MA, Volpe SL. Protein and exercise in the prevention of sarcopenia and aging. *Nutr Res.* (2017) 40:1–20. doi: 10.1016/j.nutres.2017.01.001
43. Dent E, Morley JE, Cruz-Jentoft AJ, Arai H, Kritchevsky SB, Guralnik J, et al. International clinical practice guidelines for sarcopenia (ICFSR): screening, diagnosis and management. *J Nutr Health Aging.* (2018) 22:1148–61. doi: 10.1007/s12603-018-1139-9

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A Meta-Analysis on Vitamin D Supplementation and Asthma Treatment

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Background: Vitamin D, as an immunomodulator, may be related to the therapeutic effect of asthma patients, but the research in this area is still controversial. The aim of this meta-analysis was to analyze the role of vitamin D supplementation in the treatment of asthma patients.

Materials and Methods: Randomized Controlled Trials (RCTs) of vitamin D supplementation in asthma were searched in PubMed, EMBASE, and the Cochrane library. Primary outcomes were forced expiratory volume in one second (FEV1), asthma exacerbations, Asthma Control Test scores (ACT scores), and fractional exhaled nitric oxide (FENO).

Results: A total of 10 RCTs were included, including 1,349 patients. Vitamin D supplementation didn't affect the ACT scores (SMD = 0.04, 95% CI = −0.13 to 0.21, P = 0.87), FEV1 (SMD = 0.04, 95% CI = −0.35 to 0.43, $P < 0.01$) and FENO (SMD = −0.01, 95% CI = −0.22 to 0.20, P = 0.27), but reduced the rate of asthma exacerbations (RR = 0.69, 95% CI = 0.41 to 0.88, $P < 0.01$), especially in subgroups of children (RR = 0.46, 95% CI = 0.30 to 0.70, P = 0.83) and follow up time less than 6 months (RR = 0.45, 95% CI = 0.32 to 0.63, P = 0.95). Additionally, though there was only one study included in the subgroup, it significantly enhanced FEV1 at the last visit for patients whose FEV1 baseline value was less than 70% (SMD = 0.94, 95% CI = 0.47 to 1.41).

Conclusion: Vitamin D supplementation can reduce asthma exacerbations, especially in children, and within 6 months of follow up time. In addition, vitamin D has a positive effect on improving FEV1 of patients whose FEV1 baseline value is less than 70%, but more RCTs are still needed to support this conclusion.

Systematic Review Registration: [https://inplasy.com], identifier [10.37766/inplasy2022.6.0049].

Keywords: vitamin D, asthma, FEV1, asthma exacerbations, children

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INTRODUCTION

As one of the most common chronic, non-communicable diseases, asthma is a heterogeneous clinical syndrome affecting approximately 334 million people worldwide (1). It is defined by Expert Panel Report 3 (EPR-3) as "a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role: in particular, mast cells, eosinophils, neutrophils

(especially in sudden onset, fatal exacerbations, occupational asthma, and patients who smoke), T lymphocytes, macrophages, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of coughing (particularly at night or early in the morning), wheezing, breathlessness, and chest tightness. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment" (2). The global prevalence of asthma in adults is 4.3% (3) but varies in different countries, 7.8–11.9% in the United States (4–6), 10% in Japan (7), 2.38% in India (8), and 1.2–5.8% in China (9). More than 400 thousand people were estimated by the Global Burden of Disease collaboration to die from asthma, mainly in low- and middle-income countries (10). Airflow limitation, an important feature of asthma, is more common in low-and middle-income countries due to the higher prevalence of known risk factors and poor asthma management compared to high-income countries (11). Asthma in children is predominantly male, whereas in adults it is the opposite, probably due to the effects of sex hormones (12).

The existent evidence indicates that asthma is a disease associated with various factors, including environmental factors [air pollution (13), climate change, pollen (14), microbial exposure (15), and allergic triggers (16)], host factors [nutrition state (17) and infection (18)], and genetic factors [genetic susceptibility sites of asthma (19)]. Notably, many studies have shown that dietary factors could affect the course and development of asthma. High consumption of vegetables and fruits (20–23), especially apples and oranges, could reduce the risk of asthma. Pro-inflammatory cytokines associated with fruit and vegetable intake were simultaneously decreased and anti-inflammatory factors were increased (24, 25). In addition, there was a positive association between the frequent consumption of dairy products with asthma (26) and bronchial hyperreactivity (27). However, acute effects of milk ingestion were not significant in asthma patients (28–30). A diet that emphasizes fruits, vegetables, and whole grains, but not high-fat meat and dairy products, was related to reducing the risk of asthma (31–33).

As one of the fat-soluble vitamins required by the human body, vitamin D is obtained mainly through the skin synthesis pathway after ultraviolet B (UVB) radiation, and a small part from food (oily fish, egg yolk, mushroom, liver, or organ meat) and supplements. Cholecalciferol (vitamin D3) is derived from animals and ergocalciferol (vitamin D2) is derived from plants (34). Recently, vitamin D deficiency, one of the major risk factors in asthma, has triggered more and more interest in research, which was confirmed to involve in the development and prognosis of a variety of diseases, including cancer (35), inflammatory bowel disease (36), urinary tract infection (37), respiratory infections (38), and asthma (39). It was reported that the risk of acute respiratory infection (ARI) was reduced in individuals with high serum 25(OH)D levels (40). What's more, a case-control study has reported that children who require hospitalization for acute respiratory infections had a significantly higher risk of vitamin D deficiency than children with mild acute respiratory infections (41). 1,25 (OH) 2D exerts antiviral activity and regulates inflammatory response to viral infection by stimulating cathelicidin release, regulating toll-like receptor expression, and inhibiting pro-inflammatory cytokines production (42). An RCT has proved that supplementation of vitamin D could protect against the development of acute respiratory tract infection (43). As for the rise of the COVID-19 pandemic, calcitriol non-significantly suppressed the expression of angiotensin II (Ang II) receptor type 1 (AT1) and angiotensin-converting enzyme (ACE), but markedly reduced Ang II formation, which acts as host cell receptors mediating SARS-CoV-2 infection (44). Evidence showed that vitamin D supplementation might reduce the risk of infection and death in COVID-19 (45, 46).

Furthermore, respiratory tract infection is the main cause of asthma aggravation (47). A great many studies have found that patients with low vitamin D levels were more likely to have asthma exacerbations (48–50). In addition, there is sufficient evidence that exposure to tobacco smoke and nicotine during the prenatal and postnatal periods impairs lung development, alters the immune response to viral infection, and increases the prevalence and severity of childhood wheezing (51). Chinellato I's research demonstrated that vitamin D levels were significantly higher in children with non-smoking parents than those with both smoking parents, and were intermediate in those exposed to single maternal or paternal smoking (52). It has been reported that a modest reduction in 25- hydroxyvitamin D in pregnant women exposed to cigarette smoke, is probably because of the reduced ability of the placenta of women who smoke to transport vitamin D (53). In addition, smoking in adults was associated with osteopenia and decreased serum 25(OH)D and parathyroid hormone (PTH) concentrations (54, 55). While for smokers, Ben Michael Brumpton's team found that Low serum 25(OH)D levels had a weaker correlation with greater decreases in lung function in adults with asthma, and a stronger correlation was observed in non-smokers, but not in ever smokers (56). As for the effect of vitamin D supplementation in smokers or non-smokers with asthma, Sluyter J. D.'s study demonstrated that vitamin D supplementation significantly improved the lung function of both ever-smokers and non-smokers with asthma. However, there is still a lack of RCTs on vitamin D supplementation in patients with asthma varying by smoking status (57).

However, there are contradictions between the mechanism research and clinical prognosis research on the effect of vitamin D supplementation on asthma. Some research has determined the relationship between vitamin D deficiency and the overall worsening of lung function and symptoms in patients with asthma (39, 58, 59). In terms of mechanism research, some asthma mouse model studies have indicated the protective effect of vitamin D supplementation. Serum IgE, whose elevated expression is the characteristic of active airway inflammation (60), could be reduced significantly via vitamin D supplementation. What's more, vitamin D exerted a protective effect by reducing airway remodeling and inhibited airway inflammation by reducing oxidative stress and regulating the Th17/Treg balance and the NF-κB pathway (61). The classical Wnt/β-catenin pathway plays a key role in cell proliferation, cell migration, stem cell self-renewal, organogenesis, tissue homeostasis under physiological conditions, and damaged tissue repair (62). The intracellular accumulation and nuclear transfer

of Wnt/β-catenin have a great impact on the maturation and structural adaptation of the lung, including the development of airway smooth muscle precursor cells, the maintenance of airway smooth muscle growth, and the regulation of its contraction, which was related to the pathogenesis of asthmatic airway remodeling (63–65). And the research showed that vitamin D improved airway remodeling in asthma by down-regulating the activity of the Wnt/β-catenin signaling pathway (66). In contrast, vitamin D deficiency aggravated the progression of asthma by increasing eosinophils, decreasing T regulatory cells, increasing NF-κB expression, and increasing pro-inflammatory cytokines (67). So far, there have been a number of meta-analyses regarding vitamin D supplementation in relation to asthma treatment. Some meta-analysis (68–72) have manifested that vitamin D supplementation reduced the rate of asthma exacerbations for patients with systemic corticosteroid treatment, especially in patients with vitamin D insufficiency, but didn't affect the lung function (FEV1 or FENO) and ACT scores. However, there are still a few clinical studies manifesting that vitamin D supplementation in vitamin D-deficient patients didn't improve the course and development of asthma (73, 74). Asthma control, asthma exacerbations, and lung function were all unaffected by vitamin D supplementation. The conclusions are not uniform, and some study populations only include children or adults. Therefore, a systematic meta-analysis of Randomized Controlled Trials (RCTs) was conducted to investigate the role of vitamin D supplementation and asthma treatment.

OBJECTIVES

The aim of this study was to evaluate the effect of vitamin D supplementation on clinical outcomes (Asthma Control Test scores, ACT scores; forced expiratory volume in 1 s, FEV1; fractional exhaled nitric oxide, FENO; asthma exacerbations) in asthma patients.

METHODOLOGY

Preferred reporting items (PRISMA) statements of systematic review and meta-analysis were used for the meta-analysis (75).

Search Strategy

A comprehensive literature search using predefined keywords from articles published over the last decade was conducted on PubMed, EMBASE, and the Cochrane library.

Manually search to retrieve articles using keywords: {(Asthma [Title/Abstract]) OR (asthma exacerbations [Title/Abstract])} AND (vitamin D [Title/Abstract]) AND (supplementation [Title/Abstract]) AND (RCTs [Title/Abstract]).

Inclusion Criteria

Randomized Controlled Trials published in English were included, in which vitamin D was prospectively added after the diagnosis of asthma to explore the role of vitamin D supplementation in asthmatics. The intervention group consisted of asthma patients who received any form or dose of vitamin D supplementation in addition to standard treatment, while those who did not receive vitamin D formed the control group. Then, the asthma-related outcomes were analyzed, including lung function (FEV1), FENO, ACT scores, and the rate of asthma exacerbations.

Exclusion Criteria

Retrospective and observational studies, articles or preprints not published in peer-reviewed journals, articles that did not mention the results included in our study or for which the data were incomplete, and retrospective vitamin D supplementation studies were excluded.

Study Selection

All studies selected from the database were filtered by title and abstract to exclude unrelated or duplicate articles. Two authors screened independently, and a third co-author was involved in resolving differences that arose during the literature screening process.

Data Extraction

Two authors independently extracted the relevant data from the article, including study population (age, country), intervention measures (vitamin D administration method and dose), follow-up time and outcomes (FEV1, FENO, asthma exacerbations and ACT scores), and baseline data related to the results (mean age, FEV1, ACT scores and vitamin D content).

Quality Assessment

The two authors independently evaluated the methodological quality of the included studies based on Cochrane's systematic review guidelines and resolved the differences through discussion with the third co-author. The risk of bias was plotted using Review Manager 5.4 and individual quality analysis was performed using the GRADE-PRO method.

Statistical Analysis

In this meta-analysis, we used risk ratio (RR) and standard error (SMD) as the impact measurement standards, R software version 4.1.1 (R project in Vienna, Austria) for statistical analysis and forest mapping. The methodological quality of the study was evaluated using Review Manager Version 5.4 following the Cochrane guidelines. A random effect model was used for statistical analysis due to differences in the mix of interventions and participants. The heterogeneity among studies was assessed by Cochran Q-test, and $P < 0.05$ was considered statistically significant. When data from three or more studies were available, results were summarized using either the standardized mean difference (SMD) for continuous variables or the risk ratio (RR) for dichotomized variables. Statistical analysis was performed using the Mann-Whitney *U* test, and a two-sided *P*-value of <0.05 was considered statistically significant. Using the I^2 statistic to evaluate the degree of heterogeneity between included studies. I^2 values of 25, 50, and 75% were considered low, medium, and high heterogeneity. In addition,

in order to explore the impact and heterogeneity of each outcome, prespecified subgroup analyses were stratified by FEV1 baseline values (less than 70% or greater), age (children or adults), and follow up time. The use of funnel plots failed to demonstrate potential publication bias since each result did not reach 10 studies. Sensitivity analyses were performed to check the robustness of the results by omitting one study and analyzing the remainder in each round.

RESULTS

Study Characteristics

In this review, we used database search and a comprehensive manual search strategy. A total of 259 studies was found in the initial search, and 49 RCTs were screened out. After manual deletion of duplicate references, the remaining 20 studies were selected by title and abstract. There were 15 eligible articles after excluding irrelevant articles. Among them, studies in which outcome indicators were variation quantity before and after intervention or the outcome indicators which had missing values were excluded. Eventually, 10 studies were included in the review and met the inclusion criteria through evaluating the full text (**Figure 1** and **Table 1**).

Description of the Included Studies

The characteristics and baseline data of included RCTs were presented in **Tables 1**, **2** In this review, all the included studies were RCTs, including the detailed information of 1,349 subjects, with the sample size ranging from 15 to 207, and the locations of the subjects involved in the United States (76), Holland (77), United Kingdom (78), Egypt (79), Poland (80), Pakistan (81), and India (73, 74, 82, 83).

Among the 10 included studies, one RCT (74) included patients with allergic bronchopulmonary aspergillosis (ABPA) complicating asthma, whereas the other nine RCTs included patients with asthma (73, 76–81) or moderate persistent asthma (82, 83). In addition, six studies in which participants were adults (74, 76–79, 81), while the other four RCTs were children (73, 80, 82, 83).

There was significant heterogeneity in the doses of vitamin D used in the intervention groups, with the control group receiving an equal dose of placebo, and both two groups receiving a standardized treatment, inhaled corticosteroid, according to the guidelines. The follow-up time ranged from 1 week (77) to 12 months (78).

Two RCTs (78, 82) analyzed ACT score, asthma exacerbations, FENO, and FEV1 as outcome measures. The other three studies all analyzed FEV1 as the outcome in addition to ACT scores (73), FENO (77), and asthma exacerbations (80), respectively. Asthma exacerbations were used as an outcome in Castro's (76), Dodamani's (74), Yadav's (83), and Musharraf's (81) studies. The rest of one RCT (79) used FEV1 to evaluate the outcome of the two groups.

Baseline FEV1 values were reported in seven studies in the two groups, six of which were greater than 70% (73, 76–78,

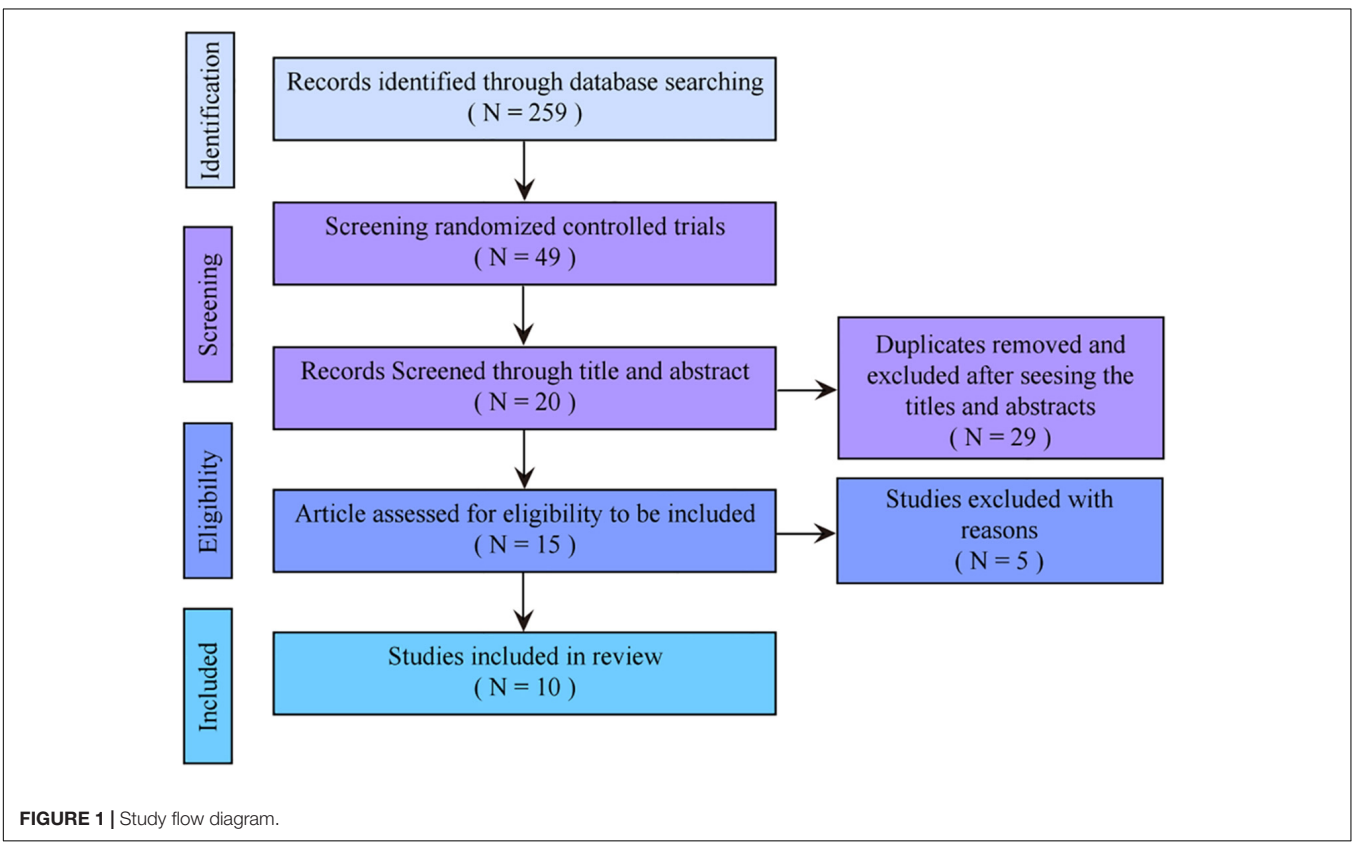

FIGURE 1 | Study flow diagram.

TABLE 1 | Summary of the included articles in this review.

References	Study design	Country	Age	Sample size (I/C)	Participants	Basic treatment	Intervention	Control/ Placebo	Follow-up time	Outcomes
Majak et al. (80)	Randomized, double-blind, parallel-group trial	Poland	5–18 years old	24/24	Patients with newly diagnosed asthma and sensitive only to house dust mites	Budesonide 800 mg/d	Vitamin D-500 IU Cholecalciferol	Placebo.	2 months 4 months	FEV1, the rate of patients with asthma exacerbations.
Castro et al. (76)	Randomized, double-blind, parallel-group study	United States	≥18 years old	201/207	Participants with asthma and a serum 25-hydroxyvitamin D level of less than 30 ng/mL	Inhaled ciclesonide (320 μg/day) and levalbuterol	Vitamin D3 100 000 IU once, then 4,000 IU/day for 28 weeks	Placebo.	3 months 5 months 7 months	The overall exacerbation rate.
Yadav et al. (83)	Randomized, double-blind, placebo-controlled trial	India	3–14 years old	50/50	Children with moderate to severe asthma as per GINA guidelines	Steroid (As one of the outcomes, the dose is not constant)	Vitamin D3 (Cholecalciferol) 60,000 IU per month	Placebo powder in the form of glucose sachet	1 month 2 months 3 months 4 months 5 months 6 months	Number of exacerbations
de Groot et al. (77)	Randomized, double-blind, placebo-controlled trial	Holland	≥18 years old	22/22	Patients with asthma	Budesonide (400–800 μg/day)	Single high dose of long-acting oral vitamin D3 preparation (400,000 IU)	Placebo	1 week 9 weeks	FEV and FENO
Martineau et al. (78)	Randomized, double-blind, placebo-controlled trial	United Kingdom	16–80 years old	125/125	Patients with asthma	Inhaled corticosteroids, long-acting β-2 agonist, oral corticosteroids (The details are unknown and the dose is not constant)	Six 2-monthly oral doses of 3 mg vitamin D3	Placebo	2 months 6 months 12 months	Severe asthma exacerbation, ACT score, FEV1, and FENO
Musharraf et al. (81)	Randomized controlled trial	Pakistan	16–46 years old	40/40	Patients were diagnosed of bronchial asthma for at least 1 year with vitamin D levels less than 30 ng/ml	Salmeterol/ fluticasone inhaler preparation Salmicort[§] 25/250 μg twice daily, Montelukast Montika[§] 10 mg at night	Vitamin D3 50,000 units fortnightly for a period of 3 months in addition to standard treatment	Standard treatment	3 months	Asthma exacerbations.

(Continued)

TABLE 1 | (Continued)

References	Study design	Country	Age	Sample size (I/C)	Participants	Basic treatment	Intervention	Control/ Placebo	Follow-up time	Outcomes
Dodamani et al. (74)	Randomized, parallel-group study	India	≥12 years old	15/15	Patients with ABPA complicating asthma	Oral prednisolone 0.5 mg/kg/day for 4 weeks. Prednisolone was then tapered by 5 mg every 2 weeks and discontinued.	Vitamin D3 60,000 IU once weekly for 8 weeks	Placebo	2 months 4 months 6 months	Number of asthma exacerbations
Shabana et al. (79)	Double blinded randomized controlled interventional study	Egypt	≥19 years old	42/37	Patients with asthma	Inhaled corticosteroids (fluticasone, budesonide, and ciclesonide), leukotriene antagonist (montelukast), long-acting beta agonists (salmeterol and formoterol), and theophylline (The details are unknown)	Single dose of 300,000 IU of vitamin D3.	Placebo	3 months	FEV1.
Jat et al. (73)	Randomized, double-blind, placebo-controlled trial	India	4–12 years old	125/125	Patients with asthma	Inhaled corticosteroids, long-acting β-2 agonist, oral corticosteroids (The details are unknown and the dose is not constant)	Vitamin D orally 1,000 IU/day for 9 months.	Placebo	9 months	CACT score, FEV1.
Thakur et al. (82)	Randomized, blinded, parallel-group, placebo-controlled trial	India	6–11 years old	30/30	Patients with moderate persistent asthma	Inhaled corticosteroids, long-acting β-2 agonist, systemic steroid, leukotriene receptor antagonist (The details are unknown)	Vitamin D orally 2,000 IU/day	Placebo	3 months	CACT score, FEV1, FeNO, and Number of patients with exacerbation

TABLE 2 | Baseline characteristics of patients in the eight studies included.

References	Age (years) Mean (SD)		FEV1% Mean (SD)		FENO Mean (SD)		ACT score Mean (SD)		25-hydroxyvitamin D Mean (SD)	
	I	C	I	C	I	C	I	C	I	C
Majak et al. (80)	10.8 (3.2)	11.1 (3.3)	94.4 (13)	98.7 (12)	NA	NA	NA	NA	NA	NA
Castro et al. (76)	39.9 (13.1)	39.5 (12.7)	91.32 (13.83)	92.09 (13.65)	NA	NA	19.33 (3.73)	19.67 (3.73)	19.8 (7.8)	18.6 (7.7)
Yadav et al. (83)	9.15 (2.444)	10.00 (1.876)	NA	NA	NA	NA	NA	NA	NA	NA
de Groot et al. (77)	59.0 (9.7)	53.6 (16.7)	99.1 (15.7)	97.6 (18.1)	26.33 (9.51)	38.33 (41.21)	NA	NA	24.9 (9.9)	22.3 (9.5)
Martineau et al. (78)	49.4 (14.8)	46.4 (13.8)	82.0 (18.7)	81.0 (20.4)	38.1 (29.1)	37 (26)	19.2 (3.9)	18.9 (3.9)	19.97 (10.10)	19.81 (9.70)
Musharraf et al. (81)	29.70 (7.74)	29.43 (8.47)	NA	NA	NA	NA	NA	NA	NA	NA
Dodamani et al. (74)	33 (12.5)	32 (12.2)	NA	NA	NA	NA	NA	NA	23.07 (29.04)	20.97 (29.2)
Shabana et al. (79)	34.00 (7.40)	35.50 (7.00)	68.38 (12.00)	67.54 (9.93)	NA	NA	NA	NA	17.56 (2.74)	18.16 (2.89)
Jat et al. (73)	8.2 (2.3)	7.8 (2.2)	92.5 (21.7)	97.0 (17.5)	NA	NA	21.7 (4.2)	21.9 (3.6)	11.6 (4.6)	10.8 (4.4)
Thakur et al. (82)	9.0 (1.7)	8.7 (1.6)	75.3 (26.5)	75.6 (15.7)	19.77 (16.11)	22.27 (24.29)	18 (2.9)	15.5 (2.7)	15.8 (8.2)	16.5 (9.9)

80, 82), whereas one of which was less than 70% (79). Three RCTs reported FENO baseline values, two of which were higher than those in the intervention group (77, 82), and the other was the opposite (78). Four RCTs counted the baseline values of ACT scores, among which the median value of three RCTs was greater than 19 points (73, 76, 78) and the other was less than 19 points (82). Baseline data for 25-hydroxyvitamin D were available for seven RCTs enrolled, with all the studies less than 30 ng/ml, and two of them more than 20 ng/ml (74, 77) and the others less than 20 ng/ml (73, 76, 78, 79, 82) (**Table 2**).

Methodological Quality of Study

According to Cochrane system evaluation guidelines, we conducted a risk bias assessment for each study included in this evaluation. A summary chart of bias risk was shown in **Figure 2**, in which red represents high deviation risk, green represents low deviation risk, and yellow represents ambiguous deviation risk. **Figure 3** showed the risk of bias graph, in which the authors expressed our judgments on various risk items of bias in each study in percentage form.

Grade summary **Table 3** gave an overall rating for the quality of evidence regarding the role of vitamin D supplementation in asthma patients. The GRADE summary demonstrated that the evidence for exacerbation of asthma (in the adult and over 6 months of follow-up subgroup) and FEV1 (in children, adults, and under 6 months of follow-up subgroup) were very low, meaning that the effect estimation was uncertain. It might be related to the significant difference in the dose and mode of vitamin D administration and the baseline data of patients across different RCTs.

Efficacy Outcomes

Asthma Control Test Scores

Asthma Control Test (ACT) scores were reported in three studies (73, 78, 82) involving 526 individuals (265 intervention and 261 placebo). The pooled data demonstrated that there was no significant difference between the placebo and vitamin D groups (SMD 0.04, 95% CI −0.13 to 0.21, low heterogeneity (I^2 = 0%, P = 0.87; **Figure 4A**).

Forced Expiratory Volume in One Second

Forced expiratory volume in one second was reported in six studies (73, 77–80, 82) involving 651 subjects (331 intervention and 320 placebo). The summary data showed that there was no significant difference between the placebo group and vitamin D group [SMD 0.04, 95% CI −0.35 to 0.43, high heterogeneity (I^2 = 78%, P < 0.01; **Figure 4D**)].

Subgroup analysis of the results for FEV1 was further performed (**Figure 5**). For the age subgroups, there was no significant difference between the placebo and vitamin D groups in adults [SMD 0.39, 95% CI −0.15 to 0.93, high heterogeneity (I^2 = 81%, P < 0.01)], while vitamin D supplementation was associated with a reduction of FEV1 at the last visit in children [SMD −0.3, 95% CI −0.54 to −0.07, low heterogeneity (I^2 = 0%, P = 0.97; **Figure 5A**)]. Regarding different FEV1 baseline values, there was no significant difference between the two groups for patients with FEV1 baseline values exceeding 70% [SMD −0.12, 95% CI −0.34 to 0.10, low heterogeneity (I^2 = 31%, P = 0.22)], while vitamin D supplementation was related to the increase of FEV1 at last visit for patients with FEV1 baseline values less than 70% (SMD 0.94, 95% CI 0.47 to 1.41, without applicable heterogeneity; **Figure 5B**). For different follow-up times, vitamin D supplementation was not associated with FEV1 when the follow-up time was less than 6 months [SMD 0.13, 95% CI −0.48 to 0.74, high heterogeneity (I^2 = 82%, P < 0.01)] or more than 6 months [SMD 0.11 95% CI −0.35 to 0.43, low heterogeneity (I^2 = 39%, P = 0.53; **Figure 5C**)].

Asthma Exacerbations

Seven studies (74, 76, 78, 80–83) reported asthma exacerbations involving 944 subjects (466 intervention and 478 placebo). The pooled data showed that vitamin D supplementation was associated with a reduced rate of asthma exacerbations (RR 0.60, 95% CI 0.41–0.88, high heterogeneity (I^2 = 64%, P < 0.01; **Figure 4B**)].

Subgroup analysis of asthma exacerbation results was complicated (**Figure 6**). In terms of different age groups, there

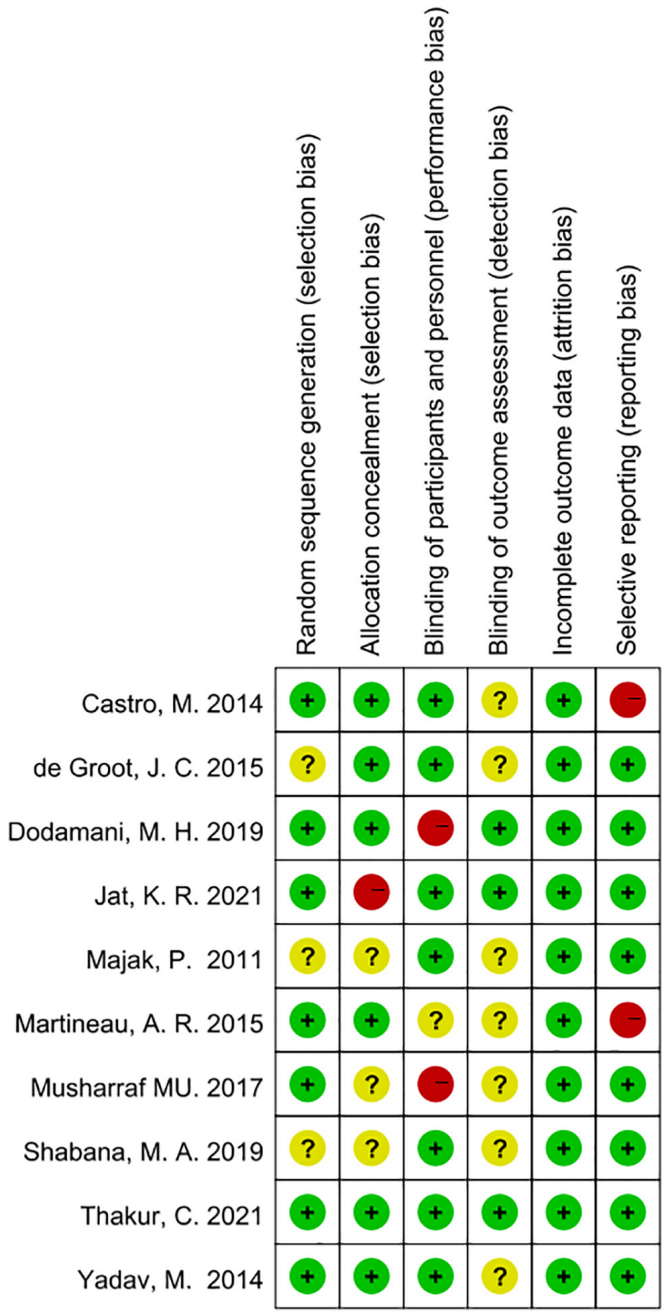

FIGURE 2 | Risk of bias summary based on Cochrane Systematic Review Guidelines for each included study included in this review (green for low risk of bias, yellow for unclear risk of bias and red for high risk of bias).

was no significant difference between the placebo and vitamin D groups in adults [RR 0.69, 95% CI 0.40 to 1.17, high heterogeneity (I^2 = 71%, P = 0.02)], while vitamin D supplementation was related to reducing the rate of asthma exacerbations in children [RR 0.46, 95% CI 0.30 to 0.70, low heterogeneity (I^2 = 0%, P = 0.83; **Figure 6A**)]. According to different follow-up time, vitamin D supplementation was related to the reduction of asthma exacerbations with less than 6 months of follow-up [RR 0.45, 95% CI 0.32 to 0.63, low heterogeneity (I^2 = 0%, P = 0.95)], but not with more than 6 months of follow-up [RR 0.87, 95% CI 0.50 to 1.50, high heterogeneity (I^2 = 77%, P = 0.04; **Figure 6B**)].

Fractional Exhaled Nitric Oxide

Fractional exhaled nitric oxide was reported in three studies (77, 78, 82) involving 350 subjects (175 intervention and 175 placebo). The pooled data indicated that there was no significant difference

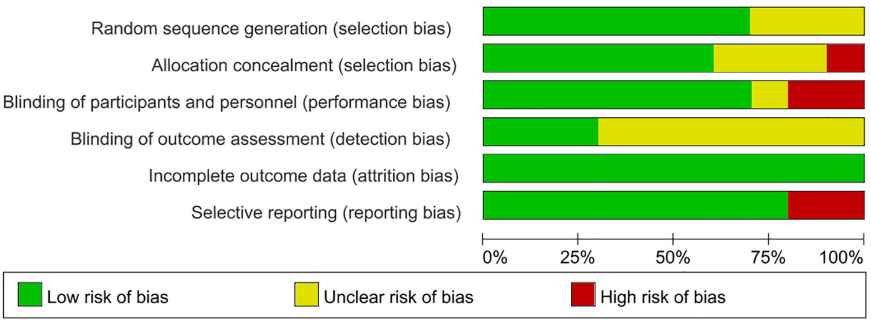

FIGURE 3 | Risk of bias graph review authors judgments about each risk of bias item presented as percentages across various study designs.

between the placebo and vitamin D groups [SMD −0.01, 95% CI −0.22 to 0.2, low heterogeneity (I^2 = 23%, P = 0.27; **Figure 4C**)].

Sensitivity Analysis

Sensitivity analysis of the outcomes using R language software (4.1.1) indicated that, after omitting each individual study, our results were consistent with the complete analysis of all endpoints, and that there was no significant correlation between vitamin D supplementation and the prognosis of patients with asthma (**Figure 7**).

DISCUSSION

In this systematic meta-analysis, vitamin D supplementation in asthmatics did not improve major health outcomes including ACT scores, FEV1, FENO, and asthma exacerbations, but contributed to increased FEV1 in subgroups with less than 70% of FEV1 baseline. In addition, enrolled RCTs showed significant baseline heterogeneity in both vitamin D dose and demographic characteristics.

There are still no objective markers to assess asthma severity. Although asthma patients have a certain degree of the inflammatory response, some severe patients may also develop exacerbation and deterioration of asthma after inflammation is controlled (84). The Primary Care Asthma Control Screening tool (adult) (85) or the Asthma Control Test [adults (86) or children (87)] can be used to quickly assess control of asthma symptoms with questionnaires. Clinical efficacy results indicated a cutoff point of 19 or lower for C-ACT or ACT (86, 87), indicating incomplete asthma control. Over the years, the definition of acute asthma or exacerbation has varied. Currently, severe exacerbation is defined as requiring corticosteroid use for at least 3 days or as an inpatient or emergency room visit due to the need for corticosteroids for asthma. Moderate deterioration was defined as an event that required modification of treatment to prevent it from becoming severe and not so severe as to require oral corticosteroids (OCS) by the American Thoracic Society/European Respiratory Society (88). The transcriptomic profile of bronchoscopy has identified high and low type 2 immunity asthma and other molecular phenotypes (89, 90). Adaptive T helper 2 cell activation produces a series of cytokines following allergen sensitization and stimulation of dendritic cells. Eosinophils are recruited to the lung mucosa by chemokine receptors and other eosinophil chemo-attractants (3). In non-eosinophilic asthma, innate lymphoid cells, macrophages, and neutrophils have an important role in stimulating the release of cytokines (interleukin-33 and interleukin-25) or chemokines (C–X–C motif chemokine ligand 8), to regulate the immune response (91, 92).

With the development of economy and medical level, vitamin D, a proline obtained from skin exposure to ultraviolet B (UVB) light and dietary intake from the liver, fish, egg yolk, and other sources, is transformed to 25-hydroxyvitamin D [25(OH)D] in liver (93, 94), which has gradually attracted the attention of the majority of domestic and foreign research scholars. Several studies have demonstrated a correlation between vitamin D deficiency and asthma prevalence and severity. Patients with vitamin D deficiency have a higher prevalence of asthma, which could be a strong prediction factor of asthma (95–97). Additionally, vitamin D deficiency was also associated with severe asthma exacerbations in multiple prospective and retrospective (98–100). Compared with children with insufficient or sufficient vitamin D, there was a correlation between vitamin D deficiency and pulmonary dysfunction in asthmatic children treated with inhaled corticosteroids (101). Although as a nutrient that regulates metabolism, vitamin D has been shown to immunomodulate various immune cells and structural cells in the airway, by activating vitamin D receptors (VDR) (102–105). Several *in vitro* and *in vivo* studies using asthma murine models have also shown that vitamin D modulated the inflammatory response. In vitamin D-treated asthmatic mice, the Penh values, type 2 cytokines, perivascular and peribronchial inflammation, goblet cell proliferation, total IgE and histamine, and mucus hypersecretion were all significantly reduced (106). Vitamin D deficiency also potentiated oxidative stress and corticosteroid resistance in severe asthma exacerbations. Vitamin D3 supplementation significantly increased the change of FEV1, and effectively alleviated ROS and DNA damage, which were related to a decrease in TNF-α and NF-κB in epithelial cells (107). Oxidative stress-activated transcription factors (TF) and signaling pathways, and partly activated the innate immune response through toll-like receptors 2 (TLR-2) and toll-like receptors 4 (TLR-4), thus promoting the release

TABLE 3 | The overall rating for the quality of evidence profile for asthma related health outcomes based on the grading of Recommendations Assessment, Development, and Evaluation (GRADE) Working group methodology.

Certainty assessment							No. of patients		Effect		Certainty	importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Vitamin D	Placebo	Relative (95% CI)	Absolute (95% CI)		
Asthma exacerbations												
7	Randomized trails	Serious[a]	Serious[b]	Serious[c]	Not serious	None	109/466 (23.4%)	163/478 (34.1%)	RR 0.59 (0.39 to 0.89)	140 fewer per 1,000 (from 208 fewer to 38 more)	Very low	Critical
Asthma exacerbations children												
3	Randomized trails	Not serious	Not serious	Serious[c]	Serious[d]	None	22/102 (21.6%)	48/102 (47.1%)	RR 0.46 (0.30–0.70)	254 fewer per 1,000 (from 329 fewer to 141 fewer)	Low	Critical
Asthma exacerbations adults												
4	Randomized trails	Serious[a]	Serious[b]	Serious[c]	Not serious	None	87/364 (23.9)	115/376 (30.6%)	RR 0.68 (0.40–1.18)	98 fewer per 1,000 (from 184 fewer to 55 more)	Very low	Critical
Asthma exacerbations follow up time <6 months												
5	Randomized trails	Serious[a]	Not serious	Serious[c]	Not serious	None	31/157 (19.7%)	71/157 (45.2%)	RR 0.45 (0.32–0.63)	249 fewer per 1,000 (from 308 fewer to 167 fewer)	Low	Critical
Asthma exacerbations follow up time >6 months												
2	Randomized trails	Serious[a]	Serious[b]	Serious[c]	Not serious	None	78/309 (25.2%)	92/321 (28.7%)	RR 0.87 (0.49–1.52)	37 fewer per 1,000 (from 146 fewer to 149 more)	Very low	Critical
ACT score												
3	Randomized trails	Serious[a]	Not serious	Serious[c]	Not serious	None	261	261	–	SMD 0.04 higher (0.13 lower to 0.21 higher)	Low	Critical
FENO												
3	Randomized trails	Serious[a]	Not serious	Serious[c]	Not serious	None	175	175	–	SMD 0.63 higher (4.77 lower to 6.03 higher)	Low	Critical
FEV1												
6	Randomized trails	Serious[a]	Serious[b]	Serious[c]	Not serious	None	331	320	–	SMD 0.04 higher (0.13 lower to 0.21 higher)	Low	Critical

(Continued)

TABLE 3 | (Continued)

Certainty assessment							No. of patients		Effect		Certainty	importance
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Vitamin D**	**Placebo**	**Relative (95% CI)**	**Absolute (95% CI)**		
FEV1 children												
3	Randomized trails	Serious[a]	Not serious	Serious[c]	Serious[d]	None	142	136	–	SMD 0.29 lower (0.52 lower to 0.05 lower)	Very low	Critical
FEV1 adults												
3	Randomized trails	Serious[a]	Serious[b]	Serious[c]	Not serious	None	189	184	–	SMD 0.39 higher (0.17 lower to 0.95 higher)	Very low	Critical
FEV1 follow up time <6 months												
4	Randomized trails	Not serious	Serious[b]	Serious[c]	Serious[d]	None	116	111	–	SMD 0.13 higher (0.51 lower to 0.77 higher)	Very low	Critical
FEV1 follow up time >6 months												
2	Randomized trails	Serious[a]	Not serious	Serious[c]	Not serious	None	215	209	–	SMD 0.12 higher (0.07 lower to 0.31 higher)	Low	Critical
FEV1 baseline <70%												
1	Randomized trails	Not serious	Not serious	Serious[c]	Not serious	None	42	37	–	SMD 0.94 higher (0.47 higher to 1.41 higher)	Moderate	Critical
FEV1 baseline =V0%												
5	Randomized trails	Serious[a]	Not serious	Serious[c]	Not serious	None	289	283	–	SMD 0.12 lower (0.33 lower to 0.10 higher)	Low	Critical

[a]*Some concern with method of randomization used, allocation concealment, binding of participants, binding of outcome assessment or selective reporting.*
[b]*Inconsistency was reported by moderate to high heterogeneity.*
[c]*There were differences in the follow up time points to measure the outcomes and vitamin D dosages and duration.*
[d]*The total sample size was less than 300.*

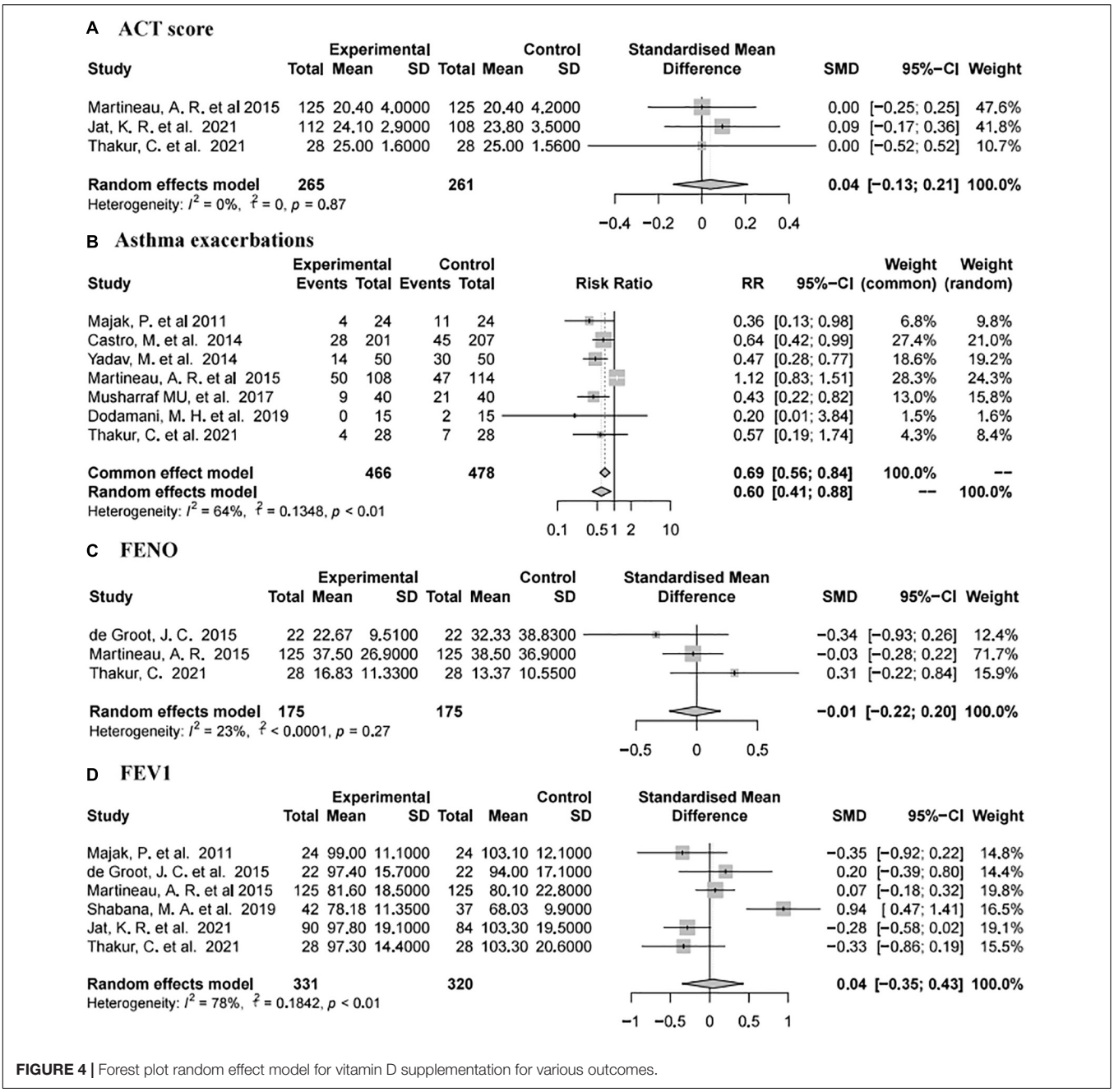

FIGURE 4 | Forest plot random effect model for vitamin D supplementation for various outcomes.

of cytokines and chemokines. In addition, oxidative stress had an important role in affecting corticosteroid insensitivity by inhibiting the activity and expression of HDAC-2 via serine hyperphosphorylation (108). Although there has been sufficient evidence that vitamin D deficiency was associated with progression and exacerbation of asthma, there are many inconsistencies in multiple prospective clinical studies. The researches indicated that vitamin D supplementation was not of use in preventing severe asthma exacerbations or control of asthma in children (73, 82, 109) or adults (76, 78). High-dose vitamin D supplementation during pregnancy did not reduce the risk and improve the allergy outcomes of asthma in children (under 6 years of age) compared to standard doses (110).

Additionally, we further confirmed that it could effectively alleviate the probability of asthma exacerbations in children and when follow-up time was less than 6 months (**Figure 6**). Noticeably, it significantly enhanced FEV1 in patients whose FEV1 baseline value was less than 70%, though there was only one study included in the subgroup. Only one former meta-analysis (111) demonstrated that vitamin D supplementation couldn't reduce asthma exacerbations and FeNO, nor could it improve lung function and asthma symptoms. Our meta-analysis offers several advantages over previous meta-analyses.

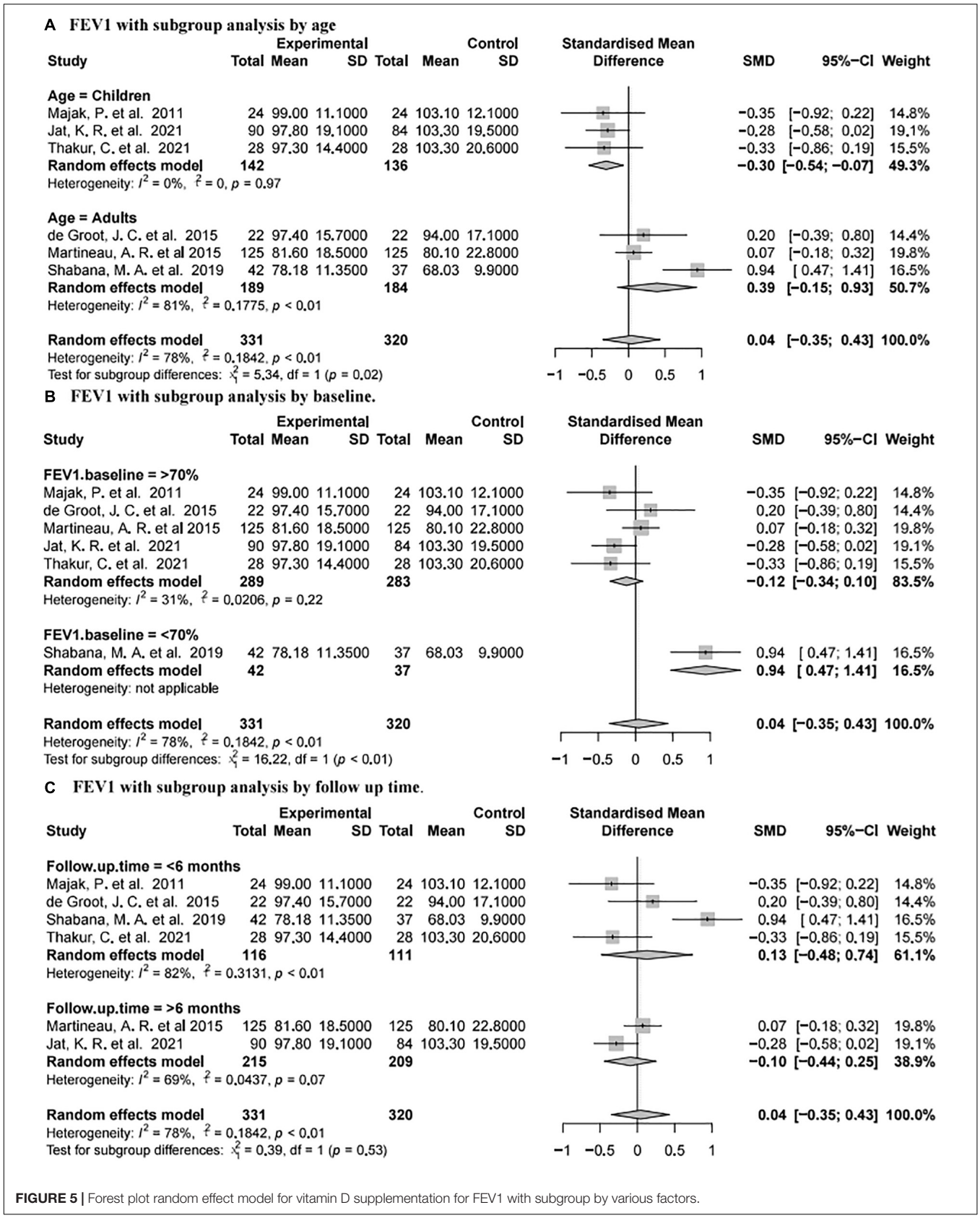

FIGURE 5 | Forest plot random effect model for vitamin D supplementation for FEV1 with subgroup by various factors.

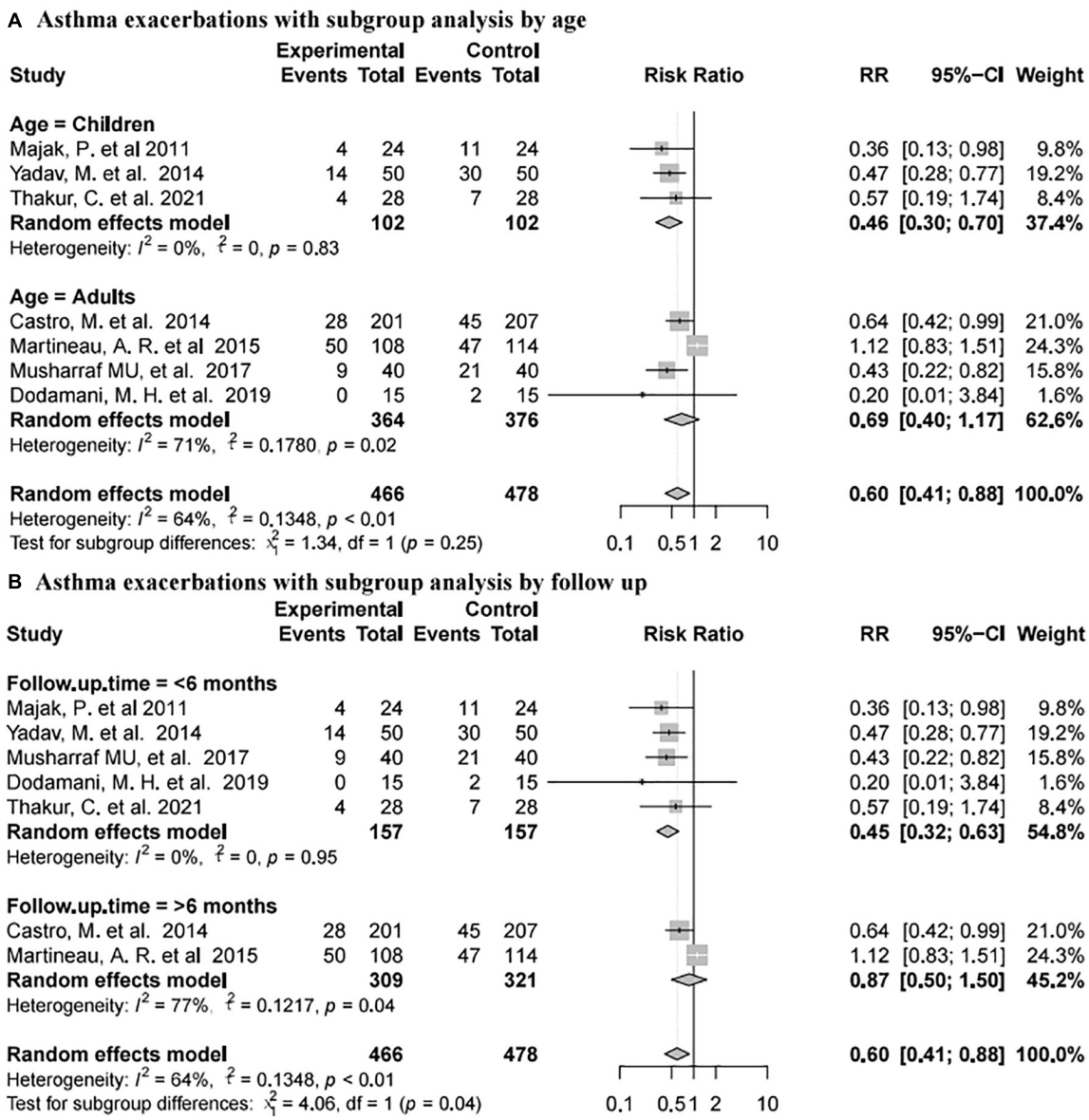

FIGURE 6 | Forest plot random effect model for vitamin D supplementation for asthma exacerbations with subgroup by various factors.

First, all included studies were RCTs, and the studies with incomplete data were strictly excluded according to the standard. Second, subgroup analyses of included studies were performed to minimize heterogeneity for baseline values including age, FEV1 values, and follow-up time in our analysis (**Table 2**). Finally, the sensitivity analysis was similar to the above results, indicating that the results of this meta-analysis were reliable (**Figure 7**). However, there are still many defects in our meta-analysis. First, heterogeneity in dose and mode of administration of vitamin D in enrolled studies was unavoidable, and not all subjects enrolled in various studies received consistent basic anti-asthma therapy. Some studies standardized the therapeutic dose of glucocorticoids for asthma (74, 76, 77, 80, 81), some observed it as an outcome variable (73, 78, 82, 83). And most studies didn't mention whether the hormone dose was changed during the follow up (73, 78, 79, 82), so we are not sure whether this will affect the accuracy of the results of RCTs. Second, the sample size of several studies included in this analysis was too small to demonstrate the reliability of the results. Finally, not all subjects enrolled in the study were asthmatics of the same severity or etiology.

In conclusion, our meta-analysis demonstrated that there was high heterogeneity in RCTs regarding improvement in exacerbation of asthma and FEV1 with vitamin D supplementation. Vitamin D supplementation led to a reduction

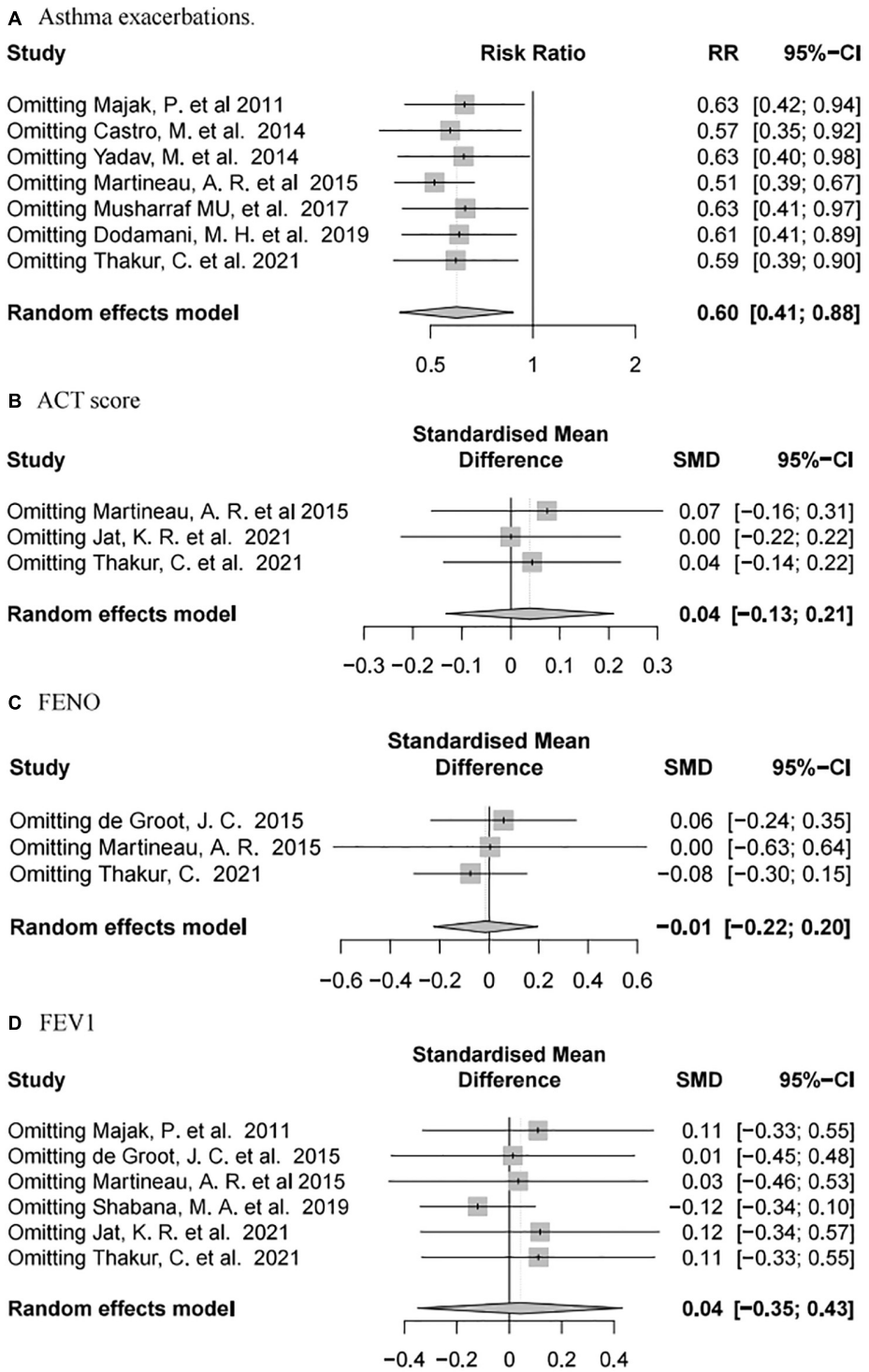

FIGURE 7 | Forest plot random effect model of sensitivity analysis for vitamin D supplementation for various outcomes.

of asthma exacerbations, especially in children and with a follow-up period of less than 6 months. In addition, it played an important role in improving FEV1 in patients with FEV1 baseline values below 70%. Though evaluating the ACT scores and FENO, we found that vitamin D worked the same way as a placebo. Based on the results of the GRADE analysis, all major findings were low or very low except for the FEV1 subgroup with baseline values below 70%. Therefore, a larger and

well-designed RCT is needed to evaluate the effect of vitamin D in the treatment of asthma, including uniform vitamin D dosing and administration mode, follow-up time, and strict inclusion and exclusion criteria. Furthermore, whether basic asthma treatment should be standardized during follow-up or used as an outcome measure of asthma treatment efficacy still needs to be further explored.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ML: data selection, data extraction, quality assessm statistical analysis, and writing – original draft. JW: data selection, data extraction, and quality assessment. XS: conceptualization, writing – review, and supervision.

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REFERENCES

1. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the global burden of disease study 2010. *Lancet.* (2012) 15:2163–96. doi: 10.1016/S0140-6736(12)61729-2
2. National Asthma Education and Prevention Program. Expert panel report 3 (EPR-3): guidelines for the diagnosis and management of asthma–summary report 2007. *J Allergy Clin Immunol.* (2007) 120:S94–138.
3. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet.* (2018) 391:783–800.
4. Stern J, Pier J, Litonjua AA. Asthma epidemiology and risk factors. *Semin Immunopathol.* (2020) 42:5–15.
5. Moorman JE, Akinbami LJ, Bailey CM, Zahran HS, King ME, Johnson CA, et al. National surveillance of asthma: United States, 2001-2010. *Vital Health Stat 3.* (2012) 35:1–58.
6. Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. *NCHS Data Brief.* (2012) 94:1–8.
7. Nakamura Y, Tamaoki J, Nagase H, Yamaguchi M, Horiguchi T, Hozawa S, et al. Japanese guidelines for adult asthma 2020. *Allergol Int.* (2020) 69:519–48. doi: 10.1016/j.alit.2020.08.001
8. Singh SK, Gupta J, Sharma H, Pedgaonkar SP, Gupta N. Socio-economic correlates and spatial heterogeneity in the prevalence of asthma among young women in India. *BMC Pulm Med.* (2020) 20:190. doi: 10.1186/s12890-020-1124-z
9. Huang K, Yang T, Xu J, Yang L, Zhao J, Zhang X, et al. Prevalence, risk factors, and management of asthma in China: a national cross-sectional study. *Lancet.* (2019) 394:407–18. doi: 10.1016/S0140-6736(19)31147-X
10. Soriano JB, Abajobir AA, Abate KH, Abera SF, Agrawal A, Ahmed MB, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the global burden of disease study 2015. *Lancet Respir Med.* (2017) 5:691–706. doi: 10.1016/S2213-2600(17)30293-X
11. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald JM, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J.* (2008) 31:143–78.
12. Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. *Front Pediatr.* (2019) 7:246. doi: 10.3389/fped.2019.00246
13. Guarnieri MBJ. Outdoor air pollution and asthma. *Lancet.* (2014) 3:1581–92.
14. D'Amato G, Chong-Neto HJ, Monge Ortega OP, Vitale C, Ansotegui I, Rosario N, et al. The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. *Allergy.* (2020) 75:2219–28. doi: 10.1111/all.14476
15. Holt PG, Sly PD. Environmental microbial exposure and protection against asthma. *N Engl J Med.* (2015) 373:2576–8. doi: 10.1056/NEJMcibr1511291
16. D Amato M, Cecchi L, Annesi-Maesano I, D Amato G. News on climate change, air pollution, and allergic triggers of asthma. *J Investig Allergol Clin Immunol.* (2018) 28:91–7. doi: 10.18176/jiaci.0228
17. Alwarith J, Kahleova H, Crosby L, Brooks A, Brandon L, Levin SM, et al. The role of nutrition in asthma prevention and treatment. *Nutr Rev.* (2020) 78:928–38.
18. Kumar S, Roy RD, Sethi GR, Saigal SR. Mycoplasma pneumoniae infection and asthma in children. *Trop Doct.* (2019) 49:117–9.
19. Bonnelykke K, Ober C. Leveraging gene-environment interactions and endotypes for asthma gene discovery. *J Allergy Clin Immunol.* (2016) 137:667–79. doi: 10.1016/j.jaci.2016.01.006
20. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr.* (2002) 76:560–8. doi: 10.1093/ajcn/76.3.560
21. Seyedrezazadeh E, Pour Moghaddam M, Ansarin K, Reza Vafa M, Sharma S, Kolahdooz F. Fruit and vegetable intake and risk of wheezing and asthma: a systematic review and meta-analysis. *Nutr Rev.* (2014) 72:411–28. doi: 10.1111/nure.12121
22. Hosseini B, Berthon BS, Wark P, Wood LG. Effects of fruit and vegetable consumption on risk of asthma, wheezing and immune responses: a systematic review and meta-analysis. *Nutrients.* (2017) 9:341. doi: 10.3390/nu9040341
23. Shaheen SO, Sterne JA, Thompson RL, Songhurst CE, Margetts BM, Burney PG. Dietary antioxidants and asthma in adults: population-based case-control study. *Am J Respir Crit Care Med.* (2001) 164:1823–8. doi: 10.1164/ajrccm.164.10.2104061
24. Wood LG, Garg ML, Powell H, Gibson PG. Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: proof of concept. *Free Radic Res.* (2008) 42:94–102. doi: 10.1080/10715760701767307
25. Baines KJ, Wood LG, Gibson PG. The nutrigenomics of asthma: molecular mechanisms of airway neutrophilia following dietary antioxidant withdrawal. *Omics.* (2009) 13:355–65. doi: 10.1089/omi.2009.0042
26. Han YY, Forno E, Brehm JM, Acosta-Pérez E, Alvarez M, Colón-Semidey A, et al. Diet, interleukin-17, and childhood asthma in Puerto Ricans. *Ann Allergy Asthma Immunol.* (2015) 115:288–293.e1. doi: 10.1016/j.anai.2015.07.020
27. Woods RK, Walters EH, Raven JM, Wolfe R, Ireland PD, Thien FC, et al. Food and nutrient intakes and asthma risk in young adults. *Am J Clin Nutr.* (2003) 78:414–21. doi: 10.1093/ajcn/78.3.414
28. Haas F, Bishop MC, Salazar-Schicchi J, Axen KV, Lieberman D, Axen K. Effect of milk ingestion on pulmonary function in healthy and asthmatic subjects. *J Asthma.* (1991) 28:349–55. doi: 10.3109/02770909109089462
29. Woods RK, Weiner JM, Abramson M, Thien F, Walters EH. Do dairy products induce bronchoconstriction in adults with asthma? *J Allergy Clin Immunol.* (1998) 101:45–50. doi: 10.1016/S0091-6749(98)70192-7

30. Nguyen MT. Effect of cow milk on pulmonary function in atopic asthmatic patients. *Ann Allergy Asthma Immunol.* (1997) 79:62–4. doi: 10.1016/S1081-1206(10)63086-4
31. Rice JL, Romero KM, Galvez Davila RM, Meza CT, Bilderback A, Williams DL, et al. Association between adherence to the mediterranean diet and asthma in Peruvian children. *Lung.* (2015) 193:893–9. doi: 10.1007/s00408-015-9792-9
32. Guglani L, Joseph CL. Asthma and diet: could food be thy medicine? *Indian Pediatr.* (2015) 52:21–2.
33. Lv N, Xiao L, Ma J. Dietary pattern and asthma: a systematic review and meta-analysis. *J Asthma Allergy.* (2014) 7:105–21. doi: 10.2147/JAA.S49960
34. Chang SW, Lee HC. Vitamin D and health – the missing vitamin in humans. *Pediatr Neonatol.* (2019) 60:237–44. doi: 10.1016/j.pedneo.2019.04.007
35. de La Puente-Yague M, Cuadrado-Cenzual MA, Ciudad-Cabanas MJ, Hernandez-Cabria M, Collado-Yurrita L. Vitamin D: and its role in breast cancer. *Kaohsiung J Med Sci.* (2018) 34:423–7. doi: 10.1016/j.kjms.2018.03.004
36. Bendix M, Dige A, Jorgensen SP, Dahlerup JF, Bibby BM, Deleuran B, et al. Seven weeks of high-dose vitamin D treatment reduces the need for infliximab dose-escalation and decreases inflammatory markers in Crohn's disease during one-year follow-up. *Nutrients.* (2021) 13:1083. doi: 10.3390/nu13041083
37. Qadir S, Memon S, Chohan MN, Memon Y. Frequency of vitamin-D deficiency in children with urinary tract infection: a descriptive cross-sectional study. *Pak J Med Sci.* (2021) 37:1058–62. doi: 10.12669/pjms.37.4.3896
38. Martineau AR, Jolliffe DA, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, et al. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. *Health Technol Assess.* (2019) 23:1–44.
39. Liu J, Dong YQ, Yin J, Yao J, Shen J, Sheng GJ, et al. Meta-analysis of vitamin D and lung function in patients with asthma. *Respir Res.* (2019) 20:161. doi: 10.1186/s12931-019-1072-4
40. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. *PLoS One.* (2010) 5:e11088. doi: 10.1371/journal.pone.0011088
41. Ingham TR, Jones B, Camargo CA Jr., Kirman J, The Whiti Te Ra Study Group. Association of vitamin D deficiency with severity of acute respiratory infection: a case-control study in New Zealand children. *Eur Respir J.* (2014) 44:439. doi: 10.1186/s13054-016-1208-6
42. Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. *J Clin Virol.* (2011) 50:194–200. doi: 10.1016/j.jcv.2010.12.006
43. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ.* (2017) 356:i6583. doi: 10.1136/bmj.i6583
44. Cui C, Xu P, Li G, Qiao Y, Han W, Geng C, et al. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and angiotensin II-exposed microglial cells: role of renin-angiotensin system. *Redox Biol.* (2019) 26:101295. doi: 10.1016/j.redox.2019.101295
45. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients.* (2020) 12:988.
46. Mendy A, Apewokin S, Wells AA, Morrow AL. Factors associated with hospitalization and disease severity in a racially and ethnically diverse population of COVID-19 patients. *medRxiv.* (2020) [Preprint]. doi: 10.1101/2020.06.25.20137323
47. Jartti T, Gern JE. Role of viral infections in the development and exacerbation of asthma in children. *J Allergy Clin Immunol.* (2017) 140:895–906. doi: 10.1016/j.jaci.2017.08.003
48. Turkeli A, Ayaz O, Uncu A, Ozhan B, Bas VN, Tufan AK, et al. Effects of vitamin D levels on asthma control and severity in pre-school children. *Eur Rev Med Pharmacol Sci.* (2016) 20:26–36.
49. Puranik S, Forno E, Bush A, Celedón JC. Predicting severe asthma exacerbations in children. *Am J Respir Crit Care Med.* (2017) 195:854–9.
50. Maes K, Serré J, Mathyssen C, Janssens W, Gayan-Ramirez G. Targeting vitamin D deficiency to limit exacerbations in respiratory diseases: utopia or strategy with potential? *Calcif Tissue Int.* (2020) 106:76–87. doi: 10.1007/s00223-019-00591-4
51. Gibbs K, Collaco JM, McGrath-Morrow SA. Impact of tobacco smoke and nicotine exposure on lung development. *Chest.* (2016) 149:552–61. doi: 10.1378/chest.15-1858
52. Chinellato I, Piazza M, Sandri M, Paiola G, Tezza G, Boner AL. Correlation between vitamin D serum levels and passive smoking exposure in children with asthma. *Allergy Asthma Proc.* (2018) 39:8–14. doi: 10.2500/aap.2018.39.4124
53. Banihosseini SZ, Baheiraei A, Shirzad N, Heshmat R, Mohsenifar A. The effect of cigarette smoke exposure on vitamin D level and biochemical parameters of mothers and neonates. *J Diabetes Metab Disord.* (2013) 12:19. doi: 10.1186/2251-6581-12-19
54. Brot C, Jorgensen NR, Sorensen OH. The influence of smoking on vitamin D status and calcium metabolism. *Eur J Clin Nutr.* (1999) 53:920–6. doi: 10.1038/sj.ejcn.1600870
55. Landin-Wilhelmsen K, Wilhelmsen L, Lappas G, Rosén T, Lindstedt G, Lundberg PA, et al. Serum intact parathyroid hormone in a random population sample of men and women: relationship to anthropometry, life-style factors, blood pressure, and vitamin D. *Calcif Tissue Int.* (1995) 56:104–8. doi: 10.1007/BF00296339
56. Brumpton BM, Langhammer A, Henriksen AH, Camargo CA Jr., Chen Y, Romundstad PR, et al. Vitamin D and lung function decline in adults with asthma: the HUNT study. *Am J Epidemiol.* (2016) 183:739–46. doi: 10.1093/aje/kwv243
57. Alsharairi NA. The effects of dietary supplements on asthma and lung cancer risk in smokers and non-smokers: a review of the literature. *Nutrients.* (2019) 11:725. doi: 10.3390/nu11040725
58. Yawn J, Lawrence LA, Carroll WW, Mulligan JK. Vitamin D for the treatment of respiratory diseases: is it the end or just the beginning? *J Steroid Biochem Mol Biol.* (2015) 148:326–37. doi: 10.1016/j.jsbmb.2015.01.017
59. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy.* (2012) 67:10–7. doi: 10.1111/j.1398-9995.2011.02711.x
60. Casaro M, Souza VR, Oliveira FA, Ferreira CM. OVA-induced allergic airway inflammation mouse model. *Methods Mol Biol.* (2019) 1916:297–301.
61. Ma JG, Wu GJ, Xiao HL, Xiao YM, Zha L. Vitamin D has an effect on airway inflammation and Th17/Treg balance in asthmatic mice. *Kaohsiung J Med Sci.* (2021) 37:1113–21. doi: 10.1002/kjm2.12441
62. Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. *Cell.* (2017) 169:985–99.
63. Cohen ED, Ihida-Stansbury K, Lu MM, Panettieri RA, Jones PL, Morrisey EE. Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. *J Clin Invest.* (2009) 119:2538–49. doi: 10.1172/JCI38079
64. Huo Y, Guan L, Xu J, Zhou L, Chen R. Tiotropium inhibits methacholine-induced extracellular matrix production via β-catenin signaling in human airway smooth muscle cells. *Int J Chron Obstruct Pulmon Dis.* (2018) 13:1469–81. doi: 10.2147/COPD.S158552
65. Hussain M, Xu C, Lu M, Wu X, Tang L, Wu X. Wnt/β-catenin signaling links embryonic lung development and asthmatic airway remodeling. *Biochim Biophys Acta Mol Basis Dis.* (2017) 1863:3226–42. doi: 10.1016/j.bbadis.2017.08.031
66. Huang Y, Wang L, Jia XX, Lin XX, Zhang WX. Vitamin D alleviates airway remodeling in asthma by down-regulating the activity of Wnt/β-catenin signaling pathway. *Int Immunopharmacol.* (2019) 68:88–94. doi: 10.1016/j.intimp.2018.12.061

67. Hall SC, Agrawal DK. Vitamin D and bronchial asthma: an overview of data from the past 5 years. *Clin Ther.* (2017) 39:917–29. doi: 10.1016/j.clinthera.2017.04.002
68. Jolliffe DA, Greenberg L, Hooper RL, Griffiths CJ, Camargo CA, Kerley CP, et al. Vitamin D supplementation to prevent asthma exacerbations: a systematic review and meta-analysis of individual participant data. *Lancet Respir Med.* (2017) 5:881–90. doi: 10.1016/S2213-2600(17)30306-5
69. Wang M, Liu M, Wang C, Xiao Y, An T, Zou M, et al. Association between vitamin D status and asthma control: a meta-analysis of randomized trials. *Respir Med.* (2019) 150:85–94. doi: 10.1016/j.rmed.2019.02.016
70. Riverin BD, Maguire JL, Li P. Vitamin D supplementation for childhood asthma: a systematic review and meta-analysis. *PLoS One.* (2015) 10:e0136841. doi: 10.1371/journal.pone.0136841
71. Jaura JKG, Safranek S. Does vitamin D supplementation reduce asthma exacerbations? *J Fam Pract.* (2020) 69:E4–6.
72. Chen Z, Peng C, Mei J, Zhu L, Kong H. Vitamin D can safely reduce asthma exacerbations among corticosteroid-using children and adults with asthma: a systematic review and meta-analysis of randomized controlled trials. *Nutr Res.* (2021) 92:49–61. doi: 10.1016/j.nutres.2021.05.010
73. Jat KR, Goel N, Gupta N, Gupta CP, Datta S, Lodha R, et al. Efficacy of vitamin D supplementation in asthmatic children with vitamin D deficiency: a randomized controlled trial (ESDAC trial). *Pediatr Allergy Immunol.* (2021) 32:479–88. doi: 10.1111/pai.13415
74. Dodamani MH, Muthu V, Thakur R, Pal A, Sehgal IS, Dhooria S, et al. A randomised trial of vitamin D in acute-stage allergic bronchopulmonary aspergillosis complicating asthma. *Mycoses.* (2019) 62:320–7. doi: 10.1111/myc.12879
75. Moher D, Liberati A, Tetzlaff J, Altman DG, Prisma Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
76. Castro M, King TS, Kunselman SJ, Cabana MD, Denlinger L, Holguin F, et al. Effect of vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *JAMA.* (2014) 311:2083–91. doi: 10.1001/jama.2014.5052
77. de Groot JC, van Roon EN, Storm H, Veeger NJ, Zwinderman AH, Hiemstra PS, et al. Vitamin D reduces eosinophilic airway inflammation in nonatopic asthma. *J Allergy Clin Immunol.* (2015) 135:670–5.e3.
78. Martineau AR, MacLaughlin BD, Hooper RL, Barnes NC, Jolliffe DA, Greiller CL, et al. Double-blind randomised placebo-controlled trial of bolus-dose vitamin D3 supplementation in adults with asthma (ViDiAs). *Thorax.* (2015) 70:451–7. doi: 10.1136/thoraxjnl-2014-206449
79. Shabana MA, Esawy MM, Ismail NA, Said AM. Predictive role of IL-17A/IL-10 ratio in persistent asthmatic patients on vitamin D supplement. *Immunobiology.* (2019) 224:721–7. doi: 10.1016/j.imbio.2019.09.005
80. Majak P, Olszowiec-Chlebna M, Smejda K, Stelmach I. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. *J Allergy Clin Immunol.* (2011) 127:1294–6. doi: 10.1016/j.jaci.2010.12.016
81. Musharraf MU, Sandhu GA, Mumtaz MU, Rashid MF. Role of vitamin d in prevention of acute exacerbation of bronchial asthma in aduLTS. *J Postgrad Med Inst.* (2017) 31:310–3. doi: 10.1002/rmv.1909
82. Thakur C, Kumar J, Kumar P, Goyal JP, Singh K, Gupta A. Vitamin-D supplementation as an adjunct to standard treatment of asthma in children: a randomized controlled trial (ViDASTA Trial). *Pediatr Pulmonol.* (2021) 56:1427–33. doi: 10.1002/ppul.25287
83. Yadav M, Mittal K. Effect of vitamin D supplementation on moderate to severe bronchial asthma. *Indian J Pediatr.* (2014) 81:650–4.
84. Wilson SJ, Ward JA, Sousa AR, Corfield J, Bansal AT, De Meulder B, et al. Severe asthma exists despite suppressed tissue inflammation: findings of the U-BIOPRED study. *Eur Respir J.* (2016) 48:1307–19. doi: 10.1183/13993003.01129-2016
85. LeMay KS, Armour CL, Reddel HK. Performance of a brief asthma control screening tool in community pharmacy: a cross-sectional and prospective longitudinal analysis. *Prim Care Respir J.* (2014) 23:79–84. doi: 10.4104/pcrj.2014.00011
86. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma control test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *J Allergy Clin Immunol.* (2006) 117:549–56. doi: 10.1016/j.jaci.2006.01.011
87. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the childhood asthma control test. *J Allergy Clin Immunol.* (2007) 119:817–25. doi: 10.1016/j.jaci.2006.12.662
88. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American thoracic society/European respiratory society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med.* (2009) 180:59–99. doi: 10.1164/rccm.200801-060ST
89. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med.* (2009) 180:388–95. doi: 10.1164/rccm.200903-0392OC
90. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. A transcriptome-driven analysis of epithelial brushings and bronchial biopsies to define asthma phenotypes in U-BIOPRED. *Am J Respir Crit Care Med.* (2017) 195:443–55. doi: 10.1164/rccm.201512-2452OC
91. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest.* (2010) 138:1140–7. doi: 10.1378/chest.09-3058
92. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol.* (2015) 16:45–56.
93. Konstantinopoulou S, Tapia IE. Vitamin D and the lung. *Paediatr Respir Rev.* (2017) 24:39–43.
94. Kerley CP, Elnazir B, Faul J, Cormican L. Vitamin D as an adjunctive therapy in asthma. Part 1: a review of potential mechanisms. *Pulm Pharmacol Ther.* (2015) 32:60–74. doi: 10.1016/j.pupt.2015.02.004
95. Walsh LJ, Wong CA, Oborne J, Cooper S, Lewis SA, Pringle M, et al. Adverse effects of oral corticosteroids in relation to dose in patients with lung disease. *Thorax.* (2001) 56:279–84. doi: 10.1136/thorax.56.4.279
96. Kaufman K, Sorensen R. An explorative study of low levels of allergen-specific IgE and clinical allergy symptoms during early childhood. *Pediatrics.* (2012) 130:S5–6. doi: 10.1111/j.1398-9995.2011.02578.x
97. Checkley W, Robinson CL, Baumann LM, Hansel NN, Romero KM, Pollard SL, et al. 25-hydroxy vitamin D levels are associated with childhood asthma in a population-based study in Peru. *Clin Exp Allergy.* (2015) 45:273–82. doi: 10.1111/cea.12311
98. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Serum vitamin D levels and severe asthma exacerbations in the childhood asthma management program study. *J Allergy Clin Immunol.* (2010) 126:52–8.e5. doi: 10.1016/j.jaci.2010.03.043
99. Brehm JM, Acosta-Perez E, Klei L, Roeder K, Barmada M, Boutaoui N, et al. Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med.* (2012) 186:140–6.
100. Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med.* (2009) 179:765–71. doi: 10.1164/rccm.200808-1361OC
101. Wu AC, Tantisira K, Li L, Fuhlbrigge AL, Weiss ST, Litonjua A, et al. Effect of vitamin D and inhaled corticosteroid treatment on lung function in children. *Am J Respir Crit Care Med.* (2012) 186:508–13. doi: 10.1164/rccm.201202-0351OC
102. Hall SC, Fischer KD, Agrawal DK. The impact of vitamin D on asthmatic human airway smooth muscle. *Expert Rev Respir Med.* (2016) 10:127–35. doi: 10.1586/17476348.2016.1128326
103. Barragan M, Good M, Kolls JK. Regulation of dendritic cell function by vitamin D. *Nutrients.* (2015) 7:8127–51. doi: 10.3390/nu7095383
104. Berraies A, Hamzaoui K, Hamzaoui A. Link between vitamin D and airway remodeling. *J Asthma Allergy.* (2014) 7:23–30. doi: 10.2147/JAA.S46944
105. Mirzakhani H, Al-Garawi A, Weiss ST, Litonjua AA. Vitamin D and the development of allergic disease: how important is it? *Clin Exp Allergy.* (2015) 45:114–25. doi: 10.1111/cea.12430
106. Feng L, Meng T, Qi Y, Athari SS, Chen X. Study effect of vitamin d on the immunopathology responses of the bronchi in murine model of asthma. *Iran J Allergy Asthma Immunol.* (2021) 20:509–19. doi: 10.18502/ijaai.v20i5.7399

107. Lan N, Luo G, Yang X, Cheng Y, Zhang Y, Wang X, et al. 25-Hydroxyvitamin D3-deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. *PLoS One*. (2014) 9:e111599. doi: 10.1371/journal.pone.0111599
108. Chung KF, Marwick JA. Molecular mechanisms of oxidative stress in airways and lungs with reference to asthma and chronic obstructive pulmonary disease. *Ann N Y Acad Sci*. (2010) 1203:85–91. doi: 10.1111/j.1749-6632.2010.05600.x
109. Forno E, Bacharier LB, Phipatanakul W, Guilbert TW, Cabana MD, Ross K, et al. Effect of vitamin D3 supplementation on severe asthma exacerbations in children with asthma and low vitamin D levels: the VDKA randomized clinical trial. *JAMA*. (2020) 324:752–60. doi: 10.1001/jama.2020.12384
110. Brustad N, Eliasen AU, Stokholm J, Bønnelykke K, Bisgaard H, Chawes BL. High-dose vitamin D supplementation during pregnancy and asthma in offspring at the age of 6 years. *JAMA*. (2019) 321:1003–5. doi: 10.1001/jama.2019.0052
111. Luo J, Liu D, Liu CT. Can vitamin D supplementation in addition to asthma controllers improve clinical outcomes in patients with asthma?: a meta-analysis. *Medicine (Baltimore)*. (2015) 94:e2185. doi: 10.1097/MD.0000000000002185

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Sarcopenia Was a Poor Prognostic Predictor for Patients With Advanced Lung Cancer Treated With Immune Checkpoint Inhibitors

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Background: It remains not well known whether skeletal muscle mass (SMM) loss has any impact on the effectiveness of immune checkpoint inhibitors (ICIs) in patients with advanced lung cancer. We aimed to evaluate the association between SMM and clinical outcome of patients with advanced lung cancer receiving ICIs as first line or second line.

Materials and Methods: From March 1st, 2019 to March 31st, 2021 at our hospital, 34 patients with advanced lung cancer treated with first-line or second-line ICIs were enrolled retrospectively. The estimation of skeletal muscle index (SMI) for sarcopenia was assessed at the level of the third lumbar vertebra (L3) on computed tomography (CT) images obtained within 4 weeks before initiation of ICIs treatment. The impact of sarcopenia (low SMI) on progression free survival (PFS) was analyzed using Kaplan-Meier method and log-rank tests. The effect of various variables on PFS was evaluated using Cox proportional hazards regression model with univariate and multivariate analysis. The impact on treatment response including objective response rate (ORR) and disease control rate (DCR) and immunotherapy related adverse events (irAEs) between patients with and without sarcopenia was compared by the chi-squared test. The comparison of SMI value between patients with objective response (OR), disease control (DC) and those without OR and DC was used student *t*-test or Mann-Whitney *U* test.

Results: Both in univariate and multivariate analysis, sarcopenia and treatment lines were the predictive factors for PFS ($p < 0.05$). Patients with sarcopenia had significantly shorter PFS than that of non-sarcopenic ones [6.57 vs. 16.2 months, hazard ratios (HR) = 2.947 and 3.542, and 95% confidence interval (CI): 1.123–13.183 and 1.11–11.308, $p = 0.022$ and 0.033]. No significant difference in ORR and irAEs was found.

Patients with sarcopenia had lower DCR than those without sarcopenia. The mean SMI value of DCR group and non-DCR group was 32.94 ± 5.49 and 44.77 ± 9.06 cm^2/m^2, respectively ($p = 0.008$).

Conclusion: Sarcopenia before immunotherapy might be a significant predictor for poor prognosis including shorter PFS and lower DCR in patients with advanced lung cancer treated with ICIs as first line or second line.

Keywords: skeletal muscle index (SMI), sarcopenia, advanced lung cancer, immune-checkpoint inhibitor, progression free survival (PFS)

INTRODUCTION

Lung cancer is the second common malignant tumor and the leading cause of cancer-related deaths worldwide (1). Nearly 70% of patients are at stage IV when initially diagnosed with lung cancer (2), with 5-year survival rate as low as 16% (3). With the introduction of immune checkpoint inhibitors (ICIs), such as PD-1 (programmed cell death protein-1) and PD-L1 (programmed death ligand 1) inhibitors, 2 to 3-year survival rates were increased by 15–20% (4, 5). However, not every eligible candidate could benefit from ICIs. Although PD-L1 expression and tumor mutational burden (TMB) have been reported as potential predictors allowing for therapeutic effect prediction for ICIs, even among lung cancer patients with positive PD-L1 (TPS ≥ 1%) or high expression (TPS ≥ 50%), only 10–20% of patients could benefit from ICIs (6, 7). Therefore, it is essential at present to find more reliable biomarkers that can identify patients who are most likely to benefit from ICIs.

Sarcopenia, characterized by loss of skeletal muscle mass and function (8), has been proposed to be associated with tumor-induced increased protein degradation and decreased protein synthesis caused by impaired Akt–mTORC1 pathway in the presence of disturbed metabolic homeostasis, malnutrition, or reduced activity (9, 10). Sarcopenia has been reported to be associated with treatment efficacy, quality of life and clinical outcomes of lung cancer patients receiving chemotherapy or surgery (11–13). However, is sarcopenia associated with the efficacy of ICIs? To investigate potential association, our team has recently performed a systematic review which showed that the PFS of patients with sarcopenia treated with ICIs was 1.46 times shorter than those without sarcopenia in various types of cancer (14), suggesting that sarcopenia may be a potential predictor for the efficacy of immunotherapy. Besides, skeletal muscle is now recognized as an immune regulatory organ that can regulates immunological processes and the inflammatory response, and the efficacy of ICIs is heavily dependent on the host's immune system (15). Therefore, sarcopenia is also very likely to be associated with the efficacy of ICIs.

Previously, several studies have attempted to investigate the potential impact of sarcopenia on the efficacy of ICIs, but with inconsistent results (16–30). These results cannot be directly compared, for their different inclusion criteria, different definition of sarcopenia [total cross-skeletal muscle (16–18, 21–25, 27, 29) vs. psoas muscle area (19, 20, 26, 28)], or different methods for measuring muscle mass [CT (16–29) vs. DXA (30)]. In addition, the majority of studies enrolled patients receiving second or more treatment lines ICIs, while only three studies enrolled a small proportion [13.4% (23), 16.5% (17), and 38.3% (30), respectively] of patients receiving ICIs as first-line treatment. Therefore, we designed a retrospective study to investigate the potential impact of sarcopenia on the efficacy of ICIs, in which sarcopenia was defined by averaging muscle area on multiple consecutive CT images at L3 level and a large proportion of patients receiving ICIs as first-line treatment were included.

MATERIALS AND METHODS

Patients

In total, 34 consecutive patients with advanced lung cancer treated with ICIs were enrolled in National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital and Shenzhen Hospital from March 1, 2019 to March 31, 2021. The inclusion criteria for eligible patients were as follows: (1) Patients were diagnosed with stage IV lung cancer including small cell lung cancer, adenocarcinoma, squamous cell carcinoma, large cell neuroendocrine carcinoma, and other types. (2) Patients were treated with ICIs in our hospital for the first time, including PD-1 or PD-L1 inhibitors or CTLA-4 inhibitors as first-line or second-line. (3) Patients received upper abdomen CT scan within 4 weeks before ICIs therapy in our hospital. And those patients with degraded CT images insufficient to perform measurements of muscle area were also excluded.

Data Collection and Follow-Up

In total, 137 consecutive patients with lung cancer who treated with ICIs from the electronic database of our hospital were identified. After selection based on inclusion and exclusion criteria, 34 eligible patients receiving first-line or second-line immunotherapy were finally included (**Figure 1**).

The data of all eligible patients were obtained, including age, sex, smoking histology, histopathology, TNM stage, ECOG PS, height and weight at the time of ICI initiation, gene expression status for EGFR and ALK, PD-L1 expression, treatment options, and CT results, treatment response, date of progression defined by CT, and date of the last follow-up. The follow-up date was ended on September 30, 2021.

The primary endpoint was progression free survival (PFS) defined as the time from initiation of immunotherapy to

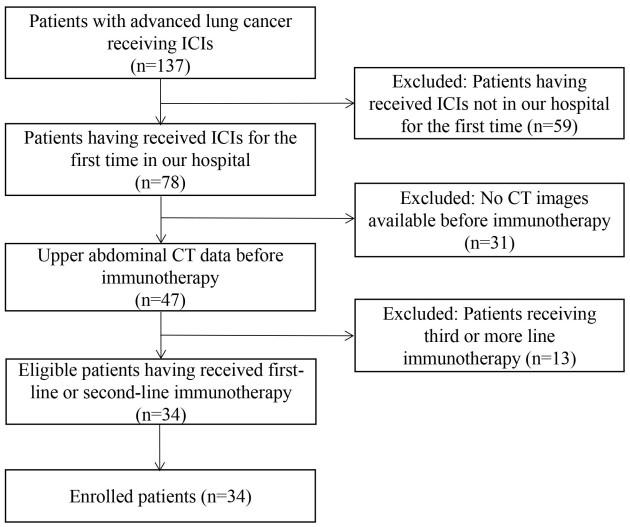

FIGURE 1 | Patient selection flow-chart. ICIs, immune checkpoint inhibitors; CT, computed tomography.

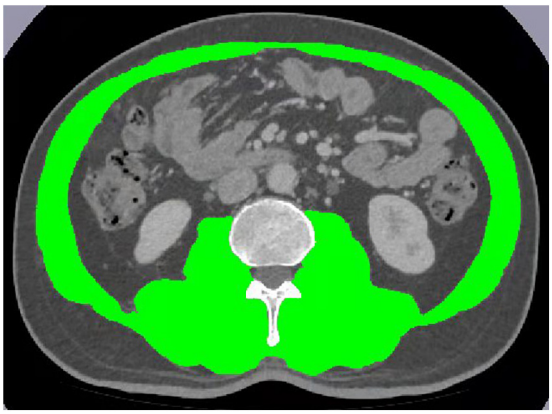

FIGURE 2 | Skeletal muscle mass analysis of computed tomography images on an L3 section by SliceOmatic.

disease progression or death. Secondary endpoints were objective response rate (ORR), disease control rate (DCR) and immunotherapy-related adverse events (irAEs). ORR was defined as the sum of proportion of complete response (CR) and partial response (PR), while DCR was defined as the sum of proportion of CR, PR, and stable disease (SD) according to iRECIST criteria (31). Any reported adverse events that might be associated with immune therapy were obtained from the medical records, mainly including immune pneumonitis, hepatotoxicity, endocrine toxicity, cardiotoxicity, etc.

Body Composition Analysis

All the enrolled patients underwent CT scan on a GE scanner (Revolution GE, United States) with following parameters: voltage of 140 kvp, tube current of 740 mA and slice thickness of 1.25 mm. The cross-sectional areas of muscle were quantified on CT images acquired within 4 weeks before immunotherapy. All consecutive axial CT slices covering the upper and lower level of the third lumbar vertebra (around 20 slices for each patient) at venous contrast-enhancement phase were chosen. On the platform of sliceOmatic (TomoVision 5.0, Magog, QC, Canada), muscle area (including the psoas, rectus abdominis, transversus abdominis, internal and external abdominal oblique muscles) were firstly manually outlined using morphology mode by YR and PH both with 4 years of experience in abdominal CT imaging and then reviewed by a senior radiologist (ZL with more than 10 years of experience in abdominal CT imaging), who were blinded for patient characteristics (**Figure 2**). The total volume of skeletal muscle at the level of the third lumbar vertebra was automatically calculated. The average skeletal muscle area of all slices at the level of the third lumbar vertebra was calculated by the following formula: Muscle area = Volume (cm^3)/(Thickness × Numbers of slice). Skeletal muscle mass (SMM) was defined as the total cross-sectional skeletal muscle area (TMA in cm^2). SMI was calculated by dividing the total cross-sectional skeletal muscle area (TMA-cm^2) at the level of lumbar vertebra L3 by height squared (m^2), which could proportionally reflect whole-body muscles of patients (32, 33). And measurements of muscle on sectional CT is considered as the gold standard method at present (34, 35). Sarcopenia was defined as low SMI as following: (1) for women, SMI $<$ 41 cm^2/m^2 regardless of their BMI; (2) for men, SMI $<$ 43 cm^2/m^2 with a BMI $<$ 25 kg/m^2, or SMI $<$ 53 cm^2/m^2 with a BMI $\geq$ 25 kg/m^2 (36).

Statistical Analysis

Statistical analysis was performed using SPSS software version 20.0 (IBM, Armonk, NY, United States). The PFS comparison between patients with and without sarcopenia was evaluated using Kaplan-Meier method and log-rank test. The impact of sarcopenia and other factors on PFS were evaluated using univariate and multivariate analysis by Cox proportional hazards regression model. Chi-squared test was used to compare the treatment response and occurrence of irAEs between the sarcopenia and non-sarcopenia groups, while student *t*-test or Mann-Whitney *U* test was used to test any difference in SMI value between patients with objective response, disease control and those without OR and DC. And two-sided $p < 0.05$ was considered to be statistically significant.

RESULTS

Patients' Characteristics

In total, 34 patients were included in the analysis. Baseline characteristics of these patients are shown in **Table 1**.

The Effect of Sarcopenia on Clinical Outcomes

Progression Free Survival

The median follow-up after immunotherapy was 11.67 months (range: 3.50–35.67 months, 95% CI: 8.032–15.308). The median

TABLE 1 | Baseline characteristics of the study population.

Characteristics	*N* = 34
Gender	
Male, *N* (%)	29 (85.3%)
Female, *N* (%)	5 (14.7%)
Median age (year), Median ± SD	63 ± 9.65
Smoking history	
Yes, *N* (%)	20 (58.8%)
No, *N* (%)	14 (41.2%)
BMI (Kg/cm^2)	
<18.5, *N* (%)	5 (14.7%)
18.5–24.9, *N* (%)	22 (64.7%)
≥25, *N* (%)	7 (20.6%)
Histopathology	
Adenocarcinoma, *N* (%)	23 (67.6%)
Squamous cell carcinoma, *N* (%)	7 (20.6%)
Small cell lung cancer, *N* (%)	3 (8.8%)
Large cell carcinoma, *N* (%)	1 (3.0%)
Driver gene expression	
EGFR mutation, *N* (%)	0 (0%)
ALK mutation, *N* (%)	0 (0%)
KRAS mutation, *N* (%)	7 (20.1%)
PD-L1 expression	
<1%, *N* (%)	5 (14.7%)
1–49%, *N* (%)	2 (5.9%)
≥50%, *N* (%)	9 (26.5%)
No record available, *N* (%)	18 (52.9%)
Treatment line	
First-line immunotherapy, *N* (%)	26 (76.5%)
Second-line immunotherapy, *N* (%)	8 (23.5%)
SMI (cm^2/m^2), mean ± SD	
Sarcopenic status	44.52 ± 9.56
Sarcopenia, *N* (%)	18 (52.9%)
Non-sarcopenia, *N* (%)	16 (47.1%)

SD, standard deviation; BMI, body mass index; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; KRAS, Kirsten Rat Sarcoma Viral Oncogene Homolog; PD-L1, programmed death-ligand 1; SMI, skeletal muscle index.

Progression Free Survival (PFS) was 9.3 months (range 2–35 months, 95% CI: 4.817–13.783) for all enrolled 34 patients. The Kaplan-Meier analysis revealed that patients with sarcopenia had a significantly shorter PFS than those without sarcopenia (6.57 vs. 16.2 months, $p = 0.022$; **Figure 3A**). Among gender, age, smoking history, BMI and PD-L1 expression, sarcopenia and received treatment line, gender, sarcopenia and treatment line were significant prognostic factors for PFS in the univariate analysis (all $p < 0.05$; **Table 2**). Patients with sarcopenia had a significantly shorter PFS than those without sarcopenia (HR: 2.947, 95% CI: 1.123–7.733, $p = 0.028$).

In multivariate analysis, among gender, PD-L1 expression, sarcopenia and treatment line, treatment line and sarcopenia were independent prognostic factors for PFS ($p < 0.05$; **Table 2**). The PFS was also significantly shorter in patients with sarcopenia than those without sarcopenia (HR: 4.268, 95% CI: 1.248–14.598, $p = 0.021$; **Figure 3B**).

Objective Response Rate and Disease Control Rate

Of 34 patients, 19 patients had objective response with Objective Response Rate (ORR) of 58.8%. There was no significant difference in ORR between sarcopenia and non-sarcopenia groups (44.4 vs. 68.8%, $p = 0.154$). In contrast, Disease Control Rate (DCR) of all patients was 85.3% (29/34). Patients with sarcopenia had a significant lower DCR than those without sarcopenia (72.2 vs. 100%, $p = 0.022$). Specifically, five patients with sarcopenia experienced progressive disease (PD), while none of the patients without sarcopenia experienced PD (**Table 3**). The mean SMI of the DCR group was significantly higher than that of the non-DCR group, with 44.77 ± 9.06 and 32.94 ± 5.49 cm^2/m^2, respectively ($p = 0.008$; **Figure 4A**). No significant difference was found in SMI between ORR group and non-ORR group (42.37 ± 9.49 vs. 43.56 ± 9.84 cm^2/m^2, $p = 0.725$; **Figure 4B**).

Immune-Related Adverse Events

In our study, 11 patients (11/34, 32.4%) experienced Immune-Related Adverse Events (irAEs). Seven cases with irAEs were in sarcopenia group and four cases in non-sarcopenia group, with no statistically significant difference ($p = 0.388$; **Table 3**). The most frequent irAEs were increased aminotransferase ($n = 3$) and pneumonitis ($n = 2$). Overall, three patients experienced ¾grade irAEs, including arthralgia and myositis ($n = 1$), pneumonitis ($n = 1$) and abnormal aminotransferase ($n = 1$).

DISCUSSION

This study investigated the prognostic value of sarcopenia in patients with advanced lung cancer treated with first-line or second-line ICIs. We found that patients with sarcopenia receiving ICIs showed significantly shorter PFS than those non-sarcopenic ones. Although ORR and irAEs were not significantly different between patients with and without sarcopenia, sarcopenic patients did show significantly lower DCR than non-sarcopenic patients. And the mean value of SMI was significantly higher in DCR group than that non-DCR group.

In our study, we found that patients with sarcopenia at the initial stage before immunotherapy had significantly shorter PFS than those without sarcopenia. Sarcopenia was shown as a poor prognosis factor of PFS both at univariate and multivariate analysis. Our results were in line with the previous reported result (17, 19, 20, 22, 26, 27, 29, 30), but not with other studies (18, 23, 24, 28). The majority of studies enrolled patients receiving second or more treatment lines ICIs, while only three studies enrolled a small proportion of patients receiving ICIs as first-line treatment (17, 23, 30). By contrast, in our study, 26 (76.5%) patients received ICIs as first-line treatment. The results we obtained were consistent with two studies focused on patients receiving ICIs as first-line treatment (17, 30), but inconsistent with another one (23). We speculate this may be due to different inclusion criteria, different definition of sarcopenia, or different methods for measuring muscle mass. Sarcopenia can be diagnosed by dual-energy X-ray absorptiometry scan, bioelectrical impedance analysis, CT, and magnetic resonance imaging (MRI) (35). In our study, we chose to measure muscle area at the level of L3 using

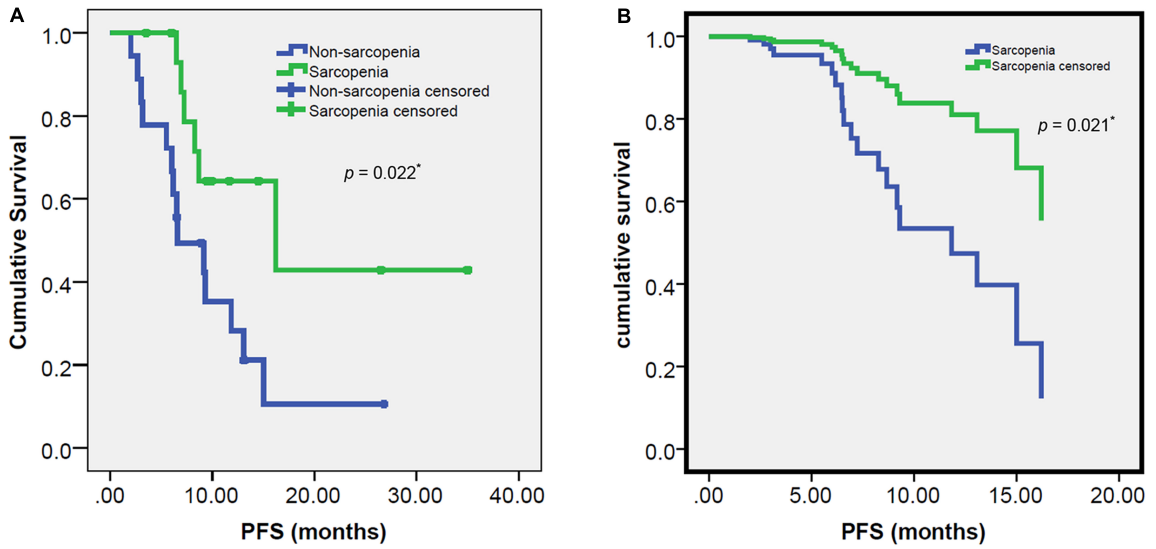

FIGURE 3 | Progression free survival (PFS) survival curves: estimated by Kaplan-Meier method and log-rank test **(A)** and estimated by multivariate analysis of Cox proportional hazards regression model **(B)** for the sarcopenia and non-sarcopenia groups. The PFS was significantly worse in the sarcopenia group than the non-sarcopenia group (6.57 vs. 16.2 months, $p = 0.022$, 0.021, respectively). *Indicates significant difference.

TABLE 2 | Univariate and multivariate cox-regression analysis of the risk of sarcopenia and clinicopathological factors on progression free survival (PFS) in patients receiving immune checkpoint inhibitors (ICIs).

Variables		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Gender	Male	1			
	Female	4.569 (1.599–13.057)	0.005*	2.120 (0.598–7.514)	0.245
Age	–	1.014 (0.970–1.030)	0.546		
Smoking history	No	1			
	Yes	0.488 (0.201–1.185)	0.113		
BMI (kg/cm^2)	<18.5	1			
	18.5–24.9	0.474 (0.146–1.540)	0.214		
	≥25	0.589 (0.147–2.366)	0.456		
PD-L1 expression	≥50%	1		1	
	<50%	3.073 (0.880–10.730)	0.078	1.947 (0.515–7.365)	0.327
	Unknown	1.24 (0.249–6.268)	0.787	0.608 (0.112–3.295)	0.564
Treatment line	First line	1		1	
	Second line	4.656 (1.698–12.764)	0.003*	9.899 (2.699–36.709)	0.001*
Sarcopenia	Non-low SMI	1		1	
	Low SMI	2.947 (1.123–7.733)	0.028*	4.268 (1.248–14.598)	0.021*

*BMI, body mass index; PD-L1, programmed death-ligand 1. *Indicates significant difference.*

TABLE 3 | Treatment response including ORR, DCR, and irAEs comparing sarcopenic vs. non-sarcopenic groups.

Variables	CR (*n*)	PR (*n*)	SD (*n*)	PD (*n*)	ORR (%)	DCR (%)	irAEs (any grade)
Sarcopenia	0	8	5	5	44.4	72.2	7
Non-sarcopenia	0	11	5	0	68.8	100	4
χ^2 value					2.03	5.211	0.747
P value					0.154	0.022*	0.388

*Chi-Square Test. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; irAEs, immunotherapy-related adverse events. *Indicates significant difference.*

CT, like the majority of studies. However, unlike previous studies using a single or two random slices at the level of L3 (16, 21, 25, 29), we measured 20 consecutive slices at the level of L3 and average the muscle area to decrease the randomness and potential selection bias. Besides, instead of using radiodensity threshold-based segmentation, we used "Morphology mode" of sliceOmatic that segment image not only based on image intensity, but also local morphology of muscle by computing the Watershed of the gradient of the image, which can avoid the bias caused by radiodensity of skeletal muscle.

Also in our study, patients receiving ICIs as first line had significantly longer PFS than those as second line in both univariate and multivariate analysis, suggesting that immunotherapy might be better prescribed as first-line treatment. The similar results have been reported in previous large cohort clinical studies (5, 37). Due to limited cases, we did not perform subgroup analysis on the potential impact of sarcopenia on the first-line treatment group and second-line treatment group, respectively, which warrants further investigation. Partially due to limited number of patients with available PD-L1 testing, our study showed that PD-L1 expression had no positive impact on PFS in the univariate analysis and multivariate analysis, which was inconsistent with previous study (38).

In addition, we found that patients with sarcopenia had significantly lower DCR than those without sarcopenia, and the mean SMI in the DCR group was significantly higher than that in the non-DCR group. The result was in accordance with the previous studies (17, 26, 30), but inconsistent with other study (19, 23, 28). However, for ORR, we found that sarcopenic patients had a trend toward lower ORR, but without significance, which was consistent with some reports (16, 17, 24, 27, 28), but inconsistent with other studies (26). Previous article showed that sarcopenia are associated with the development of hyperprogressive disease after second-line pembrolizumab in patients with non-small-cell lung cancer (39). In terms of potential impact of sarcopenia on DCR and ORR, these inconsistent results in different studies might come from different study design, different inclusion and exclusion criteria, different sample size and different way of measuring muscle area or definition of sarcopenia, etc., which needs further investigation in a prospective study with larger sample size.

In our statistical analysis, sarcopenia was not a significant factor for predicting irAEs, which was similar to our previous report (40) and other studies (16, 18, 27). However, previous study found that patients with sarcopenia experienced significantly increased risk of irAEs (22, 41). It is generally believed that sarcopenia has been associated with a greater incidence of chemotherapy toxicity, but the impact of sarcopenia on irAEs remains controversial. These controversial results from previous reports cannot be directly compared, due to varied number of patients enrolled, inconsistent tools for measuring muscle mass, different definition of sarcopenia, and different treatment regimens including ICIs. A standardized workflow of studying the impact of sarcopenia should be made before sarcopenia as a reliable prognostic biomarker could be translated into clinical practice.

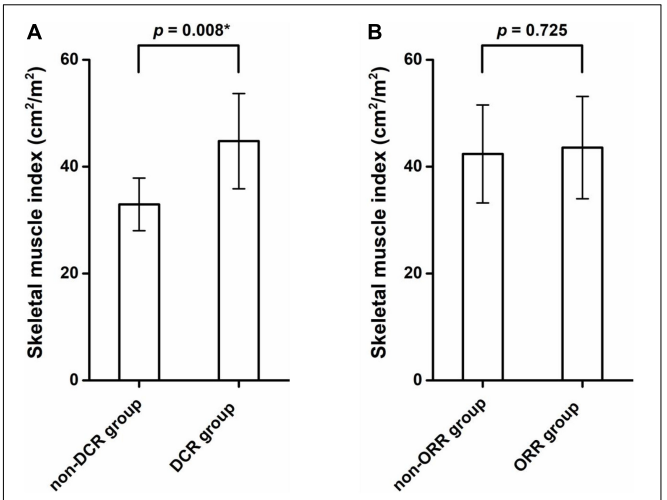

FIGURE 4 | The mean skeletal muscle index (SMI) of the disease control rate (DCR) group was significantly higher than that of the non-DCR group (44.77 ± 9.06 vs. 32.94 ± 5.49 cm^2/m^2, $p = 0.008$) **(A)**. No significant difference in SMI values was found between ORR group and non-ORR group (42.37 ± 9.49 vs. 43.56 ± 9.84 cm^2/m^2, $p = 0.725$) **(B)**. *Indicates significant difference.

With respect to the underlying mechanism of sarcopenia affecting treatment efficacy of ICIs is still not fully known, multiple studies have proposed chronic inflammation might play a central role in adverse affecting immunotherapy, such as increased neutrophil-to-lymphocyte ratio (NLR), leukocyte/lymphocyte ratio (LLR), red blood cell distribution width (RDW), TGF-α, Fibrinogen and CRP (26, 29, 30). Previous studies found that IL-15 is as a myokine expressed in skeletal muscle cells and regulates CD8 T-cell and promotes survival of T-cells (42, 43), which is important in maintaining body immune function. IL-15 serum levels decrease in older people with loss of muscle mass (44), which suggested that sarcopenia may lead to immune function impaired. And the expression of increased IL-6 and decreased IL-7 in people with loss of muscle mass effects immune system function through T-cell exhaustion (42, 44). CD4 + FoxP3 + Tregs infiltrate impaired skeletal muscle, which suggested that sarcopenia may lead to tumor immune escape (45).

Our study has several limitations. First, it is retrospective study and sample size is relatively small. Second, sarcopenia is merely defined according to SMI, without taking muscle strength and function into account, for example grip. In addition, the cut-off value of SMI was based on literature in white people instead of Asian people. Third, due to short follow-up time, the potential impact of sarcopenia on OS could not be investigated in this study.

In conclusion, sarcopenia may be a poor prognostic factor of patients with advanced lung cancer receiving ICIs as first-line or second-line treatment. Patients with sarcopenia had a significantly lower DCR and shorter PFS than those without sarcopenia, suggesting sarcopenia should be taken into consideration when using ICIs in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

SLi: idea and original draft preparation, methodology, and review and editing. ZL: methodology and review and editing. YR: visualization and review and editing. JL and YY: supervision. SLv, PH, and YS: data curation. JC: resources. DL: project administration. MC: idea and software. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 Countries. *CA Cancer J Clin.* (2021) 71:209–49. doi: 10.3322/caac.21660
2. Knight SB, Crosbie PA, Balata H, Chudziak J, Hussell T, Dive C. Progress and prospects of early detection in lung cancer. *Open Biol.* (2017) 7:170070. doi: 10.1098/rsob.170070
3. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M eds, et al. *SEER Cancer Statistics Review, 1975–2016*. Bethesda, MD: National Cancer Institute (2019). 1 p.
4. Ramalingam SS, Ciuleanu TE, Pluzanski A, Lee J-S, Schenker M, Bernabe Caro R, et al. Nivolumab + ipilimumab versus platinum-doublet chemotherapy as first-line treatment for advanced non-small cell lung cancer: three-year update from CheckMate 227 Part 1. *J Clin Oncol.* (2020) 38:9500. doi: 10.1200/JCO.2020.38.15_suppl.9500
5. Rodriguez-Abreu D, Powell SF, Hochmair M, Gadgeel SM, Esteban E, Felip E, et al. Final analysis of KEYNOTE-189: pemetrexed-platinum chemotherapy (chemo) with or without pembrolizumab (pembro) in patients (pts) with previously untreated metastatic nonsquamous non-small cell lung cancer (NSCLC). *J Clin Oncol.* (2020) 38:9582. doi: 10.1200/JCO.2020.38.15_suppl.9582
6. Reck M, Schenker M, Lee KH, Provencio M, Nishio M, Lesniewski-Kmak K, et al. Nivolumab plus ipilimumab versus chemotherapy as first-line treatment in advanced non–small-cell lung cancer with high tumour mutational burden: patient-reported outcomes results from the randomised, open-label, phase III checkmate 227 trial. *Eur J Cancer.* (2019) 116:137–47. doi: 10.1016/j.ejca.2019.05.008
7. Mok TSK, Wu Y-L, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet.* (2019) 393:1819–30. doi: 10.1016/S0140-6736(18)32409-7
8. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc.* (2011) 12:249–56. doi: 10.1016/j.jamda.2011.01.003
9. Geremia A, Sartori R, Baraldo M, Nogara L, Balmaceda V, Dumitras GA, et al. Activation of Akt-mTORC1 signalling reverts cancer-dependent muscle wasting. *J Cachexia Sarcopenia Muscle.* (2022) 13:648–61. doi: 10.1002/jcsm.12854
10. Meza-Valderrama D, Marco E, Dávalos-Yerovi V, Muns MD, Tejero-Sánchez M, Duarte E, et al. Sarcopenia, malnutrition, and cachexia: adapting definitions and terminology of nutritional disorders in older people with cancer. *Nutrients.* (2021) 13:761. doi: 10.3390/nu13030761
11. Nipp RD, Fuchs G, El-Jawahri A, Mario J, Troschel FM, Greer JA, et al. Sarcopenia is associated with quality of life and depression in patients with advanced cancer. *Oncologist.* (2018) 23:97–104. doi: 10.1634/theoncologist.2017-0255
12. Chen X, Hou L, Shen Y, Wu X, Dong B, Hao Q. The role of baseline sarcopenia index in predicting chemotherapy-induced undesirable effects and mortality in older people with stage III or IV non-small cell lung cancer. *J Nutr Health Aging.* (2021) 25:878–82. doi: 10.1007/s12603-021-1633-3
13. Takahashi Y, Suzuki S, Hamada K, Nakada T, Oya Y, Sakakura N, et al. Sarcopenia is poor risk for unfavorable short- and long-term outcomes in stage I non-small cell lung cancer. *Ann Transl Med.* (2021) 9:325. doi: 10.21037/atm-20-4380
14. Li S, Wang T, Tong G, Li X, You D, Cong M. Prognostic impact of sarcopenia on clinical outcomes in malignancies treated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Front Oncol.* (2021) 11:726257. doi: 10.3389/fonc.2021.726257
15. Afzali AM, Müntefering T, Wiendl H, Meuth SG, Ruck T. Skeletal muscle cells actively shape (auto)immune responses. *Autoimmun Rev.* (2018) 17:518–29. doi: 10.1016/j.autrev.2017.12.005
16. Cortellini A, Verna L, Porzio G, Bozzetti F, Palumbo P, Masciocchi C, et al. Predictive value of skeletal muscle mass for immunotherapy with nivolumab in non-small cell lung cancer patients: a "hypothesis-generator" preliminary report. *Thorac Cancer.* (2019) 10:347–51. doi: 10.1111/1759-7714.12965
17. Takada K, Yoneshima Y, Tanaka K, Okamoto I, Shimokawa M, Wakasu S, et al. Clinical impact of skeletal muscle area in patients with non-small cell lung cancer treated with anti-PD-1 inhibitors. *J Cancer Res Clin Oncol.* (2020) 146:1217–25. doi: 10.1007/s00432-020-03146-5
18. Haik L, Gonthier A, Quivy A, Gross-Goupil M, Veillon R, Frison E, et al. The impact of sarcopenia on the efficacy and safety of immune checkpoint inhibitors in patients with solid tumours. *Acta Oncol.* (2021) 60:1597–603. doi: 10.1080/0284186X.2021.1978540
19. Shiroyama T, Nagatomo I, Koyama S, Hirata H, Nishida S, Miyake K, et al. Impact of sarcopenia in patients with advanced non-small cell lung cancer treated with PD-1 inhibitors: a preliminary retrospective study. *Sci Rep.* (2019) 9:2447. doi: 10.1038/s41598-019-39120-6
20. Tsukagoshi M, Yokobori T, Yajima T, Maeno T, Shimizu K, Mogi A, et al. Skeletal muscle mass predicts the outcome of nivolumab treatment for non-small cell lung cancer. *Medicine.* (2020) 99:e19059.
21. Magri V, Gottfried T, Di Segni M, Urban D, Peled M, Daher S, et al. Correlation of body composition by computerized tomography and metabolic parameters with survival of nivolumab-treated lung cancer patients. *Cancer Manag Res.* (2019) 11:8201–7. doi: 10.2147/CMAR.S210958
22. Strulov Shachar S, Fried R, Shafran I, Moskovitz MT, Williams GR, Bar-Sela G, et al. Body composition as predictor of toxicity and outcomes in patients with metastatic non-small cell lung cancer (mNSCLC) receiving nivolumab (Nivo). *J Clin Oncol.* (2018) 36:e21010.
23. Roch B, Coffy A, Jean-Baptiste S, Palaysi E, Daures J-P, Pujol J-L, et al. Cachexia - sarcopenia as a determinant of disease control rate and survival in non-small lung cancer patients receiving immune-checkpoint inhibitors. *Lung Cancer.* (2020) 143:19–26. doi: 10.1016/j.lungcan.2020.03.003
24. Nishioka N, Naito T, Notsu A, Mori K, Kodama H, Miyawaki E, et al. Unfavorable impact of decreased muscle quality on the efficacy of immunotherapy for advanced non-small cell lung cancer. *Cancer Med.* (2021) 10:247–56. doi: 10.1002/cam4.3631
25. Degens JHRJ, Dingemans AMC, Willemsen ACH, Gietema HA, Hurkmans DP, Aerts JG, et al. The prognostic value of weight and body composition changes in patients with non-small-cell lung cancer treated with nivolumab. *J Cachexia Sarcopenia Muscle.* (2021) 12:657–64. doi: 10.1002/jcsm.12698

26. Nishioka N, Uchino J, Hirai S, Katayama Y, Yoshimura A, Okura N, et al. Association of sarcopenia with and efficacy of anti-PD-1/PD-L1 therapy in non-small-cell lung cancer. *J Clin Med.* (2019) 8:450. doi: 10.3390/jcm8040450
27. Cortellini A, Bozzetti F, Palumbo P, Brocco D, Di Marino P, Tinari N, et al. Weighing the role of skeletal muscle mass and muscle density in cancer patients receiving PD-1/PD-L1 checkpoint inhibitors: a multicenter real-life study. *Sci Rep.* (2020) 10:1456. doi: 10.1038/s41598-020-58498-2
28. Minami S, Ihara S, Tanaka T, Komuta K. Sarcopenia and visceral adiposity did not affect efficacy of immune-checkpoint inhibitor monotherapy for pretreated patients with advanced non-small cell lung cancer. *World J Oncol.* (2020) 11:9–22. doi: 10.14740/wjon1225
29. Wang Y, Chen P, Huang J, Liu M, Peng D, Li Z, et al. Assessment of sarcopenia as a predictor of poor overall survival for advanced non-small-cell lung cancer patients receiving salvage anti-PD-1 immunotherapy. *Ann Transl Med.* (2021) 9:1801. doi: 10.21037/atm-21-6578
30. Tenuta M, Gelibter A, Pandozzi C, Sirgiovanni G, Campolo F, Venneri MA, et al. Impact of sarcopenia and inflammation on patients with advanced non-small cell lung cancer (NCSCL) treated with immune checkpoint inhibitors (ICIs): a prospective study. *Cancers (Basel).* (2021) 13:6355. doi: 10.3390/cancers13246355
31. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol.* (2017) 18:e143–52. doi: 10.1016/S1470-2045(17)30074-8
32. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol.* (2011) 12:489–95. doi: 10.1016/S1470-2045(10)70218-7
33. Vangelov B, Bauer J, Moses D, Smee R. The effectiveness of skeletal muscle evaluation at the third cervical vertebral level for computed tomography-defined sarcopenia assessment in patients with head and neck cancer. *Head Neck.* (2022) 44:1047–56. doi: 10.1002/hed.27000
34. Martin L, Tom M, Basualdo-Hammond C, Baracos V, Gramlich L. Piloting a training program in computed tomography (CT) skeletal muscle assessment for Registered Dietitians. *JPEN J Parenter Enter Nutr.* (2022). doi: 10.1002/jpen.2348
35. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. *Age Ageing.* (2010) 39:412–23. doi: 10.1093/ageing/afq034
36. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J Clin Oncol.* (2013) 31:1539–47. doi: 10.1200/JCO.2012.45.2722
37. West H, McCleod M, Hussein M, Morabito A, Rittmeyer A, Conter HJ, et al. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 tria. *Lancet Oncol.* (2019) 20:924–37. doi: 10.1016/S1470-2045(19)30167-6
38. Nosaki K, Saka H, Hosomi Y, Baas P, de Castro G Jr., Reck M, et al. Safety and efficacy of pembrolizumab monotherapy in elderly patients with PD-L1–positive advanced non–small-cell lung cancer: pooled analysis from the KEYNOTE-010, KEYNOTE-024, and KEYNOTE-042 studies. *Lung Cancer.* (2019) 135:188–95. doi: 10.1016/j.lungcan.2019.07.004
39. Petrova MP, Donev IS, Radanova MA, Eneva MI, Dimitrova EG, Valchev GN, et al. Sarcopenia and high NLR are associated with the development of hyperprogressive disease after second-line pembrolizumab in patients with non-small-cell lung cancer. *Clin Exp Immunol.* (2020) 202:353–62. doi: 10.1111/cei.13505
40. Li S, Wang T, Lai W, Zhang M, Cheng B, Wang S, et al. Prognostic impact of sarcopenia on immune-related adverse events in malignancies received immune checkpoint inhibitors: a systematic review and meta-analysis. *Transl Cancer Res.* (2021) 10:5150–8. doi: 10.21037/tcr-21-1470
41. Hirsch L, Bellesoeur A, Boudou-Rouquette P, Arrondeau J, Thomas-Schoemann A, Kirchgesner J, et al. The impact of body composition parameters on severe toxicity of nivolumab. *Eur J Cancer.* (2020) 124:170–7. doi: 10.1016/j.ejca.2019.11.003
42. Crane JD, MacNeil LG, Lally JS, Ford RJ, Bujak AL, Brar IK, et al. Exercise-stimulated interleukin-15 is controlled by AMPK and regulates skin metabolism and aging. *Aging Cell.* (2015) 14:625–34. doi: 10.1111/acel.12341
43. Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. *J Clin Oncol.* (2015) 33:74–82. doi: 10.1200/JCO.2014.57.3329
44. Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. *Aging Cell.* (2018) 17:e12750. doi: 10.1111/acel.12750
45. Saini J, McPhee JS, Al-Dabbagh S, Stewart CE, Al-Shanti N. Regenerative function of immune system: modulation of muscle stem cells. *Ageing Res Rev.* (2016) 27:67–76. doi: 10.1016/j.arr.2016.03.006

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Adherence to a Paleolithic Diet in Combination With Lifestyle Factors Reduces the Risk for the Presence of Non-Alcoholic Fatty Liver Disease: A Case-Control Study

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Background: Evidence suggests the role of changing traditional lifestyle patterns, such as Paleolithic, to the modern lifestyle in the incidence and epidemic of chronic diseases. The purpose of this study was to investigate the associations between the Paleolithic diet (PD) and the Paleolithic-like lifestyle and the risk of non-alcoholic fatty liver disease (NAFLD) among an adult population.

Materials and methods: This case-control study was carried out among 206 patients with NAFLD and 306 healthy subjects aged >18 years. PD score was evaluated using a validated 168-item quantitative food frequency questionnaire. In addition, to calculate the Paleolithic-like lifestyle score, the components of physical activity, body mass index (BMI), and smoking status of the participants were combined with the score of the PD.

Results: The mean PD and Paleolithic-like lifestyle scores were 38.11 ± 5.63 and 48.92 ± 6.45, respectively. After adjustment for potential confounders, higher scores of adherence to the PD diet conferred a protection for the presence of NAFLD [odds ratio (OR): 0.53; 95% confidence interval (CI): 0.28–0.98; *P* for trend = 0.021]. Furthermore, PD and healthy lifestyle habits were negatively associated with NAFLD (OR = 0.42, 95% CI 0.23–0.78; *P* for trend = 0.007).

Conclusion: Our data suggest that the PD alone and in combination with lifestyle factors was associated with decreased risk of NAFLD in a significant manner in the overall population. However, prospective studies are needed to further investigate this association.

Keywords: NAFLD, Paleolithic, diet, lifestyle, chronic diseases, case-control

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive hepatic steatosis in the absence of other identifiable causes, such as excessive alcohol use and viral hepatitis (1). According to the systematic reviews and meta-analysis published in 2016, the estimated pooled prevalence of NAFLD worldwide is 25.2% (2), while in Iran, this prevalence is ∼34% (3).

Non-alcoholic fatty liver disease is considered the most common cause of chronic liver disease worldwide, as well as it can affect extra-hepatic organs, increasing the risk for type 2 diabetes mellitus, cardiovascular diseases, and chronic kidney disease (2, 4). Moreover, patients with NAFLD have an increased risk of all-cause mortality (5), highlighting the need for efforts to prevent, treat, and/or retard the progression of the disease.

The pathophysiology underlying this disorder is complex and incompletely understood. A decade ago, proposed that a "multiple parallel hits hypothesis" in which insulin resistance, lipids, mitochondrial function, innate immunity, intestinal microbiota, genetic determinants, epigenetic mechanisms, environmental factors, cytokines, and lifestyle contribute to the evolution of inflammation and fibrosis in NAFLD (6–10) so that lifestyle interventions can be important for the management of patients with NAFLD (11). An international panel of experts proposes clear and simple criteria for a diagnosis of the fatty liver. The diagnosis is based on the recognition of underlying abnormalities in metabolic health with an acceptance that "MAFLD" instead of NAFLD may commonly co-exist with other conditions (12).

Modification in diet composition and eating patterns may be a sustainable approach to NAFLD prevention and treatment (13). A dietary pattern of growing interest is the Paleolithic diet (PD) pattern, also known as the Hunter-Gatherer or Paleo diet (14). This diet pattern, modeled after diets of people who lived as hunter-gathers during the Paleolithic Era, is characterized as a predominantly plant food-based diet, with a wide diversity of fruits, nuts, and vegetables, such as wild-plant foods that with high amounts of calcium and other minerals, includes lean meat, and is low in dairy, grains, sugar, and salt (15). Based on the nutrient profile, a PD may have potential benefits for the prevention and treatment of NAFLD (13). Nevertheless, there are relatively few studies that have examined its effects on NAFLD (13, 16).

The Paleolithic diet score was constructed as discordance between diet during the Paleolithic period and the present era (17, 18). However, considering the cumulative association between diet and lifestyle factors, studies have used this score alone or in combination with lifestyle factors (Paleolithic-like lifestyle score) in relation to the incidence of some chronic diseases and mortality, showing inverse associations (17–19). However, studies investigating the relationship between these scores in the NAFLD are still scarce.

Thus, the present study aimed to investigate the associations between PD scores alone and in combination with lifestyle factors and the risk of NAFLD among Iranian adults.

SUBJECTS AND METHODS

Study Design and Population

The study protocol was ethically approved by the Regional Bioethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (No: IR.SBMU.RETECH.REC.1400.802). The required sample size for the current work was calculated based on the hypothesis of 1.5 times decreased odds of NAFLD by PD in combination with lifestyle factors. Therefore, considering a type I error of 5%, the study power of 80%, and the ratio of controls to cases as ∼1.5, we needed 206 cases and 306 controls for this project. In this case-control study, the newly diagnosed patients with NAFLD and healthy controls aged >18 years who attended the Hazrat Rasoul Hospital, Tehran, Iran between 2020 and 2021 were selected. NAFLD diagnosis (20–22) was based on the following criteria: chronic increment in liver enzymes (liver enzymes >19 U/L for women and >30 U/L for men), abstinence from alcohol intake, liver ultrasound compatible with NAFLD, having grades II and III NAFLD based on liver biopsy, and exclusion of other causes of liver disorders. Moreover, the case group was referred to our hospital for evaluation by FibroScan (20), with the FibroScan results showing a Controlled Attenuation Parameter (CAP) score of more than 237 and a fibrosis score of more than 7, and had the diagnosis of NAFLD confirmed by a gastroenterologist. Additionally, individuals without a history of NAFLD were recruited from the same hospital for the control group. The control group was selected from other outpatient clinics of our hospital, e.g., ophthalmology, otorhinolaryngology, and dermatology. The controls were required to have no history of chronic or inflammatory diseases (e.g., diabetes, cancer, gastrointestinal, or cardiovascular disorders, etc.) and to have a regular diet for the past 6 months. Two dietitians (Mh.S. and E.M.) monitored the sampling of the patients. The inclusion criteria for the control group were defined based on laboratory tests and the liver ultrasound (not suffering from any stages of hepatic steatosis). The individuals with long-term diet changes (due to a particular disease or weight loss), a history of renal and (or) hepatic disease (Wilson's disease, autoimmune liver disease, cirrhosis, non-alcoholic steatohepatitis (NASH), hemochromatosis, viral infections, or alcoholic fatty liver disease), cardiovascular disease, diabetic patients, malignancy, thyroid disorder, and autoimmune disease were not included in the study. At the beginning of the study, all the participants were asked to carefully answer the demographic, economic, and social questionnaire questions that evaluated age, employment status, education, smoking history, disease history status, use of specific medications (other than regular NAFLD drugs), and history of dieting in the past 6 months. The physical activity levels of the participants were estimated by the use of a validated short form of the International Physical Activity Questionnaire (Short IPAQ) (23). Data collection was conducted through interviews by a trained nutritionist.

Dietary Assessment

The dietary intake over the previous year was obtained using a validated semi-quantitative food-frequency questionnaire (FFQ),

which consisted of 168 food items (24). The FFQ consisted of a list of usual Iranian dietary items with standard serving sizes. For each food item, the average portion size consumed and the frequency of intake were obtained from self-reports on the FFQ. The frequency of intake for each food item included never, 2–3 times/month, 1 time/week, 2–4 times/week, 5–6 times/week, and daily. The portion sizes were reported in grams by using standard Iranian household measures (25). The daily nutrient consumption for each person were estimated by applying the United States Department of Agriculture's (USDA) National Nutrient Databank (26). The Nutritionist IV software (First Databank, San Bruno, CA, USA—modified for Iranian foods) was used to calculate the daily energy and nutrient intake of each participant.

PD and Paleolithic-Like Lifestyle Scores Measurement

The study of Whalen et al. (17) was used to calculate the score of adherence to the PD. In summary, the food items obtained from each individual using the FFQ were divided into 14 food groups (such as vegetables, fruits, fruit and vegetable diversity score, lean meat, fish, nuts, and calcium as more PD characteristics and red and processed meat, dairy foods, sugar-sweetened beverages, baked goods, grains and starches, sodium, and alcohol as less characteristic PD). The fruit and vegetable diversity score in our study was defined as the number of components of the fruit and vegetable group consumed by each person. In order to score each food group, the intake of each food component was classified as quintiles (from 1 to 5, with "1" scores the minimum consumption in each food group and "5" scores the maximum) according to the distribution of consumption of the study population. PD scores ranged from 13 to 65 and higher scores indicated higher levels of adherence to the PD.

To calculate the Paleolithic-like lifestyle score according to the study of Cheng et al. (27), the components of physical activity, body mass index (BMI), and smoking status of the participants were combined with the score of the PD. Lifestyle factors, with the exception of smoking status, were classified according to the tertile distribution. For physical activity, a score of 5 was assigned to individuals with the highest tertile, and scores of 3 and 1 were assigned to middle and lower tertiles, respectively. In contrast, the scoring was reversed for BMI. In addition, for smoking status, scores of 5, 3, and 1 were assigned to non-smokers, ex-smokers, and smokers, respectively. Finally, all diet and lifestyle values for each participant were summarized to reflect adherence to the Paleolithic-like lifestyle score. The final score range in our study ranged from 16 to 80, and higher scores indicated better adherence.

Because all participants in the study were Muslim and did not consume alcohol, alcohol intake was not considered a component of these scores in our study.

Anthropometric Measurement

An anthropometric evaluation was performed by the researchers. The patient's weight was measured with a Seca Portable Digital Scale made in Germany with an accuracy of 100 g with minimal coverage and without shoes. The patient's height needs were assessed with a Seca portable height gauge with an accuracy of 0.1 cm. In addition, the waist circumference (WC) in the middle area between the iliac crown and the last gear was determined with a Seca waist measuring device. The hip circumference was also measured in centimeters using the same measuring tape at its widest portion of the buttocks, with the tape parallel to the floor. BMI, after measuring weight and height with the mentioned method, was calculated using the formula weight (kg)/height^2 (m). All anthropometric measurements were performed by the researcher to minimize observational variations.

Biochemical Measurement

At the beginning and end of the study, after 10–12 h of fasting, 10 ml of venous blood was taken from the subjects by the laboratory technician. After clotting in the environment, the serum was isolated as soon as possible by centrifugation and kept at −70°C until sent to the laboratory for testing. Triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose (FPG) concentration were measured using the Pars Azmoon Company Kit (ParsAzmun, Tehran, Iran) and enzymatic colorimetric method. Total cholesterol (TC) concentration was measured by enzyme photometry using the Pars Test Kit (ParsAzmun, Tehran, Iran). Low-density lipoprotein cholesterol (LDL-C) concentration was also calculated using the Friedewald formula (28): LDL-C = TC–TG/6–(HDL-C). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by commercially available enzymatic reagents (Pars Azmoon, Tehran, Iran) on auto analysis (BT-3000).

Statistical Analysis

The Statistical Package Software for Social Science, version 21 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Kolmogorov-Smirnov's test and histogram charts were used to test the normality of the data. The baseline characteristics and dietary intakes were reported as mean ± standard deviation (SD) for quantitative variables, and number and percentages for qualitative variables. We compared the data between two groups using independent sample *t*-tests and chi-squared tests for continuous and categorical variables, respectively. The association of PD and Paleolithic-like lifestyle scores with the risk of NAFLD was assessed by applying logistic regression. The analyses were adjusted for probable confounders, e.g., sex and BMI, weight, WC, hip circumference, physical activity, smoking, education, and energy intake, fasting blood glucose (FBG), ALT, TG, HDL-C, and LDL-C. The odds ratio (OR) with a 95% confidence interval (CI) of NAFLD across quartiles of scores was calculated. *P*-values <0.05 were considered statistically significant.

RESULTS

The mean (±SD) age of the study population was 37.84 ± 8.70 years. The mean (±SD) BMI was 26.99 ± 4.32 kg/m^2. The mean PD and Paleolithic-like lifestyle scores were 38.11 ± 5.63 and 48.92 ± 6.45, respectively.

TABLE 1 | Demographic, anthropometric, and lifestyle characteristics of participants in the case and control groups.

Variables	Groups, mean (SD)		*P*-value[a]
	With NAFLD (*n* = 206)	**Without NAFLD (*n* =306)**	
Age, years	38.15 (8.43)	37.63 (8.88)	0.508
Female, *n* (%)	125 (60.7)	233 (76.1)	<0.001
BMI[b], kg/m^2	30.36 (3.77)	24.71 (2.97)	<0.001
Weight, kg	83.80 (10.12)	66.92 (8.87)	<0.001
Waist-circumference (cm)	102.34 (7.33)	85.17 (7.10)	<0.001
Hip-circumference (cm)	104.56 (8.36)	96.14 (5.77)	<0.001
Physical activity (Met.h/week)	1,114.57 (617.50)	1,625.59 (930.51)	<0.001
Smoking (yes), *n* (%)	16 (7.8)	8 (2.6)	0.007
Drug use (other than regular NAFLD drugs) (yes), *n* (%)	9 (4.4)	7 (2.3)	0.184
Metformin	7 (3.4)	3 (1.0)	
Atorvastatin	2 (1.0)	4 (1.3)	
ALT (mg/dl)	58.50 ±24.1	20.53 ±13.01	<0.001
AST (mg/dl)	34.88 ±17.2	23.76 ± 9.6	0.17
FPG (mg/dl)	109.28 (39.39)	92.20 (36.22)	<0.001
TC (mg/dl)	184.79 (54.93)	182.84 (39.36)	0.728
TG (mg/dl)	180.39 (123.81)	130.98 (64.29)	<0.001
LDL-C (mg/dl)	121.16 (43.03)	109.13 (31.07)	0.007
HDL-C (mg/dl)	41.26 (16.71)	48.05 (10.87)	<0.001
Paleolithic diet (PD) score	41.4 (5.5)	44.2 (5.4)	<0.001
Paleolithic-like lifestyle scores	50.4 (5.5)	56.2 (5.9)	<0.001
Education status, *n* (%)	28 (13.6)	37 (12.1)	<0.001
Lower than high school			
High school	81 (39.3)	112 (36.6)	
Higher than high school	97 (47.1)	166 (51.3)	

[a]*Obtained from ANOVA for continuous variables and chi-square for categorical variables.*

[b]*BMI: body mass index.*

FPG, fasting plasma glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

The baseline characteristics of the study subjects are shown in **Table 1**. Compared with controls, NAFLD subjects had significantly higher BMI, weight, WC, hip circumference, ALT, FBG, TG, and LDL-C concentration, but had lower physical activity and HDL-C concentration. There was also a significant difference in the level of education between the case and control groups. However, no significant differences were found for other characteristics among cases and controls.

Table 2 illustrates the macro- and micro-nutrients and the intakes of food groups in NAFLD patients vs. controls. NAFLD subjects had higher intakes of energy, carbohydrate, total fat, zinc, folate, total dairy, refined grains, and red and processed meats but lower intakes of total fiber, vitamin D, and vegetables as compared to controls. There were no significant differences between the NAFLD group and controls for all other dietary intakes.

General characteristics and dietary intake of subjects across the quartiles of PD scores are presented in **Table 3**. Compared with those in the lowest quartile of PD, subjects in the highest quartile had higher age but had lower weight and WC. No other significant difference was found in other general characteristics across quartiles of PD. In addition, individuals in the highest quartiles of PD had a higher intake of protein, saturated fatty acids (SFAs), cholesterol, potassium, iron, calcium, magnesium, zinc, vitamin C, total dairy, nut, fish, fruits, and vegetables, as well as a lower intake of sodium and refined grains.

The ORs and 95% CIs for NAFLD subjects based on quartiles of PD and Paleolithic-like lifestyle scores are reported in **Table 4**.

In crude and first adjusted model (based on age and BMI), there was a significant association for PD score in the highest quartile when compared with the lowest quartile (OR = 0.35, 95% CI 0.20–0.59; *P* for trend <0.001; OR = 0.56, 95% CI 0.29–0.95; *P* for trend = 0.018, respectively). Furthermore, after adjusting for confounders according to the final model, higher scores of adherence to the PD conferred a protection for the presence of NAFLD (OR: 0.53; 95% CI: 0.28–0.98; *P* for trend = 0.021). There was a significant relationship between reduced odds of NAFLD for those subjects with the highest score of Paleolithic-like lifestyle, when compared to subjects with the lowest score, both in the crude (OR = 0.42, 95% CI 0.24–0.73;

TABLE 2 | Dietary intakes of study participants across case and control groups.

	Groups, mean (SD)		*P*-value[a]
	With NAFLD ($n = 206$)	Without NAFLD ($n = 306$)	
Nutrients			
Energy (Kcal/day)	2,360.71 (583.87)	2,212.76 (625.54)	0.007
Carbohydrate (g/day)	338.19 (96.99)	319.69 (102.10)	0.041
Protein (g/day)	79.09 (23.12)	74.96 (23.70)	0.052
Fat (g/day)	82.76 (29.23)	76.72 (27.95)	0.019
SFA (g/day)	27.52 (10.83)	26.18 (10.62)	0.166
Cholesterol (mg/day)	225.40 (116.10)	234.43 (155.78)	0.478
Fiber (g/day)	36.33 (18.99)	40.46 (23.42)	0.029
Sodium (mg/day)	4,669.27 (4,289.44)	4,281.67 (2,609.36)	0.205
Potassium (mg/day)	3,649.90 (1,211.97)	3,539.10 (1,318.38)	0.336
Iron (mg/day)	27.07 (13.03)	25.59 (3.85)	0.226
Calcium (mg/day)	1,251.37 (453.92)	1,181.10 (447.17)	0.084
Magnesium (mg/day)	378.58 (115.44)	360.72 (132.99)	0.117
Zinc (mg/day)	11.66 (3.49)	10.95 (3.60)	0.026
Vitamin C (mg/day)	136.69 (83.83)	131.45 (88.73)	0.503
Folate (mcg/day)	552.33 (170.33)	510.68 (153.00)	0.004
Vitamin E (mg/day)	11.86 (4.50)	11.15 (5.37)	0.116
Vitamin D (mcg/day)	1.71 (1.34)	2.05 (1.66)	0.014
Caffeine (mg/day)	132.81 (118.55)	132.27 (122.22)	0.961
Food groups			
Total dairy (g/day)	481.04 (251.80)	421.68 (240.13)	0.007
Legume (g/day)	17.43 (26.09)	16.07 (18.86)	0.492
Nut (g/day)	6.60 (8.83)	6.47 (9.43)	0.873
Coffee (g/day)	17.71 (61.32)	15.63 (53.26)	0.684
Fish (g/day)	9.37 (8.96)	9.18 (7.83)	0.808
Whole grains (g/day)	91.50 (87.38)	99.54 (114.65)	0.394
Refined grains (g/day)	382.95 (219.80)	316.09 (153.74)	<0.001
Red and processed meat (g/day)	32.40 (25.12)	23.11 (19.58)	<0.001
Fruits (g/day)	355.72 (264.11)	353.06 (280.78)	0.914
Vegetables (g/day)	249.88 (129.32)	281.56 (142.78)	0.011

SFA, saturated fatty acid.
[a]*Obtained from ANOVA.*

P for trend = 0.005) and the final adjustment (OR = 0.42, 95% CI 0.23–0.78; *P* for trend = 0.007) models.

DISCUSSION

In the present study, the consumption of PD was associated with a lower chance of occurrence of NAFLD. Furthermore, the Paleolithic-type lifestyle, characterized by the combination of the PD and healthy lifestyle habits, was also negatively associated with NAFLD.

Our results suggest that adherence to the PD can protect the development of NAFLD. Recently, Fraczek et al. (29) in a meta-analysis study of randomized clinical trials evidenced the health benefits of adopting the PD. This meta-analysis found that even with short-term consumption, the PD contributed to normalizing blood pressure, improving lipid profile, with a reduction in total cholesterol (TC), TG, and LDL-C and increased HDL-C, sensitivity to insulin and glucose tolerance, and decreased body weight, fat mass, and WC. These results were also confirmed by Shah et al. (19) in a French prospective study, in which the PD and adherence to a Paleolithic lifestyle proved to be favorable alternatives for the prevention of type 2 diabetes and hypertension. Furthermore, it has been suggested that adherence to the PD was associated with a lower chance of systemic inflammation and oxidative stress (30). Considering the evidence presented and the results found in the present study, the PD can be an effective strategy for the prevention of NAFLD.

The potential benefits of PD on health may be due to a food composition that is marked by high consumption of fruits, vegetables, fish, and nuts, as well as limiting the consumption of processed and ultra-processed foods, added sugar, salt, and vegetable oils (31). As a result, a diet rich in antioxidants, fibers, monounsaturated and polyunsaturated fatty acids, potassium

TABLE 3 | Socio-demographic characteristics, anthropometric variables, and dietary intake across the quartiles of Paleolithic diet (PD) score.

	Quartiles of PD score				*P*-value
	Q1	Q2	Q3	Q4	
Demographic variables					
Age, years	36.56 (8.21)	36.92 (8.73)	37.37 (7.79)	40.51 (8.9)	0.001
Female, *n* (%)	88 (68.8)	85 (65.9)	94 (74.0)	91 (71.1)	0.537
BMI kg/m^2	27.1 (4.17)	27.09 (4.38)	27.44 (4.34)	26.30 (4.35)	0.189
Weight, kg	75.05 (11.65)	73.60 (12.86)	75.99 (12.95)	70.23 (11.93)	0.001
Waist-circumference (cm)	93.98 (9.94)	92.41 (11.76)	92.47 (11.43)	89.45 (10.72)	0.010
Hip-circumference (cm)	100.25 (7.38)	99.46 (7.67)	100.06 (9.91)	98.36 (6.96)	0.235
Physical activity (Met.h/week)	1,479.32 (856.11)	1,448.03 (903.68)	1,345.53 (782.58)	1,454.54 (863.20)	0.608
Smoking (yes), *n* (%)	6 (4.7)	3 (2.3)	11 (8.7)	4 (3.1)	0.079
Disease history (yes), *n* (%)	6 (4.7)	1 (0.8)	1 (0.8)	8 (6.3)	0.055
Drug use (yes), *n* (%)	6 (4.7)	6 (4.7)	4 (3.1)	0 (0.0)	0.104
ALT (mg/dl)	34.17 (22.25)	36.58 (52.15)	36.63 (39.12)	26.09 (38.18)	0.316
AST (mg/dl)	29.13 (20.15)	28.75 (16.91)	25.61 (17.58)	24.12 (29.17)	0.119
FPG (mg/dl)	107.51 (55.52)	98.63 (34.93)	94.30 (28.20)	91.98 (28.22)	0.077
TC (mg/dl)	189.31 (42.10)	185.43 (47.86)	181.01 (45.46)	178.81 (44.58)	0.509
TG (mg/dl)	145.26 (65.48)	155.45 (82.95)	152.36 (130.93)	134.44 (62.33)	0.510
LDL-C (mg/dl)	120.74 (40.28)	112.86 (35.81)	108.83 (30.71)	110.16 (35.52)	0.194
HDL-C (mg/dl)	44.91 (11.94)	45.36 (13.03)	46.97 (12.27)	46.87 (12.27)	0.736
Dietary intake					
Energy (Kcal/day)	2,210.16 (621.36)	2,255.54 (600.48)	2,274.48 (650.04)	2,349.11 (576.52)	0.331
Carbohydrate (g/day)	320.72 (97.08)	323.09 (106.81)	325.04 (103.38)	339.70 (93.84)	0.426
Protein (g/day)	71.56 (23.68)	74.91 (22.21)	76.49 (24.26)	83.54 (22.55)	<0.001
Fat (g/day)	75.30 (28.46)	79.33 (26.90)	80.62 (31.05)	81.36 (27.80)	0.331
SFA (g/day)	24.18 (9.50)	26.72 (9.83)	27.21 (11.12)	28.77 (11.86)	0.007
Cholesterol (mg/day)	203.50 (104.48)	228.97 (190.54)	229.86 (110.26)	260.86 (137.41)	0.014
Fiber (g/day)	38.79 (26.66)	36.60 (19.74)	37.26 (18.25)	39.31 (18.15)	0.703
Sodium (mg/day)	5,174.65 (4,737.77)	4,356.74 (2,979.33)	4,353.93 (2,670.70)	3,865.12 (2,614.64)	0.019
Potassium (mg/day)	2,897.19 (1,004.50)	3,406.99 (1,155.69)	3,627.33 (1,143.36)	4,404.92 (1,310.24)	<0.001
Iron (mg/day)	24.25 (10.95)	24.85 (12.28)	25.82 (12.58)	29.84 (17.00)	0.004
Calcium (mg/day)	1,069.75 (434.33)	1,187.25 (441.69)	1,182.44 (419.21)	1,398.01 (448.60)	<0.001
Magnesium (mg/day)	334.66 (121.38)	357.93 (133.32)	371.52 (33.78)	407.65 (105.23)	<0.001
Zinc (mg/day)	10.56 (3.44)	11.16 (3.52)	11.14 (3.66)	12.08 (3.52)	0.007
Vitamin C (mg/day)	78.89 (44.81)	123.42 (90.13)	142.08 (64.99)	189.99 (97.43)	<0.001
Folate (mcg/day)	539.98 (178.94)	517.38 (159.72)	520.56 (153.93)	531.85 (152.19)	0.660
Vitamin E (mg/day)	10.96 (4.86)	11.31 (4.42)	11.64 (5.05)	11.84 (5.77)	0.533
Vitamin D (mcg/day)	1.64 (1.42)	1.76 (1.49)	1.85 (1.39)	2.40 (1.75)	<0.001
Caffeine (mg/day)	128.76 (125.16)	124.12 (101.01)	135.15 (113.79)	142.00 (139.94)	0.661
Food groups					
Total dairy (g/day)	395.26 (245.21)	442.37 (233.14)	422.51 (213.92)	521.96 (273.79)	<0.001
Legume (g/day)	17.23 (26.11)	14.90 (17.05)	15.48 (16.63)	18.85 (26.38)	0.471
Nut (g/day)	3.54 (5.06)	5.50 (5.29)	6.95 (9.95)	10.12 (12.81)	<0.001
Coffee (g/day)	10.22 (29.65)	18.78 (69.35)	17.77 (57.45)	19.09 (61.91)	0.549
Fish (g/day)	6.49 (6.39)	7.99 (6.22)	9.50 (7.81)	13.07 (10.61)	<0.001
Whole grains (g/day)	96.30 (106.01)	102.06 (129.65)	95.44 (100.36)	91.37 (75.68)	0.877
Refined grains (g/day)	429.21 (201.38)	346.40 (166.89)	326.55 (176.91)	269.65 (161.89)	<0.001
Red and processed meat (g/day)	30.12 (20.62)	27.81 (19.95)	25.78 (17.37)	23.66 (29.57)	0.119
Fruits (g/day)	176.57 (115.13)	324.89 (258.85)	384.00 (229.34)	531.52 (322.05)	<0.001
Vegetables (g/day)	183.68 (99.48)	243.22 (122.17)	284.96 (117.37)	363.74 (144.93)	<0.001

Values are expressed as means [standard deviation (SD)] of 512 subjects.
P-values are resulted from the student's t-test.

TABLE 4 | Odds ratio (OR) and 95% confidence interval (CI) for NAFLD based on Paleolithic diet (PD score) alone and in combination with lifestyle factors (combined score).

	Quartiles of scores				*P* for trend
	Q1	Q2	Q3	Q4	
PD score					
Case/total	59/128	55/128	48/128	44/128	
Crude model	1.00 (Ref)	0.70 (0.42–1.15)	0.42 (0.25–0.70)	0.35 (0.20–0.59)	<0.001
Model 1*	1.00 (Ref)	0.74 (0.44–1.23)	0.59 (0.31–0.91)	0.56 (0.29–0.95)	0.018
Model 2	1.00 (Ref)	0.79 (0.46–1.35)	0.58 (0.31–0.94)	0.54 (0.29–0.98)	0.020
Model 3	1.00 (Ref)	0.80 (0.47–1.37)	0.55 (0.32–0.96)	0.53 (0.28–0.98)	0.021
Combined score					
Case/total	68/128	59/128	47/128	36/128	
Crude model	1.00 (Ref)	0.66 (0.38–1.14)	0.68 (0.39–1.19)	0.42 (0.24–0.73)	0.005
Model 1†	1.00 (Ref)	0.66 (0.38–1.15)	0.73 (0.41–1.28)	0.45 (0.25–0.79)	0.012
Model 2¥	1.00 (Ref)	0.77 (0.42–1.39)	0.69 (0.39–1.36)	0.44 (0.24–0.83)	0.006
Model 3	1.00 (Ref)	0.72 (0.39–1.33)	0.65 (0.35–1.20)	0.42 (0.23–0.78)	0.007

Binary logistic regression was used to obtain OR and 95% CI.

**Model 1: adjusted for sex and BMI.*

†*Model 1: adjusted for sex.*

‡*Model 2: Model 1 + weight, hip circumference, physical activity, smoking, education, and energy intake.*

¥*Model 2: Model 1 + weight, hip circumference, education, and energy intake.*

Model 3: Model 2 + fasting plasma glucose, ALT, triglyceride, HDL-C, and LDL-C.

(32), vitamins B, D, E, and K, coenzyme Q10, alpha lipoic acid, and polyphenol acid (29).

Evidence suggests that micronutrients, such as vitamins A, C, D, and E, carotenoids, zinc, copper, iron, selenium, and magnesium, may have beneficial effects on NAFLD, due to its antioxidant, antifibrotic, immunomodulatory, and lipoprotective properties (33).

The findings of the present study are consistent with recent studies that have highlighted the protective role of healthy eating against NAFLD. In a case-control study conducted with Iranian adults, it was observed that the consumption of a dietary pattern characterized by the consumption of vegetables, legumes, fruits, and dairy products with low-fat content was associated with lower chances of occurrence of NAFLD (34). Another case-control study with Iranian adults (35) found that NAFLD was inversely associated with a healthy dietary pattern, which consisted of relatively high consumption of fish, skinless poultry, low-fat dairy products, fruits, vegetables, nuts, oil, and garlic. In this study, the authors found that individuals in the highest tertile of the healthy eating pattern scores had lower odds of NAFLD than those in the lowest tertile, even after adjustment for potential confounding factors (OR: 0.30; 95% CI: 0.13–0.68).

It is already established in the literature that unhealthy eating, characterized by a diet rich in calories, sugars, saturated fats, low in polyunsaturated fatty acids, fiber, and micronutrients, is a determining factor for both the development and progression of NAFLD (36). Recently, a review article highlighted that individuals with NAFLD share a common dietary pattern, identified by low consumption of whole grains, cereals, fruits, and vegetables and high consumption of red meat, offal, refined grains, and sugars (37). In addition, evidence from a systematic review and meta-analysis demonstrated that Western dietary patterns containing high consumption of processed foods, red meats, high-fat dairy products, and refined grains can significantly increase the occurrence of NAFLD (OR: 1.56; 95% CI: 1.27–1.92). In addition, pieces of evidence showed that cooking meat at high temperatures for a long duration forms heterocyclic amines (HCAs), which are related to oxidative stress and NAFLD (38). In a systematic review study (39), in line with our results, it has been stated that the PD has beneficial effects on various risk factors related to NAFLD in various ways. So PD modulates hyperglycemic carbohydrates and, on the other hand, by eliminating insulin-tropic dairy, regulates insulin/insulin-like growth factor 1(IGF-1) signaling, which has recently been recognized as one of the risk factors associated with NAFLD (39, 40). The study also found that PD improved insulin resistance and dyslipidemia, thereby preventing the development of NAFLD (39). This diet also has a lower fructose content than other common diets, which can also prevent the development of NAFLD (39).

It is important to note that the Paleolithic lifestyle score also played a protective role against NAFLD in our study. This demonstrates that in addition to diet, each of the healthy lifestyle factors, which included physical activity, adequate BMI, and not smoking, played an important role in preventing NAFLD. It has been shown that NAFLD is affected by obesity, sedentary lifestyle, and smoking, in addition to other individual factors and environments (41).

Regarding anthropometric and metabolic markers, BMI, WC, FPG, TG, and LDL-C were significantly higher in the NAFLD group as compared to the control group, while HDL-C levels were significantly lower in cases as compared to controls. Similar

results were observed in other publications (42, 43). This finding is expected since NAFLD is closely linked to obesity, insulin resistance, and dyslipidemia (44), as well as being considered the hepatic manifestation of the metabolic syndrome (45).

This study has strengths and limitations. Among the limitations, the study design makes it impossible to assess the causal relationship. Another limitation is that despite the adjustment for several possible confounding factors, it is not possible to exclude the possibility of the existence of some potential confounding factor that has not been included in the analyses. In addition, self-reported information about food consumption can cause recall bias.

The current research has several strengths. As far as we know, this is the first study to assess the association between consumption of the PD alone and in combination with lifestyle factors and the risk of NAFLD among Iranian adults, in which trained personnel was employed to interview and collect food frequency questionnaires. Our sample size was sufficient and we tried to eliminate the impact of confounders by adjusting for a wide range of variables and by using a validated questionnaire. In addition, we could not examine the consumption of alcohol due to the fact that the participants were Muslims can be another limitation of this study. However, because both the control group and the study case group did not consume alcohol, a review of this component of the PD could not affect the results.

In conclusion, by calculating the study power by almost 80%, the present study provides pieces of evidence that PD and adherence to a healthy lifestyle were associated with decreased risk of NAFLD. Our results support previous findings of the protective role of healthy eating and living habits for NAFLD. Furthermore, they can serve as strategies for preventing and even controlling the progression of NAFLD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the research council and Ethics Committee Iran University of Medical Sciences, Tehran, Iran. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AH and MS contributed in conception, design, statistical analysis, and supervised the study. MS, BO, EM, and SF contributed to data collection and manuscript drafting. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Rinella ME, Sanyal AJ. NAFLD in 2014: genetics, diagnostics and therapeutic advances in NAFLD. *Nat Rev Gastroenterol Hepatol*. (2015) 12:65–6. doi: 10.1038/nrgastro.2014.232
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of non-alcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. (2016) 64:73–84. doi: 10.1002/hep.28431
3. Moghaddasifar I, Lankarani KB, Moosazadeh M, Afshari M, Ghaemi A, Aliramezany M, et al. Prevalence of non-alcoholic fatty liver disease and its related factors in Iran. *Int J Organ Transplant Med*. (2016) 7:149–60.
4. Mikolasevic I, Milic S, Turk Wensveen T, Grgic I, Jakopcic I, Stimac D, et al. Non-alcoholic fatty liver disease - A multisystem disease? *World J Gastroenterol*. (2016) 22:9488–505. doi: 10.3748/wjg.v22.i43.9488
5. Liu Y, Zhong GC, Tan HY, Hao FB, Hu JJ. Non-alcoholic fatty liver disease and mortality from all causes, cardiovascular disease, and cancer: a meta-analysis. *Sci Rep*. (2019) 9:11124. doi: 10.1038/s41598-019-47687-3
6. Mirmiran P, Amirhamidi Z, Ejtahed HS, Bahadoran Z, Azizi F. Relationship between diet and non-alcoholic fatty liver disease: a review article. *Iran J Public Health*. (2017) 46:1007–17.
7. Qiu S, Cai X, Sun Z, Li L, Zügel M, Steinacker JM, et al. Association between physical activity and risk of non-alcoholic fatty liver disease: a meta-analysis. *Thrapeutic Adv Gastroenterol*. (2017) 10:701–13. doi: 10.1177/1756283X17725977
8. Li L, Liu DW, Yan HY, Wang ZY, Zhao SH, Wang B. Obesity is an independent risk factor for non-alcoholic fatty liver disease: evidence from a meta-analysis of 21 cohort studies. *Obes Rev*. (2016) 17:510–9. doi: 10.1111/obr.12407
9. Akhavan Rezayat A, Dadgar Moghadam M, Ghasemi Nour M, Shirazinia M, Ghodsi H, Rouhbakhsh Zahmatkesh MR, et al. Association between smoking and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *SAGE Open Med*. (2018) 6:2050312117745223. doi: 10.1177/2050312117745223
10. Tilg H, Adolph TE, Moschen AR. Multiple parallel hits hypothesis in non-alcoholic fatty liver disease: revisited after a decade. *Hepatology*. (2021) 73:833. doi: 10.1002/hep.31518
11. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol*. (2017) 67:829–46. doi: 10.1016/j.jhep.2017.05.016
12. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol*. (2020) 73:202–9. doi: 10.1016/j.jhep.2020.07.045
13. Moore MP, Cunningham RP, Dashek RJ, Mucinski JM, Rector RS. A fad too far? Dietary strategies for the prevention and treatment of NAFLD. *Obesity*. (2020) 28:1843–52. doi: 10.1002/oby.22964
14. Konner M, Eaton SB. Paleolithic nutrition: twenty-five years later. *Nutr Clin Pract*. (2010) 25:594–602. doi: 10.1177/0884533610385702
15. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. *N Engl J Med*. (1985) 312:283–9. doi: 10.1056/NEJM198501313120505
16. Perumpail BJ, Cholankeril R, Yoo ER, Kim D, Ahmed A. An overview of dietary interventions and strategies to optimize the management of non-alcoholic fatty liver disease. *Diseases*. (2017) 5:23. doi: 10.3390/diseases5040023

17. Whalen KA, Judd S, McCullough ML, Flanders WD, Hartman TJ, Bostick RM. Paleolithic and Mediterranean diet pattern scores are inversely associated with all-cause and cause-specific mortality in adults. *J Nutr*. (2017) 147:612–20. doi: 10.3945/jn.116.241919
18. Whalen KA, McCullough M, Flanders WD, Hartman TJ, Judd S, Bostick RM. Paleolithic and Mediterranean diet pattern scores and risk of incident, sporadic colorectal adenomas. *Am J Epidemiol*. (2014) 180:1088–97. doi: 10.1093/aje/kwu235
19. Shah S, MacDonald CJ, El Fatouhi D, Mahamat-Saleh Y, Mancini FR, Fagherazzi G, et al. The associations of the palaeolithic diet alone and in combination with lifestyle factors with type 2 diabetes and hypertension risks in women in the E3N prospective cohort. *Eur J Nutr*. (2021) 60:3935–45. doi: 10.1007/s00394-021-02565-5
20. Yamamura S, Eslam M, Kawaguchi T, Tsutsumi T, Nakano D, Yoshinaga S, et al. MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. *Liver Int*. (2020) 40:3018–30. doi: 10.1111/liv.14675
21. Piazzolla VA, Mangia A. Non-invasive diagnosis of NAFLD and NASH. *Cells*. (2020) 9:1005. doi: 10.3390/cells9041005
22. Semmler G, Wöran K, Scheiner B, Unger LW, Paternostro R, Stift J, et al. Novel reliability criteria for controlled attenuation parameter assessments for non-invasive evaluation of hepatic steatosis. *United European Gastroenterol J*. (2020) 8:321–31. doi: 10.1177/2050640619900820
23. Ahmad S, Harris T, Limb E, Kerry S, Victor C, Ekelund U, et al. Evaluation of reliability and validity of the general practice physical activity questionnaire (GPPAQ) in 60–74 year old primary care patients. *BMC Fam Pract*. (2015) 16:1–9. doi: 10.1186/s12875-015-0324-8
24. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. *Public Health Nutr*. (2010) 13:654–62. doi: 10.1017/S1368980009991698
25. Ghaffarpour M, Houshiar-Rad A, Kianfar HJTNOK. The manual for household measures, cooking yields factors and edible portion of foods. (1999) 7:213.
26. Bowman SA, Friday JE, Moshfegh AJJUDoA. *MyPyramid Equivalents Database, 2.0 for USDA survey foods, 2003–2004: Documentation and User Guide*. (2008).
27. Cheng E, Um CY, Prizment AE, Lazovich D, Bostick RM. Evolutionary-concordance lifestyle and diet and Mediterranean diet pattern scores and risk of incident colorectal cancer in Iowa women. *Cancer Epidemiol Biomarkers Prev*. (2018) 27:1195–202. doi: 10.1158/1055-9965.EPI-17-1184
28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. (1972) 18:499–502. doi: 10.1093/clinchem/18.6.499
29. Fraczek B, Pieta A, Burda A, Mazur-Kurach P, Tyrała F. Paleolithic diet-effect on the health status and performance of athletes? *Nutrients*. (2021) 13:1019. doi: 10.3390/nu13031019
30. Whalen KA, McCullough ML, Flanders WD, Hartman TJ, Judd S, Bostick RM. Paleolithic and Mediterranean diet pattern scores are inversely associated with biomarkers of inflammation and oxidative balance in adults. *J Nutr*. (2016) 146:1217–26. doi: 10.3945/jn.115.224048
31. de la OV, Zazpe I, Martínez JA, Santiago S, Carlos S, Zulet M, et al. Scoping review of paleolithic dietary patterns: a definition proposal. *Nutr Res Rev*. (2021) 34:78–106. doi: 10.1017/S0954422420000153
32. Gupta L, Khandelwal D, Lal PR, Kalra S, Dutta D. Palaeolithic diet in diabesity and endocrinopathies - a vegan's perspective. *Eur Endocrinol*. (2019) 15:77–82. doi: 10.17925/EE.2019.15.2.77
33. Berná G, Romero-Gomez M. The role of nutrition in non-alcoholic fatty liver disease: pathophysiology and management. *Liver Int*. (2020) 40 Suppl 1:102–8. doi: 10.1111/liv.14360
34. Tutunchi H, Saghafi-Asl M, Asghari-Jafarabadi M, Ostadrahimi A. Association between dietary patterns and non-alcoholic fatty liver disease: results from a case-control study. *Arch Iran Med*. (2021) 24:35–42. doi: 10.34172/aim.2021.06
35. Salehi-Sahlabadi A, Sadat S, Beigrezaei S, Pourmasomi M, Feizi A, Ghiasvand R, et al. Dietary patterns and risk of non-alcoholic fatty liver disease. *BMC Gastroenterol*. (2021) 21:41. doi: 10.1186/s12876-021-01612-z
36. Vancells Lujan P, Viñas Esmel E, Sacanella Meseguer E. Overview of non-alcoholic fatty liver disease (NAFLD) and the role of sugary food consumption and other dietary components in its development. *Nutrients*. (2021) 13:1442. doi: 10.3390/nu13051442
37. Parra-Vargas M, Rodriguez-Echevarria R, Jimenez-Chillaron JC. Nutritional approaches for the management of non-alcoholic fatty liver disease: an evidence-based review. *Nutrients*. (2020) 12:3860. doi: 10.3390/nu12123860
38. Zelber-Sagi S, Ivancovsky-Wajcman D, Isakov NF, Webb M, Orenstein D, Shibolet O, et al. High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. *J Hepatol*. (2018) 68:1239–46. doi: 10.1016/j.jhep.2018.01.015
39. Tarantino G, Citro V, Finelli C. Hype or reality: should patients with metabolic syndrome-related NAFLD be on the hunter-gatherer (Paleo) diet to decrease morbidity? *J Gastrointestin Liver Dis*. (2015) 24:359–68. doi: 10.15403/jgld.2014.1121.243.gta
40. Yao Y, Miao X, Zhu D, Li D, Zhang Y, Song C, et al. Insulin-like growth factor-1 and non-alcoholic fatty liver disease: a systemic review and meta-analysis. *Endocrine*. (2019) 65:227–37. doi: 10.1007/s12020-019-01982-1
41. Juanola O, Martínez-López S, Francés R, Gómez-Hurtado I. Non-alcoholic fatty liver disease: metabolic, genetic, epigenetic and environmental risk factors. *Int J Environ Res Public Health*. (2021) 18:5227. doi: 10.3390/ijerph18105227
42. Emamat H, Ghalandari H, Totmaj AS, Tangestani H, Hekmatdoost A. Calcium to magnesium intake ratio and non-alcoholic fatty liver disease development: a case-control study. *BMC Endocr Disord*. (2021) 21:51. doi: 10.1186/s12902-021-00721-w
43. Chung GE, Youn J, Kim YS, Lee JE, Yang SY, Lim JH, et al. Dietary patterns are associated with the prevalence of non-alcoholic fatty liver disease in Korean adults. *Nutrition*. (2019) 62:32–8. doi: 10.1016/j.nut.2018.11.021
44. Reccia I, Kumar J, Akladios C, Virdis F, Pai M, Habib N, et al. Non-alcoholic fatty liver disease: a sign of systemic disease. *Metabolism*. (2017) 72:94–108. doi: 10.1016/j.metabol.2017.04.011
45. Katsiki N, Perez-Martinez P, Anagnostis P, Mikhailidis DP, Karagiannis A. Is non-alcoholic fatty liver disease indeed the hepatic manifestation of metabolic syndrome? *Curr Vasc Pharmacol*. (2018) 16:219–27. doi: 10.2174/1570161115666170621075619

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Evidence of a Causal Relationship Between Vitamin D Status and Risk of Psoriasis From the UK Biobank Study

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Background: Plenty of observational studies suggested that vitamin D concentrations were associated with psoriasis, but the causality of this relationship was elusive.

Objective: To investigate the causal relationship between vitamin D and psoriasis.

Methods: Cox proportional hazard model was used to investigate the association between vitamin D status and psoriasis in a prospective cohort study from UK Biobank. Single nucleotide polymorphisms (SNPs) that are strongly associated with circulating 25OHD were constructed as instrumental variables in Mendelian randomization (MR) to determine the causality between vitamin D and psoriasis.

Results: During a median follow-up of 10.99 years, we identified 2,856 participants with incident psoriasis. The prospective cohort study demonstrated individuals with 25OHD deficiency (< 25 nmol/L) at baseline were associated with approximately 20% increased risk of incident psoriasis in different categories of sex, age, and body mass index (BMI) after adjusting for covariates. The largest effect size was observed in the obese group (BMI > 30 kg/m^2), as 25OHD deficiency presented with 30% additional risk of incident psoriasis compared to those with 25OHD > 50 nmol/L (HR = 0.701; 95% CI: 0.583–0.843; $p < 0.001$). Additionally, 69 independent SNPs associated with circulating 25OHD level were selected for the MR analysis, and the result suggested that genetically predicted one standard deviation (SD) increment in log-transformed 25OHD was associated with 24% decreased risk of psoriasis (OR = 0.76; 95% CI: 0.60–0.98, $p = 0.020$).

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Limitations: The association of 25OHD and severity of psoriasis could not be estimated in the current study.

Conclusion: The combined prospective and MR analysis additionally provided evidence that the epidemiologically and genetically determined level of 25OHD conferred an increased risk of psoriasis.

Keywords: psoriasis, vitamin D, causality, MR, BMI

CAPSULE SUMMARY

The prospective cohort study indicated that circulating 25OHD was inversely associated with the risk of incident psoriasis, particularly in subgroups of obesity. Mendelian randomization analysis further confirmed the causal relationship between 25OHD and psoriasis genetically. Randomized controlled trials and real-world evidence are warranted to determine the efficacy of systemic management of 25OHD in the prevention of psoriasis, especially for obese individuals with 25OHD deficiency.

INTRODUCTION

Psoriasis is a common chronic, immune-mediated inflammatory skin disorder that affects approximately 2% of the world population, and markedly impaired quality of life (1, 2). It is characterized by keratinocyte hyperproliferation and dysregulated T-helper 17 immune response with markedly altered inflammatory cytokine profiles (3). Although its etiology has not yet been fully elucidated, genetic and environmental factors are thought to play a critical role in the pathogenesis of psoriasis.

To date, a large number of treatments have been used for psoriasis, including phototherapy, topical, and oral medications, conventional systemic drugs, and small molecules, and also biologics (4). The topical vitamin D analogs, either alone, or being adjunctive treatment of corticosteroids, is one of the most well-known options for the management of psoriasis (5). The compound 25-hydroxy vitamin D (25OHD) exerts immunomodulatory and anti-inflammatory effect by modulating the innate and adaptive immune system, blocking proliferation, and boosting differentiation and maturation of keratinocyte (6). It can ameliorate T-cell proliferation and promote the differentiation of regulatory T cells (Tregs), and it also can regulate macrophage response and prevent proinflammatory cytokines release from macrophages (7). And vitamin D treated *in vitro* differentiated DCs express decreased levels of costimulatory molecules and increased levels of inhibitory receptors. Moreover, they secrete lower amounts of proinflammatory and higher amounts of anti-inflammatory cytokines (8). Therefore, the deficiency of 25OHD may contribute to the development of psoriasis *via* the reduction in immunomodulatory, anti-inflammatory, and antiproliferative activities. However, the causal relationship between 25OHD and risk of psoriasis and the disease severity of psoriasis has not been truly and consistently determined from previously retrospective and cross-sectional studies (9–11).

To overcome the limitations of cross-sectional studies and resolve inconsistencies, we conducted a prospective cohort study in a large European adult population from the UK Biobank to estimate the association of serum 25OHD levels with the risk of psoriasis. We then performed a Mendelian randomization (MR) analysis to genetically uncover the causal relationship between 25OHD and psoriasis.

MATERIALS AND METHODS

Study Design and Participant

The UK Biobank is a large-scale prospective cohort study with more than 500,000 participants aged 40–69 from 22 medical centers throughout the United Kingdom, recruited between 2006 and 2010 (12). The data used in the current study was from the UK Biobank that had received an approval from the North West Multi-Center Research Ethics Committee and informed consents from all individuals prior to participation. Medical history was provided for each participant at baseline assessment through interviews, touchscreen questionnaires, and physical measurements. The participants also donated blood and urine samples for future analysis and agreed to be followed up through linkage to their health records. Analyses in this article were restricted to individuals with complete information on both 25OHD and genetic polymorphisms.

Ascertainment of Outcome

Based on the clarifications from the UK Biobank, diagnosis of psoriasis was mainly confirmed using hospital inpatient records obtained from the Hospital Episode Statistics for England, Scottish Morbidity Record data for Scotland, and the Patient Episode Database for Wales. Additional cases were complemented through linkage to self-report, primary care, and death register data. The international classification of diseases (ICD) coding system was used to record the diagnosis of psoriasis as well.

Assessment of Exposure

The biological sample was obtained from each participant at the initial assessment center visit. Serum 25OHD level was determined by chemiluminescence immunoassay with high sensitivity and precision (13).

Genetic instruments of independent common single nucleotide polymorphisms (SNPs) that being closely associated

with circulating 25OHD levels were extracted from a meta-analysis of genome-wide association studies (GWAS), including 401,460 white British participants from the UK Biobank and another dataset of 42,274 Europeans (14). Finally, 69 SNPs with significant genome-wide 25OHD level were selected as genetic instruments ($p < 6.6 \times 10^{-9}$). Only common SNPs among the conditionally independent SNPs were included for the MR analysis to ensure that our instruments were truly in linkage equilibrium, since the r^2 as a metric of linkage disequilibrium (LD) is less accurate for rare variants. It was estimated that these SNPs collectively explained about 3.1% of variance of circulating 25OHD levels, and the variance explained for a given SNP was calculated using the formula: variance explained = $2\beta\ 2\ f\ (1–f)$, where β and f denote the effect of the SNP on 25OHD level and the MAF, respectively. GWAS summary data of psoriasis from MRC-IEU, OpenGWAS data[1] were used for MR analysis (15), after excluding instrumental variables with significant genome-wide significance ($p < 5 \times 10^{-8}$) and minor allele frequency (MAF) < 5% to prevent the effects of rare variants and horizontal pleiotropy. Finally, we calculated the F statistics of all instrumental variables included in MR analyses to confirm that the F statistics of all SNPs included in the study were more than 10 (16).

Mendelian Randomization Analysis

To investigate whether there is a causal relationship between circulating 25OHD level and risk of psoriasis, a main analysis was conducted to estimate the effect of one increment in SD of natural log-transformed 25OHD on psoriasis using the inverse-variance weighted (IVW) method as previously described (17). The IVW method weighed the effect of each instrumental variable on psoriasis susceptibility by its effect on 25OHD using the Wald ratio method, and then combined these individual MR estimates into a random effect inverse-variance meta-analysis (18).

In addition, three additional methods were applied to control for pleiotropy (weighted median, MR-Egger, and mode-based estimate) and to compare the respective MR estimates. The MR-Egger method can evaluate the potential pleiotropy in MR analysis, and offset its effect by intercepts (19). The weighted median method uses weighted median of ratios of all instrumental variables, and can tolerate the weight of invalid SNPs up to 50% (20). Mode-based estimate is a method to obtain causal effect estimate from multiple genetic instruments, which can allow potential pleiotropy in majority of instrumental variables (21). In order to reduce the effect of outliers, robust regression with penalized parameter was used in the MR Egger and IVW methods.

Sensitivity analyses were conducted by excluding SNP variants associated with potential confounders. The PhenoScanner database was queried for each 25OHD-related instrumental SNP to identify genetic variants (associating variants) related to GWAS traits that are potential confounders or could introduce horizontal pleiotropy in the exposure–outcome association.

[1]https://gwas.mrcieu.ac.uk/

Statistical Analysis

We assessed the association of baseline circulating 25OHD level with the risk of incident psoriasis using Cox proportional hazard models, and estimated the hazard ratios (HRs) and 95% confidence intervals (CIs) after adjusting for covariates. The basically adjusted model included age, sex, and body mass index (BMI); and the fully adjusted model additionally included income, education, smoking status, and 25OHD supplements.

The dose–response relationship between 25OHD and incident psoriasis was plotted using cubic splines. We then standardized the serum 25OHD levels to a normal distribution and estimated the HR corresponding one SD increment. We also divided the 25OHD levels into quartiles (25th, 50th, and 75th) as well as clinical categories (deficient: < 25 nmol/L, insufficient: 25∼50 nmol/L, optimal: > 50 nmol/L). Subsequently, stratified analyses were conducted based on sex, age group, and BMI.

The cohort data was analyzed by R Version 3.6.3. The GWAS summary data was extracted by R package "TwoSampleMR" and MR, and sensitivity analyses were performed using R package "Mendelian Randomization." A p-value less than 0.05 was considered of statistical significance (two sided). We also applied the global test, outlier test, and distortion test using R package "MR-PRESSO."

RESULTS

Selection and Baseline Characteristics of Participants

In the current study, 429,681 participants were included and eligible for the analysis after excluding 9,953 participants with prevalent psoriasis, 8,320 with no genetic data, and 54,551 with missing data on serum 25OHD information (**Supplementary Figure 1**). We identified 2,856 participants with incident psoriasis during a median follow-up period of 10.99 years. Baseline characteristics including age, sex, household income, background of education, smoking status, BMI, serum 25OHD concentration, and use of vitamin D supplements are presented in **Table 1**.

Association of Observed Circulating 25OHD Levels and Incident Psoriasis

The association between observed circulating 25OHD level and risk of incident psoriasis was estimated in Cox models by including covariates (**Table 2**). One SD increment in 25OHD level was associated with 7.0% decreased risk of incident psoriasis (HR = 0.930; 95% CI: 0.896–0.965; $p < 0.001$) in basically adjusted model which included age, sex, and BMI. Similar effect (HR = 0.938; 95% CI: 0.904–0.974; $p = 0.001$) was observed in the fully adjusted model which additionally included household income, education, smoking status, and 25OHD supplements.

Then, the participants were divided into four categories by quartiles of serum 25OHD concentrations and three categories by clinical cut-offs. The risk of incident psoriasis decreased with increasing 25OHD concentration ($p_{trend} = 0.002$), and individuals in the highest quartile of 25OHD concentration had 17.3% decreased risk of incident psoriasis (HR = 0.827; 95%

TABLE 1 | Characteristics of participants from the UK Biobank.

Covariate	Total (*N* = 429,681)	Psoriasis (*N* = 2,856)	No Psoriasis (*N* = 426,825)	*p*[b]
Age at baseline (years), mean (*SD*)	56.50 (8.12)	57.17 (7.93)	56.49 (8.12)	<0.001
Age category (years), *N* (%)				<0.001
<50	101,816 (23.7)	569 (19.9)	101,247 (23.7)	
50–59	142,255 (33.1)	969 (33.9)	141,286 (33.1)	
> 60	185,610 (43.2)	1,318 (6.1)	184,292 (43.2)	
Sex, *N* (%)				0.137
Female	230,896 (53.7)	1,495 (52.3)	229,401 (53.7)	
Male	198,785 (46.3)	1,361 (47.7)	197,424 (46.3)	
Average total household income before tax (€), *N* (%)				<0.001
Less than 18,000	82,199 (19.1)	689 (24.1)	81,510 (19.1)	
18,000–30,999	93,161 (21.7)	630 (22.1)	92,531 (21.7)	
31,000–51,999	96,226 (22.4)	588 (20.6)	95,638 (22.4)	
52,000–100,000	75,872 (17.7)	433 (15.2)	75,439 (17.7)	
Greater than 100,000	20,262 (4.7)	95 (3.3)	20,167 (4.7)	
Unknown	61,961 (14.4)	421 (14.7)	61,540 (14.4)	
Education, *N* (%)				<0.001
College or university degree	139,999 (32.6)	824 (28.9)	139,175 (32.6)	
Professional qualifications	50,538 (11.8)	359 (12.6)	50,179 (11.8)	
A Levels/AS levels or equivalent	47,823 (11.1)	265 (9.3)	47,558 (11.2)	
O Levels/GCSEs or equivalent	113,917 (26.5)	748 (26.2)	113,169 (26.5)	
None of the above	76,980 (17.9)	657 (23.0)	76,323 (17.9)	
Smoking status, *N* (%)				<0.001
Never	235,288 (54.8)	1,253 (43.9)	234,035 (54.9)	
Previous	148,358 (34.6)	1,114 (39.0)	147,244 (34.5)	
Current	43,925 (10.2)	473 (16.6)	43,452 (10.2)	
Unknown	1,683 (0.4)	13 (0.5)	1,670 (0.4)	
BMI (kg/m^2), mean (SD)	27.40 (4.78)	28.37 (5.24)	27.39 (4.77)	<0.001
BMI category, *N* (%)				<0.001
Normal (<25)	142,528 (33.3)	744 (26.1)	141,784 (33.3)	
Overweight (25∼30)	181,802 (42.5)	1,234 (43.3)	180,568 (42.5)	
Obesity (> 30)	103,731 (24.2)	869 (30.5)	102,862 (24.2)	
Vitamin D concentrations (nmol/L), Mean (*SD*)	48.26 (19.69)	46.68 (20.04)	48.27 (19.69)	<0.001
Vitamin D concentrations (nmol/L)[a], *N* (%)				<0.001
12.7–32.6	106,657 (24.8)	812 (28.4)	105,845 (24.8)	
32.6–46.8	107,675 (25.1)	703 (24.6)	106,972 (25.1)	
46.8–62.0	107,454 (25.0)	697 (24.4)	106,757 (25.0)	
62.0–104.0	107,895 (25.1)	644 (22.5)	107,251 (25.1)	
Vitamin D category (nmol/L), *N* (%)				<0.001
Deficient (<25)	54,306 (12.7)	460 (16.1)	54,306 (12.7)	
Insufficient (25∼50)	183,631 (42.7)	1,210 (42.4)	182,421 (42.7)	
Optimal (> 50)	191,284 (44.6)	1,168 (41.5)	190,098 (44.6)	
Vitamin D supplements, *N* (%)				0.197
No	421,883 (98.2)	2,795 (97.9)	419,088 (98.2)	
Yes	7,798 (1.8)	61 (2.1)	7,737 (1.8)	

[a] *The data were divided by quartiles.*

[b] *Continuous data was presented as mean ± standard deviation, and between-group difference was tested using analysis of variance. Categorical data were presented as number (%), and the between-group difference was tested using chi-squared test. $p < 0.05$ was considered statistically significant for all tests.*

CI: 0.744–0.919; $p = 0.002$) compared to those in the lowest quartile, after adjustment for age, sex, and BMI. Similar effect was also observed in participants with higher 25OHD levels after additional adjustment for income, education, smoking status, and 25OHD supplements ($p_{trend} = 0.010$). Moreover, compared to 25OHD deficiency, the risk of psoriasis was decreased by 18.6% (HR = 0.814; 95% CI: 0.731–0.907; $p < 0.001$) and 20.5% (HR = 0.795; 95% CI: 0.712–0.887; $p < 0.001$) in participants with insufficient and sufficient 25OHD levels, respectively, in the fully adjusted model.

TABLE 2 | Association of vitamin D concentrations with incident psoriasis.

			Model 1[a]		Model 2[b]	
Vitamin D concentration	**N**	**Person-years**	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Per SD in concentration	2,856	4,674,462	0.930 (0.896–0.965)	<0.001	0.938 (0.904–0.974)	0.001
Quartiles						
12.7–32.6	812	1,160,748	Ref		Ref	
32.6–46.8	703	1,171,944	0.865 (0.781–0.957)	0.005	0.882 (0.797–0.977)	0.016
46.8–62.0	697	1,168,501	0.874 (0.789–0.968)	0.010	0.896 (0.809–0.993)	0.037
62.0–104.0	644	1,173,269	0.827 (0.744–0.919)	<0.001	0.841 (0.757–0.935)	0.001
			p_{trend} = 0.002		P_{trend} = 0.010	
Category						
Deficient (<25)	460	596,195	Ref		Ref	
Insufficient (25∼50)	1,210	1,998,285	0.791 (0.710–0.882)	<0.001	0.814 (0.731–0.907)	<0.001
Optimal (> 50)	1,186	2,079,982	0.770 (0.690–0.860)	<0.001	0.795 (0.712–0.887)	<0.001
			p_{trend} < 0.001		p_{trend} < 0.001	

[a]*Model 1 was adjusted for age, sex, and BMI.*
[b]*Model 2 was additionally adjusted for income, education, smoking status, and vitamin D supplements.*

Subgroup Analysis for the Association of Observed 25OHD Level With Psoriasis

Stratified analysis based on sex (**Supplementary Table 1**), age group (**Supplementary Table 2**), and BMI (**Table 3**) categories were estimated by HR in fully adjusted model to determine the relationship between serum 25OHD concentration and risk of incident psoriasis. Overall, per one SD decrease of 25OHD level was associated with increased risk of psoriasis. The prospective cohort study demonstrated individuals with 25OHD deficiency (<25 nmol/L) at baseline were associated with approximately 20% increased risk of incident psoriasis when compared to individuals with optimal 25OHD (>50 nmol/L) in different categories of sex, age, and BMI after adjusting for covariates. The largest effect size was observed in the obese group (BMI > 30 kg/m^2), as 25OHD deficiency presented with 30% additional risk of incident psoriasis compared to those with 25OHD > 50 nmol/L (HR = 0.701; 95% CI: 0.583–0.843; $p < 0.001$, **Table 3**). However, the interaction effect between BMI and 25OHD was calculated, and no interaction effects were observed between BMI and 25OHD (**Supplementary Table 3**). Effect size observed in male and female groups were similar. And in the age stratified analysis, the effect seems more obvious in the population less than 50 years old.

Dose-Response Pattern Between 25OHD and Incident Psoriasis

The cubic splines show a non-linear relationship between 25OHD concentration and risk of incident psoriasis (**Figure 1**). The risk of incident psoriasis decreased when the circulating 25OHD concentration increased from 0 to 50 nmol/L, but thereafter the effect reduced. The analysis was also conducted by subgroups of sex, age, and BMI, and the effect of 25OHD was modified by obesity since the curves were almost linear in participants having BMI < 30 kg/m^2.

Mendelian Randomization Analysis for Genetically Determined 25OHD Level With Psoriasis Risk

Sixty-nine common and conditionally independent SNPs (**Supplementary Table 4**) explaining 3.1% of the variation of circulating 25OHD levels were included for the MR analysis. We identified no significant associations with known potentially pleiotropic confounders influencing the exposure–outcome association based on PhenoScanner search, at a Bonferroni-correction threshold of 5×10^{-8}. However, an enrichment in SNPs associated with certain trait categories, including body composition and serum lipid traits, was observed; and this may be associated with the risk of psoriasis. A detailed description of the SNP-trait association is provided in **Supplementary Table 5**. Among 462,933 participants in the cohort, one genetically predicted increment in the SD of 25OHD was associated with 24% decreased risk of psoriasis (OR_{IVW} = 0.76; 95% CI 0.60–0.96; p = 0.02) according to the IVW method. In addition, MR-Egger method suggested a significant association ($OR_{MR-Egger}$ = 0.79; 95% CI 0.63–1.00; p = 0.05), while the weighted median and mode-based estimates presented with a comparable effect size (OR = 0.87) with no statistical significance. Additionally, sensitivity analysis was conducted to exclude proxy SNPs and covariate-associated SNPs, and the results also showed a causality between the genetically predicted level of 25OHD and risk of psoriasis after excluding proxy SNPs (OR_{IVW} = 0.71; 95% CI: 0.55–0.92; p = 0.01), body-composition associated SNPs (OR_{IVW} = 0.74, 95% CI: 0.55–0.98; p = 0.04), and diabetes-associated SNPs (OR_{IVW} = 0.76, 95% CI: 0.59–0.98; p = 0.03) (**Figure 2**). However, when all comorbidity-associated SNPs were excluded, the result changed to negative; but the trend still remains (OR_{IVW} = 0.79, 95% CI: 0.56–1.13; p = 0.20). A scatter plot of the MR analysis investigating the effect of 25OHD on psoriasis was presented in **Supplementary Figure 2**. Meanwhile, MR-PRESSO method was used to find evidence for pleiotropy

TABLE 3 | Association of vitamin D concentrations with incident psoriasis in different BMI categories.

Vitamin D concentration, nmol/L	Normal (BMI < 25)				Overweight (25 < BMI < 30)				Obesity (BMI > 30)			
	N	Person-years	HR (95%CI)	*p*	*N*	Person-years	HR (95% CI)	*p*	*N*	Person-years	HR (95% CI)	*p*
Per SD in concentration	744	1,551,468	0.945 (0.880–1.015)	0.119	1,234	1,978,052	0.951 (0.898–1.006)	0.082	869	1,127,639	0.895 (0.838–0.956)	0.001
Quartiles												
12.7–32.6	181	330,338	Ref		303	455,722	Ref		326	367,308	Ref	
32.6–46.8	152	352,207	0.802 (0.646–0.995)	0.045	320	498,491	0.963 (0.823–1.128)	0.643	228	316,920	0.826 (0.697–0.978)	0.027
46.8–62.0	199	391,839	0.941 (0.768–1.153)	0.559	309	516,263	0.887 (0.757–1.041)	0.142	187	257,300	0.841 (0.702–1.007)	0.059
62.0–104.0	212	477,084	0.812 (0.664–0.992)	0.042	302	507,576	0.869 (0.740–1.020)	0.086	128	186,111	0.789 (0.643–0.968)	0.023
			p_{trend} = 0.091				p_{trend} = 0.261				p_{trend} = 0.045	
Category												
Deficient (<25)	96	168,700	Ref		161	226,571	Ref		202	196,235	Ref	
Insufficient (25∼50)	274	596,057	0.833 (0.659–1.052)	0.125	531	843,323	0.887 (0.743–1.059)	0.184	401	551,175	0.724 (0.611–0.858)	<0.001
Optimal (> 50)	374	786,711	0.853 (0.680–1.071)	0.170	542	908,158	0.823 (0.689–0.983)	0.031	266	380,229	0.701 (0.583–0.843)	<0.001
			p_{trend} = 0.294				p_{trend} = 0.086				p_{trend} < 0.001	

All models were adjusted for sex, age, income, education, smoking status, and vitamin D supplements.

(1,000 simulations). The MR estimates for psoriasis did not alter the conclusion after removing one outlier SNP with increased evidence of pleiotropic effects (**Supplementary Table 6**).

DISCUSSION

We first examined the association of circulating 25OHD level with the risk of incident psoriasis in a large-scale longitudinal data from the UK Biobank, and then investigated the causal relationship of genetically predicted level of 25OHD and psoriasis based on MR analysis. Individuals with 25OHD deficiency were more likely to have psoriasis, especially for obese subjects or aged under 50 years. This relationship was additionally confirmed by MR analysis.

Previously, a large number of observational studies tried to determine the association of circulating 25OHD with the risk of psoriasis, and most reported a lower serum level of 25OHD in patients with psoriasis compared to controls. A meta-analysis by Lee and Song found that psoriatic patients had a lower level of serum 25OHD compared to healthy controls, and revealed a small but significant correlation between circulating 25OHD level and the severity of psoriasis (22). Pitukweerakul et al. found the relationship between hypovitaminosis D and psoriasis, but did not establish a causal relationship (23). In the current study, we first used a large-scale prospective cohort and confirmed that 25OHD deficiency could increase the incidence of psoriasis in the following 11 years. A non-linear relationship between serum 25OHD concentration and the incidence of psoriasis was found. Results showed that the protective effect of vitamin D on incident psoriasis was quite obvious, but it seems to work under a specific threshold, which has not been reported in previous studies. However, we would refrain from suggesting that the high serum 25OHD concentration had opposite effect because of the large CI. The relatively small sample size of people with high serum 25OHD concentration might account for this result. The existence of the threshold and the probable mechanisms were still unclear, which need further confirmations.

The effect of 25OHD on incident psoriasis was also modified by obesity. The protective effect of vitamin D on incident psoriasis seem to be larger in obese individuals. This is consistent with previous finding that serum 25OHD was significantly associated with the risk of psoriasis only in patients with central obesity (24). These findings suggested that the effect of decreased 25OHD on the pathogenesis of psoriasis might be closely related to obesity, a well-known comorbidity of psoriasis. However, the role obesity played in the relationships and the specific associations among obesity, 25OHD, and psoriasis were seldom discussed in previous studies. Several hypotheses have been proposed such as the influence of metabolic disorders of obese patients, and there might be a mediating effect of serum 25OHD between obesity and psoriasis, but no further confirmation (24, 25). Previous evidences suggested that vitamin D also has beneficial effects on serum lipid profiles and thus cardiovascular health and other comorbidities (26, 27). Thus, it might suggest that vitamin D reduce the risk of psoriasis and other comorbidities at the same time and result in synergy effects in obese people with more complications. Meanwhile, as a fat-soluble vitamin, the absorption and metabolism of vitamin D might be affected by fat distribution (28). The changes of

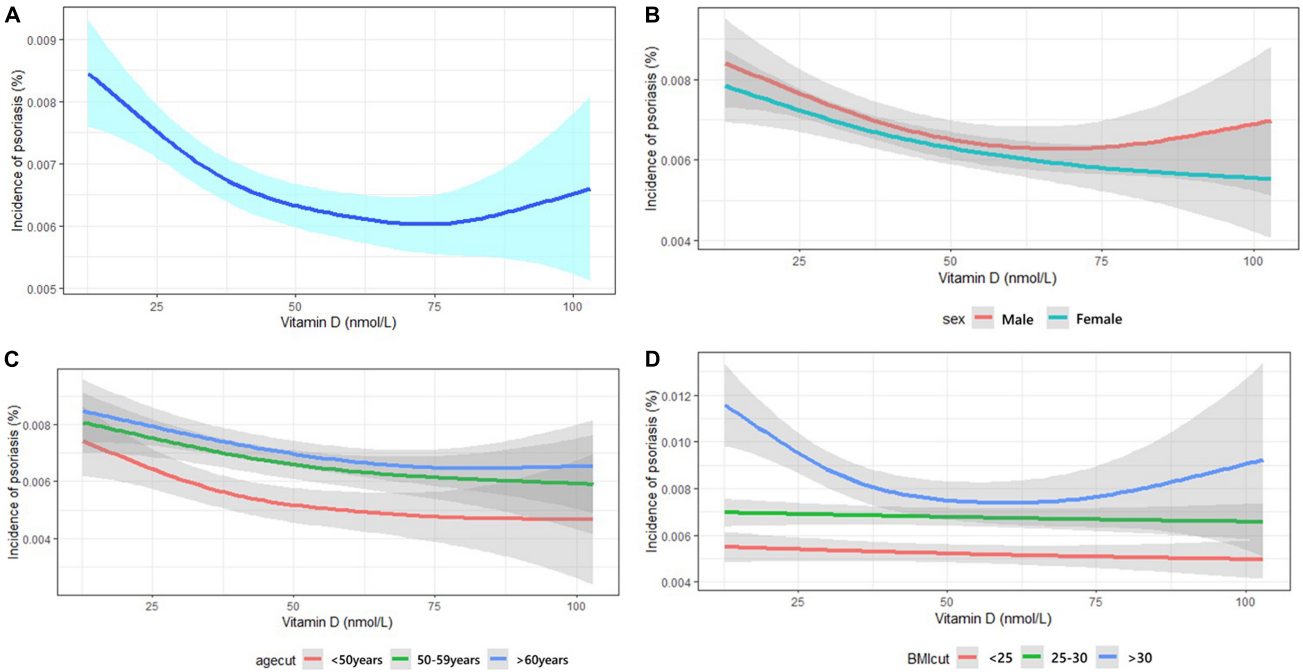

FIGURE 1 | Dose-effect relationship between vitamin D and psoriasis.

distribution and metabolic function of fat in obese people might directly or indirectly influence the effect of vitamin D. And these might be reasons that obesity modify the association between vitamin D and psoriasis. However, further studies are still needed to verify these hypotheses.

We used 69 common and conditionally independent SNPs from the newly published GWAS summary datasets of vitamin D in our MR analysis, and further confirmed the causal relationship between 25OHD with psoriasis at genetic level. The MR is an approach that uses genetic variants associated with a modifiable exposure or biological intermediate to estimate the causal relationship between these variables and a medically relevant outcome (29). The random allocation of genetic variants at conception reduces confounding from environmental factors and thus strengthens causal inference (30). Therefore, our results of MR further supported vitamin D deficiency as a possible cause of incident psoriasis. However, results of additional sensitivity analysis were negative after all SNPs associated with lipid, BMI, and diabetes were excluded, while the effect size did not change evidently. The reason might be the limitation of strength of these included SNPs, which explains less variation of circulating 25OHD levels. Further GWASs might be needed to expand the range of genetic instruments and obtain more reliable results.

25OHD is produced by the skin under the action of ultraviolet light and dietary intake. While topical 25OHD analogs exert various anti-inflammatory, anti-oxidant, and immunomodulatory actions by modulating innate and adaptive immune system, blocking proliferation, and promoting differentiation and maturation of keratinocyte is recommended as a treatment option by the National Psoriasis Foundation; the application of oral 25OHD supplementation as an adjunctive therapeutic options for psoriasis is still discussed, and its clinical use is predominantly based on experience of dermatologists and nutritionists (31). However, compared to patients with single psoriasis, patients of psoriasis with comorbidities such as psoriatic arthritis and obesity were more commonly recommend vitamin D supplementation (32). And it has been reported that this supplementation may be useful for comorbidity prevention in some cases (33). Previous studies that tried to determine the association between circulating 25OHD and psoriasis severity presented inconsistent findings. Mattozzi et al. showed that serum 25OHD level was significantly associated with the psoriasis area and severity index (PASI) score in patients with psoriasis (9). However, a randomized, double-blind, placebo-controlled trial (RCT) found that oral 25OHD3 supplementation did not significantly alleviate plaque psoriasis compared to the placebo (10). Another RCT conducted by the same research team found that monthly use of 25OHD3 supplementation did not reduce the severity of mild psoriasis compared to placebo as well (34). A small-scale RCT in Asians indicated clinical efficacy of oral 25OHD2 in the treatment of psoriasis without increasing adverse events (11). However, a recent meta-analysis study of RCTs did not support the efficacy of oral vitamin D supplementation in lessening the severity of psoriasis (35). Additionally, no studies confirmed the optimal dose of systemic 25OHD supplement in the treatment of psoriasis, although most of them did not observe adverse effects within a relatively narrow range of dosages (0.25–2 μg/d) (36). Combined with our finding, we deliberately concluded that 25OHD has a causal relationship with psoriasis, and this effect is modified by obesity. Cluster

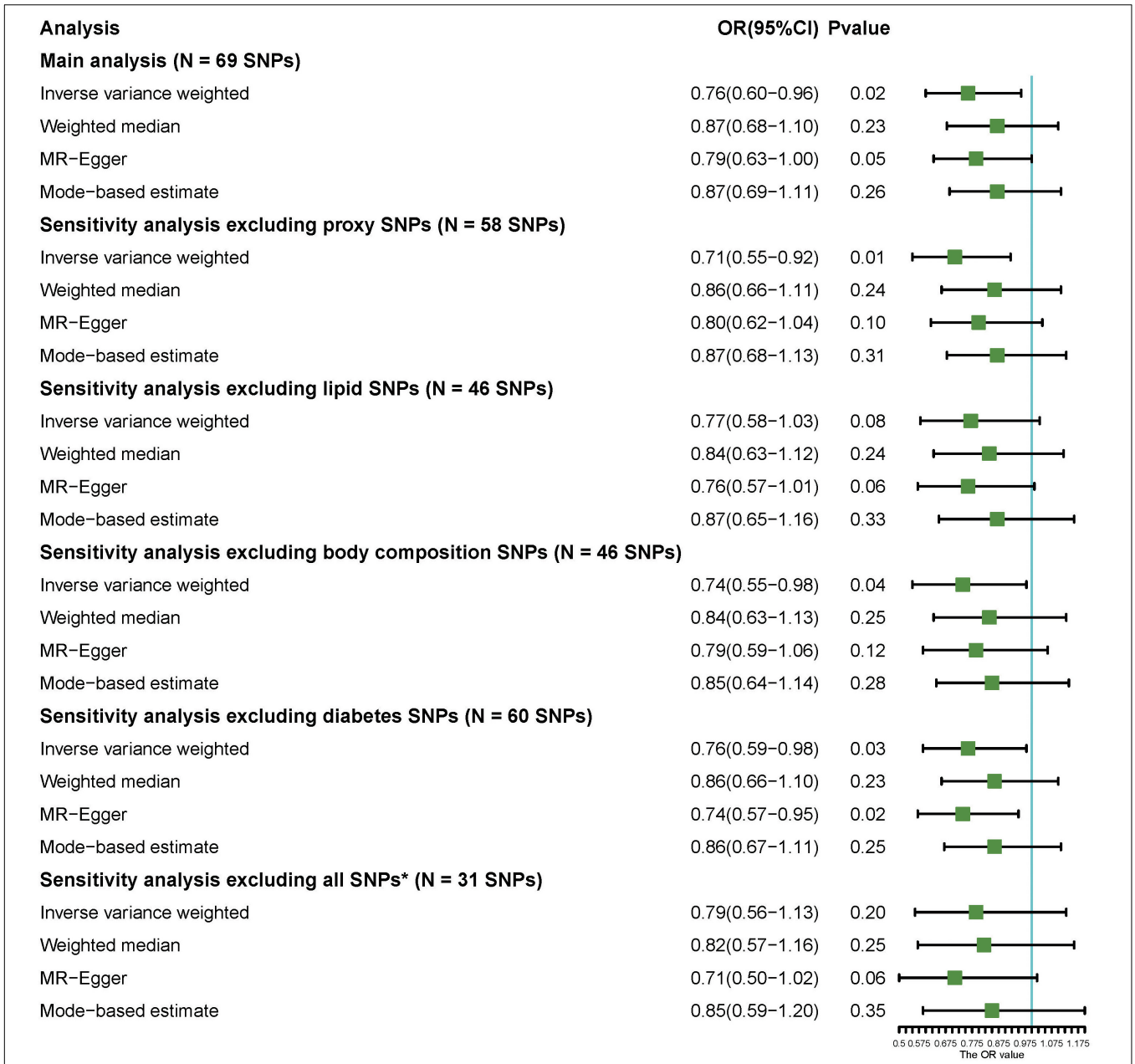

FIGURE 2 | Results of Mendelian randomization analysis.

RCT studies in a general population with a high incidence rate of psoriasis would be warranted to confirm the effectiveness of systemic 25OHD supplementation in the prevention of psoriasis, especially for obese individuals with a deficient level of 25OHD, and to determine the optimal dose and adverse effects of the supplementation.

Strengths and Limitations

This study has several strengths. First, the large sample size in addition to a prospective study design provide a higher level of evidence than retrospective studies. Second, a non-linear association and effect modification by obesity were identified, and this may explain some of the discrepancies among previous studies. Third, the MR analysis further enhanced our findings at genetic level, by constructing instrumental variables as a measure of exposure.

Nevertheless, this study also has limitations. The study population came from Europe, and the findings should be validated in different races and regions. Additionally, although some confounders were adjusted, unknown and unmeasured confounders and effect modifiers might be ignored. Also, the potential bias due to participant overlap have not been calculated

because the conditions about the overlap of participants from these two published GWAS summary datasets could not been obtained. Last but not the least, the association of 25OHD and severity of psoriasis could not be estimated in the current study since relevant information such as PASI score was not available.

CONCLUSION

This prospective cohort study shows that 25OHD was inversely associated with the risk of incident psoriasis, and effect modification by obesity was identified. MR analysis further confirmed the causality at genetic level.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://biobank.ctsu.ox.ac.uk/crystal/index.cgi.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the North West Multi-Center Research Ethics Committee (UK biobank application ID #55257). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YZ: conception, bibliographical research, manuscript development, and writing. DJ and GZ: bibliographical research, manuscript development, and writing. YX: bibliographical research and supervision of the manuscript. MS, XC, and HL: critical revision and supervision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.807344/full#supplementary-material

REFERENCES

1. Michalek I, Loring B, John S. A systematic review of worldwide epidemiology of psoriasis. *J Eur Acad Dermatol Venereol.* (2017) 31:205–12. doi: 10.1111/jdv.13854
2. Nestle F, Kaplan D, Barker J. Psoriasis. *N Engl J Med.* (2009) 361:496–509.
3. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. *Cell Mol Immunol.* (2012) 9:302–9. doi: 10.1038/cmi.2012.15
4. Florek A, Wang C, Armstrong A. Treatment preferences and treatment satisfaction among psoriasis patients: a systematic review. *Arch Dermatol Res.* (2018) 310:271–319. doi: 10.1007/s00403-018-1808-x
5. Barrea L, Savanelli M, Di Somma C, Napolitano M, Megna M, Colao A, et al. Vitamin D and its role in psoriasis: an overview of the dermatologist and nutritionist. *Rev Endocr Metab Disord.* (2017) 18:195–205. doi: 10.1007/s11154-017-9411-6
6. Cantorna M. Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? *Proc Soc Exp Biol Med.* (2000) 223:230–3. doi: 10.1046/j.1525-1373.2000.22333.x
7. Cyprian F, Lefkou E, Varoudi K, Girardi G. Immunomodulatory effects of vitamin D in pregnancy and beyond. *Front Immunol.* (2019) 10:2739. doi: 10.3389/fimmu.2019.02739
8. Karthaus N, van Spriel A, Looman M, Chen S, Spilgies L, Lieben L, et al. Vitamin D controls murine and human plasmacytoid dendritic cell function. *J Investig Dermatol.* (2014) 134:1255–64. doi: 10.1038/jid.2013.501
9. Mattozzi C, Paolino G, Salvi M, Macaluso L, Scarnò M, De Vita G, et al. Correlation between plasmatic levels of vitamin D and PASI score. *G Ital Dermatol Venereol.* (2018) 153:155–60. doi: 10.23736/S0392-0488.17.05622-X
10. Ingram M, Jones M, Stonehouse W, Jarrett P, Scragg R, Mugridge O, et al. Oral vitamin D supplementation for chronic plaque psoriasis: a randomized, double-blind, placebo-controlled trial. *J Dermatol Treat.* (2018) 29:648–57. doi: 10.1080/09546634.2018.1444728
11. Disphanurat W, Viarasilpa W, Chakkavittumrong P, Pongcharoen P. The clinical effect of oral vitamin D2 supplementation on psoriasis: a double-blind, randomized, placebo-controlled study. *Dermatol Res Pract.* (2019) 2019:5237642. doi: 10.1155/2019/5237642
12. Trehearne A. Genetics, lifestyle and environment. UK Biobank is an open access resource following the lives of 500,000 participants to improve the health of future generations. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* (2016) 59:361–7. doi: 10.1007/s00103-015-2297-0
13. Lin L, Smeeth L, Langan S, Warren-Gash C. Distribution of vitamin D status in the UK: a cross-sectional analysis of UK Biobank. *BMJ Open.* (2021) 11:e038503. doi: 10.1136/bmjopen-2020-038503
14. Manousaki D, Mitchell R, Dudding T, Haworth S, Harroud A, Forgetta V, et al. Genome-wide association study for vitamin D levels reveals 69 independent loci. *Am J Hum Genet.* (2020) 106:327–37. doi: 10.1016/j.ajhg.2020.01.017
15. Hemani G, Zheng J, Elsworth B, Wade K, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife.* (2018) 7:e34408. doi: 10.7554/eLife.34408
16. Efstathiadou A, Gill D, McGrane F, Quinn T, Dawson J. Genetically determined uric acid and the risk of cardiovascular and neurovascular diseases: a mendelian randomization study of outcomes investigated in

randomized trials. *J Am Heart Assoc.* (2019) 8:e012738. doi: 10.1161/JAHA.119.012738
17. Lawlor D, Harbord R, Sterne J, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med.* (2008) 27:1133–63. doi: 10.1002/sim.3034
18. Burgess S, Butterworth A, Thompson S. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol.* (2013) 37:658–65. doi: 10.1002/gepi.21758
19. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080
20. Bowden J, Davey Smith G, Haycock P, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965
21. Hartwig F, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization *via* the zero modal pleiotropy assumption. *Int J Epidemiol.* (2017) 46:1985–98. doi: 10.1093/ije/dyx102
22. Lee Y, Song G. Association between circulating 25-hydroxyvitamin D levels and psoriasis, and correlation with disease severity: a meta-analysis. *Clin Exp Dermatol.* (2018) 43:529–35. doi: 10.1111/ced.13381
23. Pitukweerakul S, Thavaraputta S, Prachuapthunyachart S, Karnchanasorn R. Hypovitaminosis D is associated with psoriasis: a systematic review and meta-analysis. *Kansas J Med.* (2019) 12:103–8. doi: 10.17161/kjm.v12i4.13255
24. Kuang Y, Xiao Y, Fang Z, Zhang Y, Shen M, Chen X, et al. Association of serum vitamin D with psoriasis and effect modification by central obesity. *Front Med.* (2020) 7:236. doi: 10.3389/fmed.2020.00236
25. Kanda N, Hoashi T, Saeki H. Nutrition and psoriasis. *Int J Mol Sci.* (2020) 21:5405.
26. Dibaba D. Effect of vitamin D supplementation on serum lipid profiles: a systematic review and meta-analysis. *Nutr Rev.* (2019) 77:890–902. doi: 10.1093/nutrit/nuz037
27. Jafari T, Fallah A, Barani A. Effects of vitamin D on serum lipid profile in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *Clin Nutr (Edinburgh Scotland).* (2016) 35:1259–68. doi: 10.1016/j.clnu.2016.03.001
28. Borel P, Caillaud D, Cano N. Vitamin D bioavailability: state of the art. *Crit Rev Food Sci Nutr.* (2015) 55:1193–205. doi: 10.1080/10408398.2012.688897
29. Evans D, Davey Smith G. Mendelian randomization: new applications in the coming age of hypothesis-free causality. *Annu Rev Genomics Hum Genet.* (2015) 16:327–50. doi: 10.1146/annurev-genom-090314-050016
30. Georgakis M, Gill D. Mendelian randomization studies in stroke: exploration of risk factors and drug targets with human genetic data. *Stroke.* (2021) 52:2992–3003. doi: 10.1161/strokeaha.120.032617
31. Megna M, Ferrillo M, Barrea L, Patruno C, Muscogiuri G, Savastano S, et al. Vitamin D and psoriasis: an update for dermatologists and nutritionists. *Minerva Endocrinol.* (2020) 45:138–47. doi: 10.23736/S0391-1977.20.03190-9
32. Ford A, Siegel M, Bagel J, Cordoro K, Garg A, Gottlieb A, et al. Dietary recommendations for adults with psoriasis or psoriatic arthritis from the medical board of the national psoriasis foundation: a systematic review. *JAMA Dermatol.* (2018) 154:934–50. doi: 10.1001/jamadermatol.2018.1412
33. Zuccotti E, Oliveri M, Girometta C, Ratto D, Di Iorio C, Occhinegro A, et al. Nutritional strategies for psoriasis: current scientific evidence in clinical trials. *Eur Rev Med Pharmacol Sci.* (2018) 22:8537–51. doi: 10.26355/eurrev_201812_16554
34. Jarrett P, Camargo C, Coomarasamy C, Scragg R. A randomized, double-blind, placebo-controlled trial of the effect of monthly vitamin D supplementation in mild psoriasis. *J Dermatol Treat.* (2018) 29:324–8. doi: 10.1080/09546634.2017.1373735
35. Theodoridis X, Grammatikopoulou M, Stamouli E, Talimtzi P, Pagkalidou E, Zafiriou E, et al. Effectiveness of oral vitamin D supplementation in lessening disease severity among patients with psoriasis: a systematic review and meta-analysis of randomized controlled trials. *Nutrition.* (2021) 82:111024. doi: 10.1016/j.nut.2020.111024
36. Stanescu A, Simionescu A, Diaconu C. Oral vitamin D therapy in patients with psoriasis. *Nutrients.* (2021) 13:163. doi: 10.3390/nu13010163

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Visceral adiposity-related dietary patterns and the risk of cardiovascular disease in Iranian adults: A population-based cohort study

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Background: Visceral obesity is a significant predictor of cardiovascular disease (CVD). Diet may associate with CVD risk through its effects on visceral adiposity. This study aimed to find dietary patterns (DPs) related to indicators of visceral adiposity and to determine whether the DPs were associated with CVD risk.

Methods: This prospective study included 2,496 participants of the Tehran Lipid and Glucose Study (TLGS) without CVD, who were followed from the third study examination (2005–2008; baseline) to March 2018. DPs at baseline were determined using reduced rank regression (RRR) and partial least squares regression (PLS). The response variables were age and BMI-adjusted waist circumference (WC) and age-adjusted visceral adiposity index (VAI).

Results: Two and three DPs were retained with RRR and PLS, respectively. The first patterns of each method were mainly characterized by adjusted-WC (RRR: 10.8%, PLS: 8.6%); none of them were associated with CVD risk. The second pattern of RRR and the third pattern of PLS were mainly explained by adjusted-VAI (RRR: 3.3, PLS: 2.1%). After adjusting for CVD risk factors, the hazard ratios [95% confidence intervals (CI)] for CVD in the second and third tertiles of the RRR-pattern 2 were 1.76 (1.15, 2.69) and 1.55 (1.00, 2.43) vs. the first tertile (*p*-trend: 0.058). This pattern had high positive loadings for non-leafy vegetables, pickled vegetables, fried vegetables, and bread and high negative loadings for eggs, cakes, butter, jam-honey, red meat, poultry, fish, juice, non-fermented dairy, and fruits. Per one SD increase in PLS-pattern 3 score, the risk of CVD was 19% higher (95%CI = 3–38%). This positive association was also observed across tertiles of the pattern (p-trend: 0.032). This pattern was characterized by high intakes of leafy vegetables, non-leafy vegetables, organ meat, soft drinks, olive oil, pickled vegetables, fried

vegetables, and bread and low intakes of biscuits, cakes, butter, eggs, and non-fermented dairy.

Conclusion: For each of the RRR and PLS approaches, a visceral-related DP that was positively linked to CVD was identified. These two patterns had a modest correlation. The pattern generated by PLS explained more variations in food groups and offered stronger evidence of association with CVD than the RRR-derived pattern.

KEYWORDS

reduced rank regression, partial least squares regression, dietary pattern, cardiovascular disease, hybrid method

Introduction

Cardiovascular disease (CVD) is the most common cause of mortality worldwide. According to World Health Organization estimates, 17.9 million individuals died from CVD in 2019 (1). Dietary risk factors showed the highest contributions to CVD deaths and CVD disability-adjusted life years among other non-communicable diseases worldwide. In 2017, 10 million CVD-related deaths and 207 million CVD disability-adjusted life years globally were attributed to an unsatisfactory diet (2). The majority of the dietary risk factors for CVD were proposed based on the findings of researches examining the associations between individual foods and nutrients (3, 4). Recent scientific statements intended to improve cardiovascular health, on the contrary, highlight the significance of dietary patterns (DPs) beyond individual foods or nutrients. According to the statements, cardio-protective DPs are characterized by high intakes of fruits, vegetables, whole grains, and healthy sources of protein and low intakes of processed foods and added sugar and salt beverages and foods (5, 6). However, the majority of the information comes from studies conducted on Western populations; hence, non-Western studies would aid in the development of future dietary recommendations (6).

Dietary pattern analysis is a supplementary approach that allows researchers to look at the whole diet rather than just a single item or nutrient. The approach captures the cumulative impact of dietary components by accounting for food-nutrient interactions (7). Dietary pattern analysis is classified into three types, namely, hypothesis-driven approaches, exploratory approaches, and hybrid approaches combining the two (8, 9). Reduced rank regression (RRR) and partial least squares (PLS) are two-hybrid methods in which DPs are derived based on intermediate response variables. The response variables are biomarkers of exposure or disease or other mediating variables thought to be essential for the development of the disease. The methods enable food pattern detection to include *a priori* assumptions about possible pathophysiological pathways (8, 9). To date, limited prospective studies investigate the association between RRR-derived DPs and risk of CVD morbidity and mortality (10–17), and only two studies employed the PLS approach in addition to the RRR method (13, 17). In these studies, dietary intakes of nutrients (e.g., nutrients related to excess energy intake), body mass index (BMI), blood pressure, lipid profiles, and inflammatory markers were selected as response variables (10–17). Visceral adiposity may be an important mediator in the relationship between diet and CVD risk (18, 19). However, the association between visceral adiposity-related DPs and CVD risk has yet to be investigated. To address this, we determined DPs related to waist circumference (WC) and visceral adiposity index (VAI), the two visceral adiposity indices. We then examined the association between the adiposity-related patterns and incident CVD over a mean of 10.2 years of follow-up.

Materials and methods

Study population

This longitudinal study was conducted within the Tehran Lipid and Glucose Study (TLGS) framework, a large-scale population-based prospective study being performed on a representative sample of Tehran, the capital of Iran. Participants were recruited from residents of District 13 of Tehran. Details, rationale, sampling, and data collection methods of TLGS have been mentioned previously (20). In brief, the TLGS began in 1999–2001; the data gathering process is ongoing at 3-year intervals to update the health-related baseline measurements of participants. Since the third examination cycle (2005–2008), dietary information has been gathered using a food frequency questionnaire (FFQ). Of the 3,687 participants in the third examination cycle who had complete dietary data, which we used as the baseline for the current analyses, those aged $\geq$ 19 years (n = 3,086) were selected. Participants with

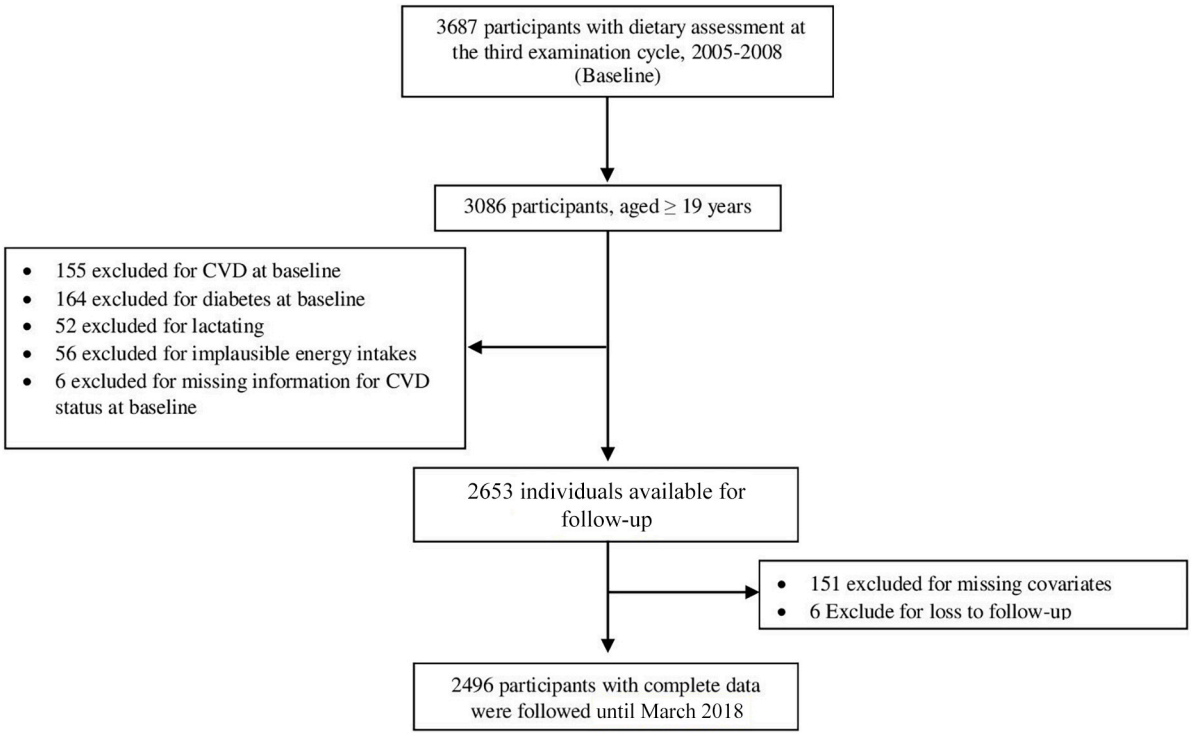

FIGURE 1
Diagram showing the selection process of the study participants.

prevalent CVD or diabetes, lactating women, those who had reported implausible energy intakes [<814 (first percentile) or > 5,320 (99th percentile) for women; <915 (first percentile) or > 6,641 kcal (99th percentile) for men], and those with missing data about CVD status at baseline or follow-ups or missing covariates were excluded. A total of 2,496 adults free of CVD at the third examination cycle (as baseline) were finally included in this study, who were followed until March 2018 (**Figure 1**).

This study was conducted based on the guidelines of the Declaration of Helsinki; the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, approved the study design. All participants provided written informed consent before participation in the study.

Demographic and anthropometric measurements

Information on age, sex, education, smoking habits, past medical history of CVD, drug usage, and family history of premature CVD of participants was collected by trained investigators using a pretested questionnaire in private and face-to-face interviews. The participants were weighed using a digital scale while wearing less clothing and no shoes, and their height was measured in a standing position with no shoes using a non-stretched tape measure. BMI was calculated by dividing weight in kilograms by height squares in meters. WC was measured by using a non-stretched tape with no pressure on the body surface. Leisure time, job, and household activities in the past year were assessed using the Persian-translated Modifiable Activities Questionnaire (21), and total physical activity was reported as metabolic equivalent minutes per week (MET-min/wk).

Clinical and biochemical measurements

Systolic and diastolic blood pressure was measured two times in a seated position on the right arm after 15 min of rest, using a standardized mercury sphygmomanometer (Riester, Jungingen, Germany). The mean of the two measurements was reported as the participants' blood pressure.

Fasting blood samples were collected in the morning after 12–14-h overnight fasting based on the standard protocol. All biochemical measurements were assessed at the TLGS research laboratory on the same day of blood collection. Fasting plasma glucose (FPG), triglycerides (TGs), total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured using laboratory kits (Pars Azmun, Tehran, Iran) (22).

Dietary assessment

Dietary data at baseline were collected using a validated and reliable semi-quantitative FFQ (23, 24). The questionnaire had 168 food items with predefined portion sizes. A trained dietitian asked participants to report the consumption of each food item according to its predefined portion size during the preceding year on a daily, weekly, or monthly basis as appropriate. The portion size of consumed foods was converted to daily frequency and then converted to grams. On the basis of their nutrient profiles, the ingested items were classified into 34 food groups for DP analysis (**Supplementary Table 1**).

Intermediate (response) variables for dietary pattern extraction

The intermediate variables to extract DPs were WC and VAI. The VAI is a sex-specific index that incorporates anthropometric (BMI and WC) and biochemical variables (TG and HDL) to indirectly assess visceral adiposity (25). It can be calculated using the following formulas (25):

$$\text{Male} : [\text{WC}/(39.68 + (1.88 \times \text{BMI}))] \times (\text{TG}/1.03) \times (1.31/\text{HDL})$$

$$\text{Female} : [\text{WC}/(36.58 + (1.89 \times \text{BMI}))] \times (\text{TG}/0.81) \times (1.52/\text{HDL})$$

To control the effects of age and BMI on the visceral adiposity variables, WC was adjusted for age and BMI, and VAI was adjusted for age by the residual method.

Definition of outcome and terms

The data collection process for CVD outcomes was previously addressed (26). Participants were followed annually to identify medical events. After reporting a medical event by participants or their relatives, an expert panel evaluated the medical documents to confirm the incidence of a specific outcome. The CVD incidence was defined as myocardial infarction (definite or probable), angiographically proven coronary heart disease, stroke, and death due to cardiovascular causes. Any CVD events in first-grade female family members aged 65 years or lower or first-grade male family members aged 55 years or lower were considered a premature family history of CVD (27). Based on the American Diabetes Association's guidelines, diabetes mellitus was defined as FPG $\geq$ 126 mg/dl or 2-hPG $\geq$ 200 mg/dl or glucose-lowering medical drug usage (28). Hypertension was defined as having a systolic blood pressure $\geq$ 140 mmHg or a diastolic blood pressure $\geq$ 90 mmHg or being on antihypertensive medication (29). Hypercholesterolemia was defined as serum total cholesterol $\geq$ 200 mg/dl or the use of any lipid-lowering medical drug. We categorized participants according to their smoking habits into two groups, namely, ever-smokers (current smokers or ex-smokers) and non-smokers. We divided education status into $\leq$ 12 years and $>$ 12 years (academic education).

Statistical methods

Energy-adjusted food groups, age and BMI-adjusted WC, and age-adjusted VAI were determined using the residual method. Since the distribution of food groups and VAI were skewed, the natural logarithmic transformation was performed on the variables before residual adjusting. DPs were obtained by two statistical methods, namely, RRR and PLS, using PROC PLS in SAS version 9.1.3. Energy-adjusted intakes of the 34 groups were selected as predictors, and adjusted-WC and adjusted-VAI were selected as intermediate variables. For further statistical analyses, the two patterns of RRR and the first three patterns of PLS that explained the most variations in intermediate variables were retained.

Participants were divided into three groups based on the tertiles of dietary pattern score derived by the first pattern obtained for each method. The characteristics of participants across the tertiles of the pattern scores were compared using ANOVA for normally distributed continuous variables, the Kruskal-Wallis test for non-normally distributed continuous variables, and chi-square for categorical variables.

We used the Cox proportional hazards regression model to estimate CVD risk per one SD increase in each dietary pattern score. CVD risk was also assessed for the tertile of each pattern, considering the first group as the reference category. A linear trend (p-trend) was obtained by assigning the median value to each tertile and treating this as a continuous variable. Statistical tests and graphical diagnostics based on scaled Schoenfeld residuals were used to assess the proportional hazards (PH) assumption. All proportionality assumptions were generally satisfied. The time of follow-up was calculated from the date of entrance to the study to the first occurrence of CVD events or the last follow-up date. Regression models were adjusted for potential covariates related to the risk of CVD. In the first adjusted model, age (continuous), sex, premature family history of CVD (yes/no), and energy intake (continuous) were included as covariates. The second model was additionally adjusted for academic education (yes/no), ever-smoking (yes/no), physical activity (continuous), prevalent hypertension (yes/no/missing),

prevalent hypercholesterolemia (yes/no), and baseline fasting plasma glucose (continuous). Six individuals lacked the information necessary to assess their hypertension status at baseline, and thus, we created an additional category of "missing" for them. Results were reported as hazard ratios (HR) and 95% confidence intervals (95%CI).

In sensitivity analyses, the risk of CVD was recalculated after excluding the following: 1, those with early CVD events (< 2 years); 2, excluding participants aged < 30 years; 3, those with early CVD events and aged < 30 years. Statistical analyses were performed using SPSS version 20; a p-value < 0.05 was considered statistically significant.

Results

Visceral adiposity-dietary patterns

Two patterns were obtained from RRR, which explained 7.8% of the variations in both the food group intake and the response variables. The first RRR-derived dietary pattern was defined mainly by adjusted-WC (10.8%) and, to a lesser extent, by adjusted-VAI (1.2%). This pattern was characterized by a high intake of soft drinks, organ meat, bread, pasta-rice, and sugars and a low intake of non-leafy vegetables, rye-bulgur, fried vegetables, fruits, leafy vegetables, dried fruits, and non-fermented dairy. The second RRR-derived dietary pattern was explained by more variation in adjusted-VAI (3.3%) but far less variation in adjusted-WC than the first RRR-derived pattern. This pattern had high positive loads on non-leafy vegetables, pickled vegetables, fried vegetables, and bread and high negative loads on eggs, cakes, butter, jam-honey, red meat, poultry, fish, juice, non-fermented dairy, and fruits.

Three DPs were retained by PLS, explaining 17.0% variation in food intake and 7.3% variation in the two response variables. Among the three patterns, most of the adjusted-WC and adjusted-VAI variances were defined by the first and third DPs, respectively. The first pattern represented a higher intake of soft drinks, pasta-rice, bread, and organ meat and a lower intake of non-leafy vegetables, fruits, fried and leafy vegetables, dried fruits, rye-bulgur, legumes, olive oil, fermented dairy, and jam-honey. The second pattern was positively related to the high intake of juice, organ meat, fish, jam-honey, olive oil, canned fruits, nuts, poultry, eggs, dried fruits, non-fermented dairy, fruits, sugars, and red meat and low intake of vegetable fat. The third pattern was a diet with high positive loads on leafy vegetables, non-leafy vegetables, organ meat, soft drinks, olive oil, pickled vegetables, fried vegetables, and bread and high negative loads on biscuits, cakes, butter, eggs, and non-fermented dairy. Factor loading and variances explained by each dietary pattern are presented in Table 1. The median intakes of food groups with factor loading ≥ 0.15 across tertiles of each DP are shown in Supplementary Tables 2, 3.

TABLE 1 Factor loadings of food groups in visceral adiposity-related dietary patterns identified by RRR and PLS.

Food groups[a]	RRR-derived		PLS-derived		
	Pattern 1	Pattern 2	Pattern 1	Pattern 2	Pattern 3
Soft drinks	**0.42**	0.13	**0.31**	0.09	**0.24**
Organ meat	**0.34**	−0.08	**0.17**	**0.29**	**0.29**
Non-leafy vegetables	**−0.30**	**0.40**	**−0.37**	−0.04	**0.35**
Fried vegetables	**−0.28**	**0.15**	**−0.31**	0.00	**0.18**
Rye-bulgur	**−0.28**	0.13	**−0.25**	−0.01	0.04
Breads	**0.27**	**0.15**	**0.19**	−0.11	**0.15**
Pasta-rice	**0.26**	−0.13	**0.22**	0.02	−0.04
Fruits	**−0.26**	**−0.15**	**−0.33**	**0.19**	0.12
Sugars	**0.21**	−0.12	0.13	**0.19**	0.05
Leafy vegetables	**−0.20**	0.08	**−0.31**	0.08	**0.37**
Dried fruits	**−0.19**	−0.10	**−0.26**	**0.22**	0.10
Non-fermented dairy	**−0.17**	**−0.17**	−0.14	**0.19**	**−0.17**
Biscuits	−0.13	−0.13	−0.04	0.07	**−0.29**
Fast foods	0.11	0.13	0.13	0.02	0.005
Pickled vegetables	−0.11	**0.22**	−0.12	−0.06	**0.20**
Jam-honey	−0.11	**−0.24**	**−0.15**	**0.27**	−0.10
Legumes	−0.10	0.05	**−0.17**	0.04	0.10
Red meat	−0.09	**−0.22**	−0.03	**0.17**	−0.12
Olive oil	−0.07	−0.08	**−0.17**	**0.26**	**0.22**
Fermented dairy	−0.07	−0.04	**−0.15**	0.15	0.14
Juice	0.08	**−0.18**	−0.01	**0.32**	0.12
Canned fruits	0.06	−0.14	0.03	**0.25**	0.05
Fish	0.06	**−0.20**	−0.02	**0.27**	0.10
Animal fat	−0.06	−0.03	−0.07	0.07	0.02
Potatoes	0.05	−0.05	0.004	0.12	0.09
Nuts	−0.05	−0.09	−0.13	**0.24**	0.14
Eggs	−0.05	**−0.37**	−0.02	**0.23**	**−0.19**
Tea-coffee	−0.03	−0.06	−0.03	0.09	0.02
Butter	−0.03	**−0.25**	0.03	0.14	**−0.29**
Cakes	0.02	**−0.28**	0.06	**0.21**	**−0.20**
Poultry	0.02	**−0.20**	−0.02	**0.23**	0.01
Snacks	0.01	0.12	0.13	−0.04	−0.10
Vegetable fat	−0.01	0.14	0.04	**−0.17**	−0.09
Vegetable oil	−0.003	0.04	−0.07	0.08	0.13
Explained variations (%)					
Food groups	4.7	3.1	7.3	5.8	3.9
Adjusted_WC[b]	10.8	0.36	8.6	1.2	0.9
Adjusted_VAI[c]	1.2	3.32	0.69	1.2	2.1
Total responses	6.0	1.84	4.6	1.2	1.5

Factor loadings ≥ 0.15 were bolded for ease of reading. [a]Adjusted for energy intakes using the residual method. [b]Adjusted for age and BMI using the residual method. [c]Adjusted for age using the residual method.

Association between dietary patterns

In Figure 2, correlations between DPs and response variables are shown. The first pattern derived by each method had a high correlation (r = 0.88; p < 0.001). RRR-pattern 2 was negatively correlated with the PLS-pattern 2 (r = −0.73; p < 0.001) and positively correlated with the PLS-pattern 3 (r = 0.56; p < 0.001). RRR-pattern 2 had a modest inverse correlation with adjusted-WC (r = −0.06; p = 0.003), and PLS-pattern 2 was inversely correlated with adjusted-VAI (r = −0.11; p < 0.001). The other patterns were positively correlated with the response variables. It should be mentioned that patterns identified by the same statistical method were uncorrelated.

Characteristics of participants

The mean age of the 2,496 participants was 38.1 ± 13.3 years at baseline; 1,340 participants were female (53.7%). Characteristics of participants based on tertiles of the first patterns obtained by each method are reported in Table 2. The number of men and smokers increased from tertile 1 to tertile 3 in both patterns. In addition, with increasing tertiles of RRR-pattern 1, participants had higher WC, FPG, and TGs but lower physical activity and HDL-C at baseline. Those with higher adherence to the PLS-pattern 1 were younger and had lower physical activity, BMI, HDL-C, and total cholesterol.

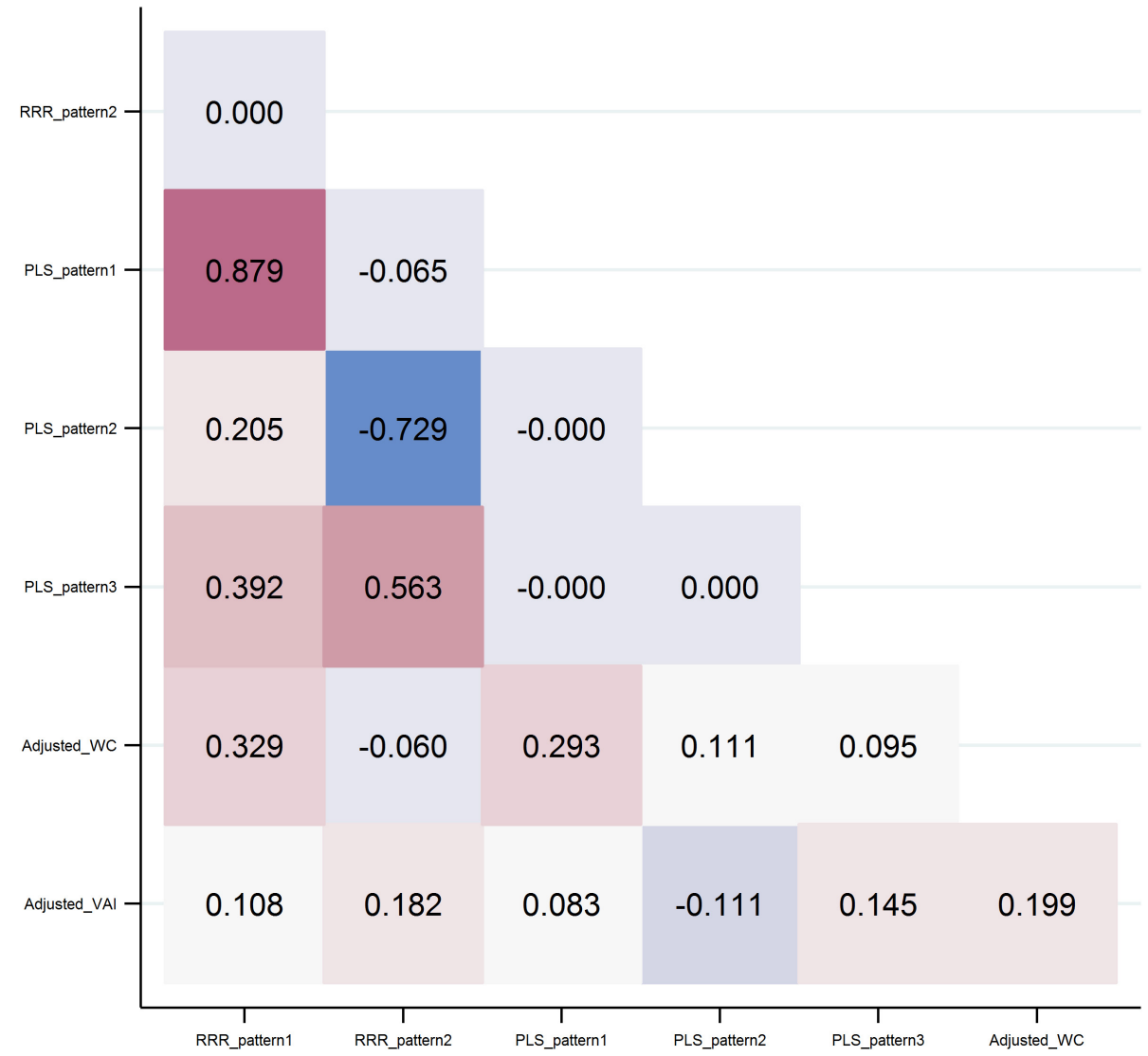

FIGURE 2
Pearson correlations between dietary patterns and response variables.

Association between dietary patterns and cardiovascular disease events

During a mean of 10.2 (*SD* = 1.94) years of follow-up (25,522 person-years), 141 incidence cases of CVD were identified. HRs (95%CI) for CVD events according to the tertiles of visceral adiposity DPs are shown in Table 3. After adjustment for age, sex, family history of CVD, and energy intake, the risk of CVD was marginally increased per one SD change in the RRR-pattern 2, but the association became non-significant after adjusting for further covariates in model 2. The risk of CVD events was significantly higher in tertiles 2 and 3 of the RRR-pattern 2 than in the first tertile in both adjusted models. One SD increase in the PLS-pattern 3 was associated with a 51% (95%CI = 30–74%) increased risk of CVD events. After controlling for all potential covariates, the estimate was reduced to 19% significantly higher CVD risk per one SD (95%CI = 3–38%; model 2). Consistent with the findings for the continuous score, the risk of CVD events increased by tertiles of PLS-pattern 3 and remained significant after adjusting for all potential covariates (p-trend = 0.032). No associations were observed between adherence to the other visceral-adiposity pattern and CVD events.

Sensitivity analysis

In sensitivity analysis (Table 4), after excluding those with an early occurrence of CVD (<2 years), the risk of CVD events was significantly increased per one SD increase of RRR-pattern 2 and PLS-pattern 3. When we excluded those with early CVD (<2 years) and those younger than 30, the risk of CVD occurrence was 25% (95% CI = 2–53%) and 22% (95% CI = 4–44%) with higher RRR-pattern 2 and PLS-pattern 3, respectively, in the fully adjusted model.

Discussion

In this prospective study, we identified DPs related to visceral adiposity markers using the two methods of RRR and PLS, and then we demonstrated the DPs associations with the risk of CVD in Iranian adults. The first pattern determined by both methods was mainly explained by inter-individual variations in adjusted-WC, explaining 10.8% variation in RRR and 8.6% variation in PLS. These two patterns were positively correlated with the two response variables and were highly correlated with each other (r = 0.88). Despite some differences in the magnitude of factor loadings and characteristics of the DPs, both were characterized by

TABLE 2 Baseline characteristics of participants according to the tertiles of the first dietary patterns obtained by RRR and PLS.

Variables	Dietary pattern scores tertiles							
	RRR-pattern 1				PLS-pattern 1			
	1st	2nd	3rd	p−value[a]	1st	2nd	3rd	p−value[a]
Age, years	38.8 (13.7)	37.8 (13.0)	37.8 (13.2)	0.236	41.0 (13.8)	38.1 (13.1)	35.3 (12.3)	< 0.001
Female, *n* (%)	626 (75.3)	477 (57.3)	237 (28.5)	< 0.00	605 (72.8)	457 (54.9)	278 (33.4)	< 0.001
Academic education, *n* (%)	226 (27.2)	234 (28.1)	198 (23.8)	0.111	220 (26.5)	227 (27.3)	211 (25.3)	0.662
Ever−smokers, *n* (%)	104 (12.5)	167 (20.0)	270 (32.5)	< 0.001	109 (13.1)	175 (21.0)	257 (30.9)	< 0.001
Premature Family history of CVD, *n* (%)	85 (10.2)	93 (11.2)	75 (9.0)	0.346	83 (10.0)	83 (10.0)	87 (10.4)	0.937
Physical activity, MET-min/wk	1,134 (357, 2,456)	878 (257, 2,292)	834 (119, 2,501)	0.011	1,191 (405, 2,501)	917 (238, 2,233)	792 (99.9, 2,536)	< 0.001
BMI, kg/m2	26.8 (4.97)	26.7 (4.91)	26.7 (4.56)	0.873	27.3 (4.86)	26.7 (4.81)	26.3 (4.73)	< 0.001
WC, cm	86.5 (13.5)	88.6 (13.3)	91.4 (12.4)	< 0.001	88.2 (13.4)	88.5 (13.1)	89.8 (13.1)	0.034
VAI	1.81 (1.12, 2.87)	1.91 (1.27, 3.08)	2.06 (1.27, 3.25)	< 0.001	1.93 (1.20, 3.05)	1.91 (1.18, 3.01)	1.94 (1.25, 3.07)	0.806
FPG, mg/dl	86.3 (8.62)	86.9 (8.61)	87.6 (8.60)	0.007	86.9 (9.01)	86.6 (8.40)	87.3 (8.43)	0.256
TGs, mg/dl	108 (77.0, 153)	117 (83.0, 167)	129 (86.0, 182)	< 0.001	114 (81.0, 164)	116 (79, 169)	119 (84, 174)	0.129
HDL-C, mg/dl	44.9 (10.6)	42.9 (9.92)	40.5 (9.31)	< 0.001	44.4 (10.4)	43.0 (10.1)	40.9 (9.59)	< 0.001
Total cholesterol, mg/dl	185 (38.5)	184 (37.8)	185 (37.7)	0.910	188 (38.8)	184 (36.3)	182 (38.7)	0.007
Hypertension[b], *n* (%)	77 (9.3)	86 (10.3)	93 (11.2)	0.441	94 (11.4)	91 (10.9)	71 (8.6)	0.129
Hypercholesterolemia, *n* (%)	279 (33.6)	273 (32.8)	290 (34.9)	0.663	309 (37.2)	279 (33.5)	254 (30.5)	0.015

Data are shown as mean (SD), median (25th, 75th percentile), or No. (%). [a]Based on ANOVA for normally distributed continuous variables, the Kruskal-Wallis test for non-normally distributed variables, and chi-square for categorical variables. [b]Missing for n = 6. BMI, body mass index; CVD, cardiovascular disease; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein-C; PLS, partial least squares; RRR, reduced rank regression; TGs, triglycerides; VAI, visceral adiposity index; WC, waist circumference.

TABLE 3 Hazard ratio (HR) and 95% confidence intervals (CIs) for cardiovascular events after a mean follow-up of 10.2 years based on visceral adiposity-related dietary patterns.

Dietary pattern scores	Continuous		Tertile			
	Per one *SD*	*p*-value	1st	2nd	3rd	*p*-trend
RRR-dietary patterns						
Pattern 1						
Incidence cases	141		46	39	56	
Person-years	25,522		8,486	8,610	8,426	
Incidence per 10,000	55.2		54.3	45.3	66.5	
Unadjusted	1.13 (0.97, 1.31)	0.120	1.00	0.83 (0.55, 1.28)	1.22 (0.83, 1.80)	0.312
Model 1	1.07 (0.91, 1.27)	0.375	1.00	0.77 (0.50, 1.19)	1.00 (0.66, 1.52)	0.939
Model 2	1.06 (0.89, 1.25)	0.531	1.00	0.72 (0.47, 1.13)	0.95 (0.62, 1.45)	0.951
Pattern 2						
Incidence cases	141		35	57	49	
Person-years	25,522		8,529	8,456	8,536	
Incidence per 10,000	55.2		41.0	67.4	57.4	
Unadjusted	1.09 (0.91, 1.31)	0.329	1.00	1.64 (1.08, 2.50)	1.40 (0.91, 2.16)	0.148
Model 1	1.20 (1.00, 1.44)	0.054	1.00	1.71 (1.12, 2.61)	1.68 (1.09, 2.60)	0.021
Model 2	1.15 (0.96, 1.38)	0.135	1.00	1.76 (1.15, 2.69)	1.55 (1.00, 2.43)	0.058
PLS-dietary patterns						
Pattern 1						
Incidence cases	141		57	38	46	
Person-years	25,522		8,485	8,546	8,490	
Incidence per 10,000	55.2		67.2	44.5	54.2	
Unadjusted	0.95 (0.86, 1.06)	0.339	1.00	0.66 (0.44, 0.99)	0.80 (0.54, 1.18)	0.220
Model 1	1.02 (0.91, 1.14)	0.766	1.00	0.69 (0.45, 1.06)	1.09 (0.72, 1.65)	0.821
Model 2	0.99 (0.88, 1.11)	0.812	1.00	0.65 (0.43, 1.00)	1.03 (0.67, 1.54)	0.959
Pattern 2						
Incidence cases	141		45	42	54	
Person-years	25,522		8,517	8,529	8,476	
Incidence per 10,000	55.2		52.8	49.2	63.7	
Unadjusted	1.04 (0.92, 1.17)	0.512	1.00	0.93 (0.61, 1.42)	1.21 (0.81, 1.79)	0.355
Model 1	0.95 (0.85, 1.07)	0.395	1.00	0.84 (0.55, 1.28)	0.91 (0.61, 1.37)	0.674
Model 2	0.99 (0.88, 1.12)	0.913	1.00	0.88 (0.57, 1.35)	1.01 (0.67, 1.52)	0.970
Pattern 3						
Incidence cases	141		25	43	73	
Person-years	25,522		8,601	8,515	8,405	
Incidence per 10,000	55.2		29.1	50.5	86.8	
Unadjusted	1.51 (1.30, 1.74)	< 0.001	1.00	1.74 (1.06, 2.85)	3.00 (1.90, 4.72)	<0.001
Model 1	1.20 (1.04, 1.40)	0.015	1.00	1.41 (0.86, 2.31)	1.77 (1.07, 1.10)	0.014
Model 2	1.19 (1.03, 1.38)	0.022	1.00	1.34 (0.81, 2.20)	1.64 (1.03, 2.60)	0.032

Model 1: Adjusted for age (continuous), sex, premature family history of CVD (yes/no), and energy intake (continuous). Model 2: Additionally adjusted for academic education (yes/no), ever-smokers (yes/no), physical activity (continuous), baseline prevalent hypertension (yes/no/unclear), baseline prevalent hypercholesterolemia (yes/no), and baseline fasting plasma glucose (continuous).

higher intakes of soft drinks, bread, pasta-rice, and organ meat and lower intakes of vegetables (fried, leafy, and non-leafy), fruits, dried fruits, and rye-bulgur. After taking into account traditional risk factors for CVD, there was no significant association between these patterns and the development of CVD.

RRR-pattern 2 and PLS-pattern 3 determined more variation in adjusted-VAI than the other patterns. Adjusted VAI was positively correlated with RRR-pattern 2 and PLS-pattern 3, but adjusted-WC showed a small inverse correlation with RRR-pattern 2 ($R = -0.06$) and a positive correlation with PLS-pattern 3 ($r = 0.10$). A moderate positive correlation was

TABLE 4 Sensitivity analysis on prospective associations between visceral adiposity-related dietary patterns and risk of cardiovascular events.

	RRR-dietary patterns				PLS-dietary patterns					
	Pattern 1	***p*-value**	**Pattern 2**	***p*-value**	**Pattern 1**	***p*-value**	**Pattern 2**	***p*-value**	**Pattern 3**	***p*-value**
Excluding early CVD (< 2 years)										
Incidence cases = 119										
Model 1	1.05 (0.88, 1.26)	0.569	1.32 (1.08, 1.61)	0.006	1.02 (0.89, 1.16)	0.808	0.89 (0.79, 1.00)	0.057	1.22 (1.04, 1.43)	0.014
Model 2	1.04 (0.87, 1.24)	0.691	1.25 (1.02, 1.53)	0.029	0.99 (0.87, 1.13)	0.993	0.92 (0.81,1.05)	0.923	1.22 (1.04,1.43)	0.017
Excluding adults < 30 years										
Incidence cases = 139										
Model 1	1.08 (0.91, 1.28)	0.377	1.19 (0.99, 1.43)	0.066	1.01 (0.89, 1.14)	0.901	0.96 (0.86, 1.08)	0.517	1.20 (1.04,1.39)	0.015
Model 2	1.06 (0.89, 1.25)	0.515	1.12 (0.93, 1.35)	0.234	0.98 (0.87, 1.10)	0.767	1.00 (0.89, 1.13)	0.942	1.19 (1.03, 1.38)	0.023
Excluding early CVD and aged < 30 years										
Incidence cases = 117										
Model 1	1.05 (0.88, 1.27)	0.573	1.31 (1.07, 1.60)	0.008	1.01 (0.88, 1.15)	0.900	0.90 (0.79, 1.02)	0.092	1.23 (1.04, 1.44)	0.013
Model 2	1.04 (0.87, 1.25)	0.673	1.25 (1.02, 1.53)	0.035	0.99 (0.87, 1.13)	0.861	0.93 (0.82, 1.06)	0.286	1.22 (1.04, 1.44)	0.017

Data are shown as hazard ratio (95% confidence intervals) estimated per one SD of dietary pattern scores. Model 1: Adjusted for age (continuous), sex, premature family history of CVD (yes/no), and energy intake (continuous). Model 2: Additionally adjusted for academic education (yes/no), ever-smokers (yes/no), physical activity (continuous), baseline prevalent hypertension (yes/no/unclear), baseline prevalent hypercholesterolemia (yes/no), and baseline fasting plasma glucose (continuous).

found between the two patterns ($r = 0.56$). CVD risk was higher across the tertiles of RRR-pattern 2, but the association of CVD with the score as a continuous variable was not significant. In the fully adjusted model, the risk of CVD was 19% higher per one SD increase in the score of PLS-pattern 3, and the risk of CVD was significantly increased from tertile 1 to tertile 3 of the score. VAI is a lipid combined anthropometric index that takes into consideration BMI, TGs, and HDL-C in addition to WC. Therefore, it is not unexpected that the variable did not exhibit the same correlation with DPs as WC alone.

Limited prospective studies have investigated the association between DPs extracted with RRR and the risk of CVD (10–17); among them, PLS has been used in only two studies (13, 17). The intermediate response variables in these studies were different from ours. Intakes of nutrients were mainly used, and inflammatory markers and some traditional CVD risk factors, including BMI, total cholesterol/HDL-C, and systolic blood pressure, were defined as mediating variables in single studies. In this study, we used surrogates of visceral adiposity as intermediate response variables because of the deleterious effect of visceral adiposity on cardio-metabolic health that has been proposed (19). Since WC has been shown to be ineffective in distinguishing between subcutaneous and visceral fat, we employed two surrogates of visceral adiposity (30). In addition, there is a growing interest in employing the combined indices as compared with the separate anthropometric measures since some research indicates that the combined indices give better insights into the visceral adiposity function than the single variables (31, 32).

The PLS-pattern 3 could explain the 0.9% variation in WC and 2.1% variation in VAI. Therefore, the pattern cannot be considered a major determinant pattern of VAI or WC. However, the effect size of the association was remarkable and increased gradually from tertile 1 to 3 and was significant after controlling for all important traditional risk factors for CVD. The findings imply that even a small variation in visceral adiposity attributed to dietary intake is significantly associated with CVD development. Indeed, in contrast to principal component analysis, both RRR and PLS do not characterize major eating patterns in a population but discover what diet variety is crucial for illness development (33). In this pattern, both healthy (including vegetables and olive oil) and unhealthy foods (including organ meat and soft drinks) were loaded, so the pattern can be considered as a mixed DP. High intake of organ meat, soft drinks, salty vegetables, and fried vegetables may decrease the health benefits of consuming leafy vegetables and olive oil. The study's findings suggest that the health benefits of consuming vegetables and olive oil depend on the contribution of the other food groups in a dietary pattern. Previous research has also demonstrated the counteractive effects of various foods in a DP, which may modify its overall health benefits or detriments (34).

Taking into account the intermediate variables in the RRR and PLS approaches to studying diet-disease association increases the chance of identifying DPs related to a condition (8, 9). Although the two procedures are conceptually similar, RRR extracts factors explaining the maximal response variation. PLS constructs factors based on maximal variations in both predictors (food groups) and responses (9, 35). It is not well defined which method has the most capability to identify DPs

associated with a disease. As expected, in our study, the food group variations were explained more in PLS than in RRR, but the differences in defining the response variables were small. In addition, the number of patterns related to CVD was similar, but the association was more robust and significant for one SD increase in pattern scores derived by PLS. Most previous studies suggest that RRR produces more patterns related to an outcome than PLS (13, 33, 36, 37). However, in a case-control study, PLS could identify more patterns that were significantly associated with CVD than RRR (35). A recent prospective study suggested that deriving a DP for RRR and PLS is similarly efficient (17).

The study's strengths include a prospective design, a relatively long period of follow-up, administration of a valid FFQ, use of trained interviewers to fill the FFQ, accurate detection of CVD mortality and morbidity with medical document evaluation by a trained health professional, and adjusting for the most important CVD risk factors. Another advantage of the study is the use of the PLS method in addition to RRR. PLS is rarely implemented in nutritional epidemiology. As a result, the study's findings give some evidence for comparing the ability of the two distinct hybrid methods of RRR and PLS to detect DPs linked to CVD. Another strength of the research is including two different visceral fat surrogates as response variables. Moreover, to reflect visceral adiposity independent of age and BMI, the residuals of WC regressed on age and BMI, and VAI regressed on age were calculated.

It is also necessary to acknowledge some of the study's limitations. First, accurate reporting of the food items of FFQ relies on participants' memory. In addition, participants may add bias by over-reporting eating "good" foods or under-reporting eating "bad" foods. Therefore, the FFQ-assessed food intakes are subject to measurement errors (38). Second, DPs were extracted based on dietary intake data collected at baseline, which could not reflect the long-term dietary intake. Third, visceral fat was not directly measured. Magnetic resonance imaging (MRI) and computed tomography (CT) are the gold standards for measuring visceral adiposity; however, cost and participant burden prevent the use of MRI and CT in population-based studies (30). Fourth, we could not analyze CVD mortality and stroke separately because of the low number of cases and power consideration. Fifth, the division of the original food items into food groups is arbitrary, and changes in food grouping may impact the patterns found (8). We categorized grains into three groups, namely, rye-bulgur, pasta-rice, and bread. Because the fiber of bread typically consumed by Iranians differs less, we combine their intakes into a single category. In addition, we divided dairy intakes into two groups according to the fermentation process and not their fat contents due to the likelihood of underestimation for high-fat dairy intakes. Sixth, the *post hoc* power calculation showed that the power of the study in DPs that we did not find significant associations was insufficient. Therefore, the null hypothesis may be incorrectly accepted, leading to false-negative results.

Conclusion

In conclusion, using RRR and PLS approaches, we identified two DPs positively associated with CVD development. The pattern obtained by RRR with a high intake of non-leafy vegetables, pickled vegetables, fried vegetables, and bread and a low intake of eggs, cakes, butter, jam-honey, red meat, poultry, fish, juice, non-fermented dairy, and fruits showed an inverse correlation with adjusted-WC and a positive correlation with adjusted-VAI. The risk of CVD was substantially greater in the second and third tertiles of the pattern. The PLS-derived pattern with positive loadings on leafy vegetables, non-leafy vegetables, organ meat, soft drinks, olive oil, pickled vegetables, fried vegetables, and bread and high negative loads on biscuits, cakes, butter, eggs, and non-fermented dairy was positively correlated with both response variables. The risk of CVD increased with each SD increase in the score and throughout its tertiles. Our findings implicate that all DPs with high intakes of vegetables are not necessarily cardio-protective diets. Identification of DPs related to visceral adiposity improves our understanding about the association between diet and risk of CVD, which merits more investigation in future prospective studies.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

This study was designed according to the Declaration of Helsinki principles; the Ethics Committee of the Research Institute for Endocrine Sciences at the Shahid Beheshti University of Medical Sciences confirmed the study design (IR.SBMU.ENDOCRINE.REC.1400.056). The patients/participants provided their written informed consent to participate in this study.

Author contributions

NM and FR conceptualized the study. NM analyzed and interpreted the data and wrote the first draft of the manuscript. FR contributed to the literature review and helped in preparing the first draft of the manuscript. MM contributed to the data analysis. PM and FA supervised the analyses and contributed to the critical revision of the

manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The reviewer GA declared a shared affiliation with the authors to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.812701/full#supplementary-material

References

1. World Health organization. (2022) Available online at: https://www.who.int/en/news-room/fact sheets/detail/cardiovascular-diseases-(cvds) (Accessed June 7, 2022)

2. Gbd 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the global burden of disease study 2017. *Lancet.* (2019) 393:1958–72. doi: 10.1016/S0140-6736(19)30041-8

3. Violi F, Pastori D, Pignatelli P, Carnevale R. Nutrition, thrombosis, and cardiovascular disease. *Circ Res.* (2020) 126:1415–42. doi: 10.1161/CIRCRESAHA.120.315892

4. Gomez-Delgado F, Katsiki N, Lopez-Miranda J, Perez-Martinez P. Dietary habits, lipoprotein metabolism and cardiovascular disease: from individual foods to dietary patterns. *Crit Rev Food Sci Nutr* . (2021) 61:1651–69. doi: 10.1080/10408398.2020.1764487

5. Visseren FL, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice: developed by the task force for cardiovascular disease prevention in clinical practice with representatives of the European society of cardiology and 12 medical societies with the special contribution of the European association of preventive cardiology (EAPC). *Eur Heart J.* (2021) 42:3227–337. doi: 10.1093/eurheartj/ehab484

6. Lichtenstein AH, Appel LJ, Vadiveloo M, Hu FB, Kris-Etherton PM. Rebholz CM, et al. 2021 dietary guidance to improve cardiovascular health: a scientific statement from the American heart association. *Circulation.* (2021) 144:e472–87. doi: 10.1161/CIR.0000000000001031

7. Schulze MB, Hoffmann K. Methodological approaches to study dietary patterns in relation to risk of coronary heart disease and stroke. *Br J Nutr.* (2006) 95:860–9. doi: 10.1079/bjn20061731

8. Schulz C-A, Oluwagbemigun K, Nöthlings U. Advances in dietary pattern analysis in nutritional epidemiology. *Eur J Nutr.* (2021) 60:4115–30. doi: 10.1007/s00394-021-02545-9

9. Zhao J, Li Z, Gao Q, Zhao H, Chen S, Huang L, et al. A review of statistical methods for dietary pattern analysis. *Nutr J.* (2021) 20:37. doi: 10.1186/s12937-021-00692-7

10. Drogan D, Hoffmann K, Schulz M, Bergmann MM, Boeing H, Weikert C. A food pattern predicting prospective weight change is associated with risk of fatal but not with nonfatal cardiovascular disease. *Nutr J.* (2021) 20:37. doi: 10.1186/s12937-021-00692-7

11. Johns DJ, Lindroos AK, Jebb SA, Sjöström L, Carlsson LM, Ambrosini GL. Dietary patterns, cardiometabolic risk factors, and the incidence of cardiovascular disease in severe obesity. *Obesity.* (2015) 23:1063–70. doi: 10.1002/oby.20920

12. Biesbroek S, Van Der ADL, Brosens MC, Beulens JW, Verschuren WM, Van Der Schouw YT, et al. Identifying cardiovascular risk factor–related dietary patterns with reduced rank regression and random forest in the EPIC-NL cohort. *Am J Clin Nutr.* (2015) 102:146–54. doi: 10.3945/ajcn.114.092288

13. Nazari SSH, Mokhayeri Y, Mansournia MA, Khodakarim S, Soori H. Associations between dietary risk factors and ischemic stroke: a comparison of regression methods using data from the multi-ethnic study of atherosclerosis. *Epidemiol Health.* (2018) 40:e2018021. doi: 10.4178/epih.e2018021

14. Shi Z, Ganji V. Dietary patterns and cardiovascular disease risk among Chinese adults: a prospective cohort study. *Eur J Clin Nutr.* (2020) 74:1725–35. doi: 10.1038/s41430-020-0668-6

15. Lee HA, An H, Lee E. Dietary patterns related to cardiovascular disease based on reduced rank regression analysis of healthy middle-aged Koreans: data from the community-based Korean genome and epidemiology study (KoGES) cohort. *Am J Clin Nutr.* (2020) 111:1159–69. doi: 10.1093/ajcn/nqaa078

16. Gao M, Jebb SA, Aveyard P, Ambrosini GL, Perez-Cornago A, Carter J, et al. Associations between dietary patterns and the incidence of total and fatal cardiovascular disease and all-cause mortality in 116,806 individuals from the UK Biobank: a prospective cohort study. *BMC Med.* (2021) 19:83. doi: 10.1186/s12916-021-01958-x

17. Lazarova SV, Jessri M. Associations between dietary patterns and cardiovascular disease risk in canadian adults: a comparison of partial least squares, reduced rank regression and the simplified dietary pattern technique. *Am J Clin Nutr.* (2022):nqac117. doi: 10.1093/ajcn/nqac117 [Epub ahead of print].

18. Kammerlander AA, Lyass A, Mahoney TF, Massaro JM, Long MT, Vasan RS, et al. Sex differences in the associations of visceral adipose tissue and

cardiometabolic and cardiovascular disease risk: the framingham heart study. *J Am Heart Assoc.* (2021) 10:e019968. doi: 10.1161/JAHA.120.019968

19. Chartrand DJ, Murphy-Després A, Alméras N, Lemieux I, Larose E, Després J-P. Overweight, obesity, and CVD risk: a focus on visceral/ectopic fat. *Curr Atheroscler Rep*. (2022) 24:185–95. doi: 10.1007/s11883-022-00996-x

20. Azizi F, Zadeh-Vakili A, Takyar M. Review of rationale, design, and initial findings: tehran lipid and glucose study. *Int J Endocrinol Metab.* (2018) 16:e84777. doi: 10.5812/ijem.84777

21. Momenan AA, Delshad M, Sarbazi N, Rezaei Ghaleh N, Ghanbarian A, Azizi F. Reliability and validity of the modifiable activity questionnaire (MAQ) in an Iranian urban adult population. *Arch Iranian Med.* (2012) 15:279–82.

22. Azizi F, Ghanbarian A, Momenan AA, Hadaegh F, Mirmiran P, Hedayati M, et al. Prevention of non-communicable disease in a population in nutrition transition: tehran lipid and glucose study phase II. *Trials.* (2009) 10:5. doi: 10.1186/1745-6215-10-5

23. Esfahani FH, Asghari G, Mirmiran P, Azizi F. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the tehran lipid and glucose study. *J Epidemiol.* (2010) 20:150–8. doi: 10.2188/jea.je20090083

24. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the tehran lipid and glucose study. *Public Health Nutr.* (2010) 13:654–62. doi: 10.1017/S1368980009991698

25. Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, Midiri M, et al. Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk. *Diabetes Care.* (2010) 33:920–2. doi: 10.2337/dc09-1825

26. Hadaegh F, Harati H, Ghanbarian A, Azizi F. Association of total cholesterol versus other serum lipid parameters with the short-term prediction of cardiovascular outcomes: tehran lipid and glucose study. *Eur J Cardiovasc Prev Rehabil.* (2006) 13:571–7. doi: 10.1097/01.hjr.0000216552.81882.ca

27. Goff DC Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB Sr., Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American college of cardiology/American heart association task force on practice guidelines. *J Am Coll Cardiol.* (2014) 63:2935–59. doi: 10.1016/j.jacc.2013.11.005

28. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2010) 33:S62–9. doi: 10.2337/dc10-S062

29. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA*. (2014) 311:507–20. doi: 10.1001/jama.2013.284427

30. Shuster A, Patlas M, Pinthus J, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. *Br J Radiol.* (2012) 85:1–10. doi: 10.1259/bjr/38447238

31. Ejtahed H-S, Kelishadi R, Hasani-Ranjbar S, Angoorani P, Motlagh ME, Shafiee G, et al. Discriminatory ability of visceral adiposity index as an indicator for modeling cardio-metabolic risk factors in pediatric population: the CASPIAN-V study. *J Cardiovasc Thorac Res.* (2019) 11:280–6. doi: 10.15171/jcvtr.2019.46

32. Bijari M, Jangjoo S, Emami N, Raji S, Mottaghi M, Moallem R, et al. The Accuracy of visceral adiposity index for the screening of metabolic syndrome: a systematic review and meta-analysis. *Int J Endocrinol.* (2021) 2021:6684627. doi: 10.1155/2021/6684627

33. Hoffmann K, Schulze MB, Schienkiewitz A, Nöthlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. *Am J Epidemiol.* (2004) 159:935–44. doi: 10.1093/aje/kwh134

34. Mertens E, Markey O, Geleijnse JM, Givens DI, Lovegrove JA. Dietary patterns in relation to cardiovascular disease incidence and risk markers in a middle-aged British male population: data from the caerphilly prospective study. *Nutrients.* (2017) 9:75. doi: 10.3390/nu9010075

35. DiBello JR, Kraft P, McGarvey ST, Goldberg R, Campos H, Baylin A. Comparison of 3 methods for identifying dietary patterns associated with risk of disease. *Am J Epidemiol.* (2008) 168:1433–43. doi: 10.1093/aje/kwn274

36. Melaku YA, Gill TK, Taylor AW, Adams R, Shi Z. A comparison of principal component analysis, partial least-squares and reduced-rank regressions in the identification of dietary patterns associated with bone mass in ageing Australians. *Eur J Nutr.* (2018) 57:1969–83. doi: 10.1007/s00394-017-1478-z

37. Naja F, Itani L, Hwalla N, Sibai AM, Kharroubi SA. Identification of dietary patterns associated with elevated blood pressure among lebanese men: a comparison of principal component analysis with reduced rank regression and partial least square methods. *PLoS One.* (2019) 14:e0220942. doi: 10.1371/journal.pone.0220942

38. Kipnis V, Midthune D, Freedman L, Bingham S, Day NE, Riboli E, et al. Bias in dietary-report instruments and its implications for nutritional epidemiology. *Public Health Nutr.* (2002) 5:915–23. doi: 10.1079/PHN2002383

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Nutritional aspects in chronic non-cancer pain: A systematic review

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Objectives: Chronic pain (CP) is an unpleasant emotional and sensory experience that can be accompanied by tissue damage that persists for more than 3 months. Recent studies show that certain nutritional strategies can help to improve pain, so this study is aimed to systematically review scientific evidence to understand and map the effect of the use of nutritional strategies on the presence or intensity of chronic non-cancer pain (CNCP) and the association of these nutritional aspects with the presence or intensity of CNCP.

Study design: A systematic review.

Methods: Two independent researchers searched for randomized clinical trials (RCTs) and observational studies that explored the relationship between nutrition and CNCP in adults from 2010 to 2020 in PubMed, Web of Science, Scopus, and Cochrane Library databases. A total of 24 studies were included, of which 20 were RCTs and 4 were observational studies. They are classified into the administration of nutritional supplements, dietary modification, and incorporation of food.

Results: Of these studies, those that have a significant effect on pain are dietary modification and the use of nutritional supplements. On the other hand, the main results from the few observational studies included in this review point to the existence of an association relationship between less pain and a ketogenic or hypocaloric diet or adherence to the Mediterranean diet.

Conclusion: Dietary modification seems to be one plausible therapeutic option to improve and relieve CNCP. However, more research is needed in this regard to obtain better conclusions.

Systematic Review Registration: [www.crd.york.ac.uk/prospero], identifier [CRD42021226431].

KEYWORDS

chronic pain, diet, feeding, nutrients, nutrition, supplements

Introduction

According to the International Association for the Study of Pain, chronic pain (CP) is defined as an unpleasant emotional and sensory experience that may or may not be accompanied by tissue damage that persists for more than 3 months (1, 2). When pain is not a consequence of an oncological process, it is called chronic non-cancer pain (CNCP) (3).

It is estimated that one in five people in the world suffers from CP and one in three cannot maintain an independent lifestyle due to pain (4).

It produces consequences in the performance of daily activities, in the practice of physical exercise (3), and poor quality sleep (5), and it is difficult to participate in social activities (6) with significant social and health costs (1, 7).

The main intervention for CP relief is the use of antalgic drugs, which gives rise to numerous adverse effects (7). Nevertheless, there are currently other approaches to pain, such as psychosocial strategies, physical activity interventions (2), or nutritional care (8), which seem to show positive results in pain relief.

Recent studies show the potential use of nutritional strategies to decrease pain sensation or reduce the risk of suffering from CP since it is cheaper than analgesic drugs and is less likely to produce adverse effects. That is why some researchers have tried to shed light on the role of nutritional elements in CP. Thus, our objective was to systematically review scientific evidence based on clinical and observational studies to understand and map the effect of the use of different nutrients, foods, or food supplements on the presence or intensity of CNCP, and the association of these nutritional aspects with the presence or intensity of CNCP.

Materials and methods

Search strategy and data sources

Between March and April 2020, a search was carried out for documents published in the last 10 years in the PubMed, Web of Science, Scopus, and Cochrane Library databases.

The search equation was as follows: (diet OR antioxidants OR micronutrients OR nutrition OR "integrative pain medicine" OR healing OR eating OR "nutritional status" OR "anti-inflammatory diet" OR food OR eating OR appetite OR "food habits" OR "food preferences" OR nutrient OR "diet therapy") AND ("chronic pain" OR "persistent pain" OR "long term pain" OR pain OR "back pain" OR neuralgia OR "trigeminal neuralgia" OR hyperalgesia OR fibromyalgia OR "phantom limb" OR "complex regional pain syndromes" OR "nociceptive pain" OR headache OR endometriosis OR migraine OR arthritis) NOT (cancer OR tumor OR oncolog*).

Inclusion criteria

The selected documents were (1) original articles or systematic reviews that explored the relationship between nutrition and CNCP; (2) published between 2010 and 2020; (3) in English or Spanish; (4) with experimental (randomized clinical trials; RCTs) or observational epidemiological design; (5) implemented in over 18 years old population, men, and/or women; (6) full text available, and (7) with sufficient methodological quality. Specifically, only those observational studies that had a high or acceptable methodological quality according to the Scottish Intercollegiate Guidelines Network (SIGN) tool (9) and experimental studies with a score greater than 3 on the Jadad scale (10) were included in the present review.

Exclusion criteria

The exclusion criteria were (1) documents that studied pharmacological and surgical treatments with no nutritional approach for CNCP, (2) acute pain, and (3) as this systematic review is focused only on nutritional interventions, pain derived from surgical interventions or oncological processes was also excluded.

The search and screening of documents were carried out by two researchers independently and the discrepancies regarding the selected documents were resolved by consensus of the researchers. Registration was made in the International Prospective Register of Systematic Reviews (PROSPERO) with the code CRD42021226431.

A data extraction table was created for the documents included in the review (**Table 1**), with the following items: first author, year, type of pain, objectives, method, sample, duration, measuring instruments, intervention design, and results.

Results

Study characteristics

A total of 17,295 documents were found. Of these, 64 articles were selected for full-text reading of which 24 documents were finally included. **Figure 1** summarizes the selection process of the studies included in this review.

Regarding the epidemiological design of the studies included, 20 studies were experimental (RCTs), 2 studies were prospective cohort observational studies, 1 was the retrospective cohort, and 1 was case-control. The most common etiology of pain in the studies was osteoarthritis (n = 10), followed by rheumatoid arthritis (n = 7). **Table 1** describes the main characteristics of the studies included in this systematic review.

TABLE 1 Main characteristics of the studies included in this systematic review.

First author et. al./type of pain	Objectives	Method/ Sample/ Duration	Measuring instruments	Intervention design	Results
Abbasnezhad et al. (11) Irritable bowel syndrome (IBS)	To explore the effects of vitamin D supplementation on symptoms, severity score, and quality of life in patients with IBS	RCT $N = 90$ 6 months	DS, IBS, VAS	50,000IU vitamin D_3 ($n = 45$) Placebo ($n = 45$)	IBS symptoms improved in the two groups. Abdominal pain significantly improved in the vitamin D group ($p < 0.007$).
Cordero et al. (20) Fibromyalgia (FM)	To evaluate the effect of CoQ_{10} on clinical symptoms in FM patients.	RCT $N = 20$ 70 days	FIQ, VAS	CoQ_{10} 300mg/day ($n = 10$) Placebo ($n = 10$).	Reduction in pain in CoQ_{10} compared to placebo (56%) and reduction in tender joints (44%) ($p < 0.01$).
Sawaddiruk et al. (21) FM	To study whether supplementing CoQ_{10} with pregabalin can reduce pain better than pregabalin alone in FM patients.	RCT $N = 11$ 40 days	FIQ, VAS, Pressure Pain Limit	300mg/day CoQ_{10}+150mg/day pregabalin. Placebo	VAS and FIQ decreased in CoQ_{10} compared to placebo ($p < 0.05$). Pregabalin + CoQ_{10} reduced pain more than placebo.
Dunn-Lewis et al. (13) OA	To examine the effect of multinutrient supplementation on physical capacity, fatigue, mood, and other factors in active men and women of ages 40-70.	RCT $N = 31$ 63 days	PROMIS-57, Lequesne Knee Index, KOOS	0.25mg vitamin B_{12} + 6mg vitamin B_6 + 0.40mg folic acid + 20mg pantothenic acid + 500mg taurine + 2000mg leucine + 500mg isoleucine + 500mg valine + 50mg green tea	Men show improvement in fatigue, pain and joint pain, although it does not occur in women.
Fukumitsu et al. (17) OA	To investigate the effect of administering olive extract containing maslinic acid (MA) over a 12-week period in elderly patients with mild knee joint pain, especially when climbing stairs.	RCT $N = 20$ 12 weeks	VAS, SF-8	50 mg MA ($n = 12$) Placebo (n = 8)	Pain VAS does not change between the two groups ($p = 0.65$).
Malek et al. (18) OA	To assess the anti-inflammatory effects of L-carnitine supplementation in women with knee OA.	RCT $N = 72$ women 8 weeks	DS, VAS	750 mg L-carnitine tartrate ($n = 36$) Placebo ($n = 36$)	Difference in pain severity according to VAS ($p < 0.05$).
Rondanelli et al. (19) OA	To investigate the short-term anti-inflammatory and anti-pain potential of non-animal chondroitin sulfate (CS) supplementation in obese patients with OA.	RCT $N = 60$ 12 weeks	VAS, WOMAC, SF36	600 mg Chondroitin sulfate/day ($n = 30$) Placebo ($n = 30$).	Improvement in WOMAC and VAS in CS in both knees ($p = 0.001$)
Shell et al. (14) OA	To examine the efficacy and tolerability of theramine (AAB) in patients with chronic back pain compared to or in combination with ibuprofen.	RCT $N = 122$ 28 days	VAS, Roland-Morris Disability Questionnaire (RMDQ), Oswestry Low Back Pain Scale (OLBPS)	Ibuprofen 400mg-day Theramine 710mg/day Ibuprofen 400mg/day + theramine 10mg/day	In AAB group and the combined group there was significant improvement. In the AAB group, the RMDQ decreased by 50.3% and in the OLBPS, by 41.91%.
Ghavipour et al. (15) RA	To investigate the effect of POMx on disease activity and biomarkers of inflammation in patients with rheumatoid arthritis (RA).	RCT $N = 55$ 8 weeks	DAS28, VAS, FCFQ	POMx (250mg/day with a concentration of 40% ellagicacid) (n = 30) placebo (n = 25)	Reduction of DAS28 score ($p < 0.001$), related to decrease in swelling ($p < 0.001$), tender joint count ($p = 0.001$), pain intensity ($p = 0.003$).

(Continued)

TABLE 1 (Continued)

First author et. al./type of pain	Objectives	Method/ Sample/ Duration	Measuring instruments	Intervention design	Results
Helli et al. (16) RA	To examine the effect of sesamin on inflammatory markers and clinical indices in patients with RA.	RCT $N = 44$ women 6 weeks	DS, DAS28, VAS	200mg sesamin/day ($n = 22$) Placebo ($n = 22$)	Reduction of the number of tender joints and severity of pain compared to placebo ($p < 0.05$).
Santanam et al. (22) Endometriosis	To investigate whether the administration of antioxidants in patients with endometriosis can affect pelvic pain in women.	RCT $N = 59$ women 8 weeks	VAS	Vitamin E 1200IU + vitamin C 1000mg ($n = 46$) Placebo ($n = 13$)	Improvement of dysmenorrhea in antioxidant group (37%). Chronic pelvic pain improved in 43%.
Singh et al. (12) Pancreatitis	To evaluate the effect of antioxidant supplementation compared with placebo on pain and quality of life.	RCT $N = 107$ 6 months	VAS	600µg selenium, 0.54g vitamin C, 9000IU b-carotene, 270IU vitamin E and 2g methionine (n = 54) Placebo ($n = 53$)	Reduction of pain intensity with VAS in both groups ($p < 0.05$).
Schell et al. (30) OA	To examine the effect of dehydrated strawberries on pain and biomarkers of inflammation in obese adults with knee OA.	RCT $N = 17$ 26 weeks	ICOAP, HAQ, VAS, DS	50g dehydrated strawberries Placebo	Pain score and HAQ are lower in strawberries. Knee pain and total pain, using ICOAP, lower in strawberries ($p < 0.05$). No differences in VAS.
Schumacher et al. (28) OA	To evaluate the effect of cherry juice on the improvement of knee OA.	RCT $N = 59$ 13 weeks	WOMAC	470 ml/day Cherry juice ($n = 27$) Placebo ($n = 32$)	WOMAC improvement ($p = 0.002$) and pain ($p = 0.042$) in cherry juice.
Hashempur et al. (29) OA	To evaluate the efficacy of green tea extract in patients with knee OA.	RCT $N = 50$ 1 month	VAS, WOMAC	Green tea 1,500 mg/day + diclofenac 100 mg/day ($n = 25$) Diclofenac ($n = 25$)	Improvement in knee pain, functional capacity and joint stiffness in green tea group. VAS ($p = 0.038$).
Lindqvist et al. (25) RA	To investigate whether a diet rich in mussels, together with additional treatment, can reduce pathological activity in patients with RA.	RCT $N = 39$ 30 weeks	DS, DAS28, VAS, HAQ, SF36	75g/day mussels ($n = 20$) Control ($n = 19$)	No difference was observed between both groups.
Pirouzpanah et al. (26) RA	To study the possible beneficial effects of chamomile tea consumption on DAS-28, VAS and symptoms in patients with RA.	RCT $N = 44$ women 42 days	VAS, DS, DAS28	6g/day chamomile ($n = 22$) Placebo ($n = 22$)	Number of tender joints changed significantly ($p = 0.000$). DAS-28, number of swollen joints and VAS did not change.
Thimóteo et al. (27) RA	To evaluate the effects of cranberry juice on biomarkers of inflammation and pathological activity in patients with RA.	RCT N = 41 90 days	DAS28, VAS	500ml/day Cranberry juice ($n = 23$) Control ($n = 18$)	Reduction ($p = 0.048$) in the perception of pain with DAS28.
Messier et al. (24) OA	To compare the effects of diet + physical exercise, diet alone, or physical exercise alone on pain, function, mobility, quality of life in overweight and obese patients with knee OA.	RCT $N = 454$ 18 months	WOMAC, SF-36	Diet + exercise Diet (hypocaloric, low in fat and high in vegetables) Exercise (1h/day, 3 days/week)	D + E greater decrease in pain, according to WOMAC, compared to E ($p = 0.004$) and D ($p = 0.001$).

(Continued)

TABLE 1 (Continued)

First author et. al./type of pain	Objectives	Method/ Sample/ Duration	Measuring instruments	Intervention design	Results
Zamani et al. (23) RA	To determine the symbiotic supplementation effects on clinical and metabolic parameters of patients with RA.	RCT $N = 54$ 8 weeks	DS, DAS28, VAS	Symbiotic Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum + 800 mg inulin ($n = 27$) Placebo ($n = 27$)	Improvement of DAS28 ($p = 0.004$) and VAS pain ($p < 0.001$).
Di-Lorenzo et al. (31) Migraine	Assess whether a ketogenic diet has an effect on the clinical parameters of migraine.	OS $N = 96$ women 6 months	Headache frequency	Ketogenic diet ($n = 45$) Hypocaloric diet ($n = 51$).	The number of days with headache, frequency of headache attacks and consumption of drugs for headaches decreased in both groups ($p < 0.0001$).
Lourdudoss et al. (34) RA	To investigate potential associations between dietary intake of fatty acids and different pain patterns after treatment with antirheumatic drugs in early RA.	OS $N = 591$ 3 months	FCFQ, VAS, DAS28	Fatty acids omega 3 and omega 6	No statistically significant association.
Shmagel et al. (33) OA	To investigate the association between magnesium intake and knee pain score in a prospective cohort of patients with knee OA.	Cohort study $N = 2548$ 48 months	FCFQ, WOMAC, KOOS	Magnesium	Patients with lower magnesium intake had worse WOMAC and KOOS than those with higher magnesium intake ($p < 0.001$).
Veronese et al. (32) OA	To observe if a high adherence to the Mediterranean diet pattern is associated with a lower frequency of pain, stiffness, disability and depression.	OS cohort $N = 4470$	FCFQ, SF-12, WOMAC	Mediterranean diet	Greater adherence to a Mediterranean diet had a lower score in WOMAC ($p < 0.0001$), less pain and disability. Lower adherence to vegetables had a worse score in SF-12 ($p = 0.01$).

Antiox, antioxidants; DAS28, Disease Activity Score 28; DS, dietary survey; FCFQ, food consumption frequency questionnaire; FIQ, Fibromyalgia Impact Questionnaire; HAQ, Health Assessment Questionnaire; ICOAP, Intermittent and Constant Osteoarthritis Pain; KOOS, Knee Injury and Osteoarthritis Outcome Score; OA, osteoarthritis; OS, observational study; PROMIS-57, Patient-Reported Outcomes Measurement Information System-57; RA, rheumatoid osteoarthritis; RCT, randomized clinical trial; SF-36, short form health survey-36; SF-8, Short Form Health Survey-8; VAS, visual analog scale; vit, vitamin; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Main results from the experimental studies

The nutritional interventions evaluated for CNCP in the studies included the administration of nutritional supplements, dietary modification, and incorporation of food.

Administration of nutritional supplements

Regarding the studies carried out on pain caused by chronic pancreatitis, Abbasnezhad et al. (11) reported a significant improvement in pain during 6 months with the administration of 50,000 IU of vitamin D ($p < 0.007$). In addition, Singh et al. (12) described a significant reduction in the number of days with pain caused by chronic pancreatitis ($p < 0.05$) and a significant decrease in the intensity of the pain ($p = 0.001$) evaluated with the visual analog scale (VAS) after 3 months under treatment with an antioxidant compound of 600 μg of selenium, 0.54 g of vitamin C, 9,000 IU of beta carotene, 270 IU of vitamin E, and 2 g of methionine.

Regarding chronic back pain, Dunn-Lewis et al. (13) found a significant decrease ($p < 0.05$) in the intensity of back pain measured with the Patient-Reported Outcomes Measurement Information System–57 (PROMIS-57) and Knee Injury and Osteoarthritis Outcome Score (KOOS) instruments in men after supplementing the diet for 63 days with a multi-nutrient complex containing 0.25 mg of vitamin B_{12}, 6 mg of vitamin B_6, 0.40 mg of folic acid, 20 mg of pantothenic acid, 500 mg of taurine, 2,000 mg of leucine, 500 mg of isoleucine, 500 mg of valine, and 50 mg of green tea per supplement unit. However, no change in the intensity of pain was detected in women. Shell et al. (14) found a decrease in back pain intensity measured with

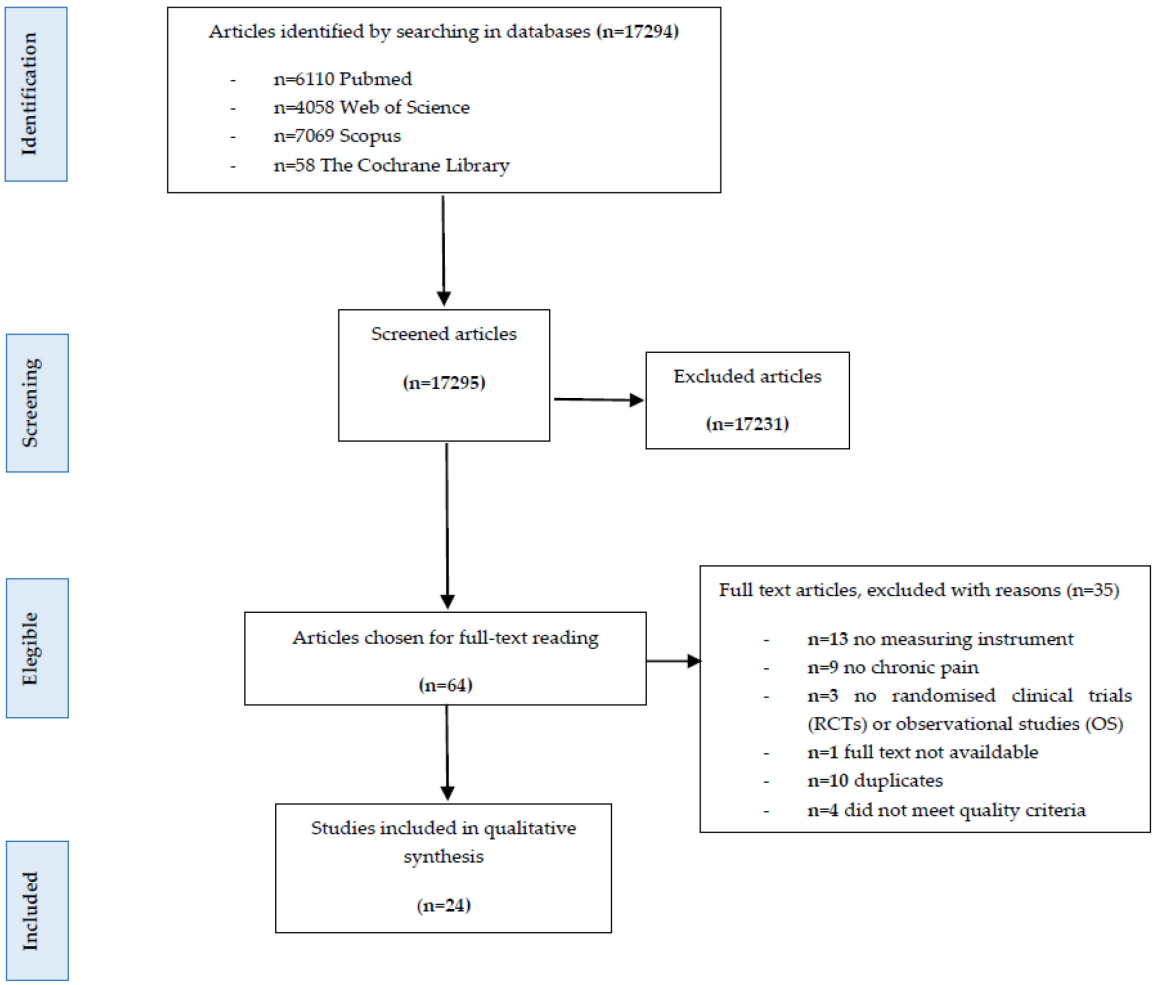

FIGURE 1
Preferred reporting items for systematic reviews (PRISMA) flow diagram.

the Roland Morris and Oswestry Disability Scales after 28 days of intervention with the combined administration of theramine (710 mg/day) and ibuprofen ($p < 0.05$).

Concerning patients with CP due to rheumatoid arthritis, Ghavipour et al. (15) supplemented the diet of the participants with two daily capsules of POMx (250 mg/day with a concentration of 40% ellagic acid) for 8 weeks and observed a significant reduction in rheumatoid arthritis pain perception measured with disease activity score-28 (DAS28; $p < 0.001$) and a decrease in the number of tender joints ($p = 0.001$) that also reduced pain intensity ($p = 0.003$). Helli et al. (16) observed that when 200 mg/day of sesamin was administered for 6 weeks, the number of tender joints and the intensity of pain evaluated with DAS28 and VAS were significantly reduced ($p < 0.05$ for both of them).

In the case of pain caused by osteoarthritis, Fukumitsu et al. (17) performed an intervention with maslinic acid with a dose of 50 mg/day for 12 weeks and found no significant difference in pain intensity measured with VAS when compared with the placebo group. However, Malek et al. (18), after using L-carnitine with a dose of 750 mg/day for 8 weeks, did find significantly lower pain intensity levels assessed by the VAS in the intervention group as compared to the control group ($p = 0.019$). Analogously, Rondanelli et al. (19) found that the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score in the group that had consumed chondroitin sulfate for 12 weeks at a dose of 600 mg/day had decreased significantly by 8.70 points, compared to the placebo group ($p = 0.001$).

On the other hand, Cordero et al. (20) evaluated pain in patients with fibromyalgia using Fibromyalgia Impact Questionnaire (FIQ). After the administration of 300 mg/day of CoQ_{10} for 70 days, they found a significant reduction in pain intensity ($p < 0.01$) and a significantly lower number of tender joints ($p < 0.01$) in comparison with the placebo. Furthermore, Sawaddiruk et al. (21) studied the effect of CoQ_{10} at a dose of

300 mg/day for 40 days in fibromyalgia and observed that the VAS and FIQ values decreased significantly in the CoQ_{10} group as compared to the placebo ($p < 0.05$).

Regarding dysmenorrhea, Santanam et al. (22) found a significant decrease in the number of painful days of the menstrual cycle in the group of participants who had ingested 1,200 IU of vitamin E and 1,000 mg of vitamin C for 8 weeks ($p < 0.05$). After the antioxidant intervention, chronic pelvic pain was decreased in 43% of the patients, and dysmenorrhea was descended in 37%.

For their part, Zamani et al. (23) carried out a clinical trial administering symbiotic supplements (Symbiotic *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and 800 mg inulin) for 8 weeks to patients with rheumatoid arthritis. A significant improvement was observed in scores measured with the DAS28 and VAS scales in this group ($p = 0.004$ and $p < 0.001$, respectively).

Dietary modification

In other research studies, diet intervention is accompanied by physical exercise. Messier et al. (24) carried out an intervention with a hypocaloric diet (low in fat and high in vegetables) combined with 1 h per day of physical training for 3 days a week, alternating aerobic and strength exercises in patients with osteoarthritis. The results showed that the compressive strength in the knee was decreased by 5% in the group that only did physical exercise (E), 10% in the group where only the diet was modified (D), and 9% in the diet group accompanied by physical exercise (D + E) at 18 months. However, in the D + E group, a greater decrease in pain was found, according to WOMAC, at 18 months when compared to E ($p = 0.004$) and D ($p = 0.001$).

Incorporation of food

The addition of foods, such as mussels, chamomile tea, blueberry or cherry juice, green tea, and strawberries, has been studied to evaluate the reduction of osteoarticular pain.

With respect to rheumatoid arthritis, Lindqvist et al. (25) observed that the group that consumed 75 g/day of mussels showed a lower intensity of perceived pain measured with DAS28 ($p = 0.017$). However, this difference was not observed when compared with the group that consumed meat ($p = 0.200$). Likewise, no statistically significant difference was obtained when comparing the number of tender joints and the assessment of pain intensity using the VAS tool when comparing the intervention group with the control group ($p = 0.48$). For their part, Pirouzpanah et al. (26) analyzed the effect of chamomile tea (6 g/day) on rheumatoid arthritis. The number of tender joints was decreased significantly ($p < 0.001$), although this change was not observed in the score measured by DAS28, the number of swollen joints, or the perception of pain. Regarding the consumption of blueberry juice (500 ml/day) at 90 days, Thimóteo et al. (27) stated that there was a significant reduction ($p = 0.048$) in the perception of pain when compared with the control group measured with the DAS28 instrument.

For osteoarthritis, Schumacher et al. (28) observed a significant improvement in the WOMAC score at 13 weeks in the group that consumed 470 ml/day of cherry juice ($p = 0.002$) when compared with the placebo group. The same relationship was observed by Hashempur et al. (29) in all the variables analyzed (knee pain, functional capacity, and joint stiffness) that included the VAS score ($p = 0.038$) in the group that consumed green tea (1,500 ml/day) during 30 days. In the control group, pain intensity only significantly descended when measured with WOMAC but not when measured with VAS.

Regarding knee pain, Schell et al. (30) described that in the group that consumed 50 g/day of strawberries, the intensity of pain was significantly lower at 12 weeks ($p < 0.05$) for both constant pain and intermittent pain, measured with the Intermittent and Constant Osteoarthritis Pain (ICOAP), although there were no differences in VAS for pain at the end of the 26 weeks of intervention.

Main results of observational studies

Nutritional aspects, such as the type of diet or some supplements, have been evaluated from observational studies for their plausible relation to pain. Concerning diet modification studies, Di Lorenzo et al. (31) observed that the number of days with headache was decreased in the two groups that followed hypocaloric or ketogenic diet ($p < 0.0001$). However, this improvement had occurred earlier in the group with a hypocaloric diet, from the second month, while in the group with a ketogenic diet, it had occurred from the sixth month. On the other hand, other clinical variables, such as frequency of headache attacks or consumption of drugs for headaches, were decreased equally in the two groups from the sixth month ($p < 0.0001$). Furthermore, Veronese et al. (32) found that patients who had greater adherence to the Mediterranean diet had better scores in WOMAC ($p < 0.0001$) and less general pain evaluated by WOMAC ($p < 0.05$).

Regarding the observational studies about nutritional supplements, Shmagel et al. (33) focused on knee pain in patients with osteoarthritis and observed that a lower intake of magnesium in the diet was associated with worse scores on WOMAC and KOOS than those with higher magnesium intake ($p < 0.001$). Likewise, they found a relationship between people who had low magnesium intakes in their diet and greater intensity of knee pain due to osteoarthritis at 48 months of follow-up. However, Lourdudoss et al. (34) did not find a statistically significant association between the consumption of omega 3 fatty acids within the diet and pain due to rheumatoid arthritis nor did they found an association between

supplementation with omega 3, omega 6, and the omega 6:omega 3 ratios with DAS28 scores.

Discussion

The aim of this study was to review the scientific literature on the impact of the use of nutritional strategies among people with CNCP. We found that most of the interventions with nutritional supplements collected in our study show improvement and relief in CP (11, 13, 20, 21). This is also the case when it is modified to a hypocaloric, Mediterranean, or with a healthier profile diet (24, 31, 32). However, the use of stand-alone foods, such as fruit juices, yields few hopeful results (26, 30).

We found a few studies whose intervention was the modification of the diet, and it was easier to find studies whose intervention was by using a capsule or pill. This could be due to the ease of applicability of the second one, while the modification in diet requires more effort both in patients and researchers. That is why we understand the nutritional education of special relevance in these patients, highlighting above all the main difficulties they may go through, such as lack of knowledge, lack of interest, or rigidity in the face of change (35).

The use of nutritional interventions to relieve pain in clinical practice has numerous benefits, such as fewer adverse effects than drugs, being more economical methods, or increasing patient autonomy (7, 8, 36).

We observe that the intervention that offers the best results is diet modification. This is also confirmed by Brain et al. (8), Clinton et al. (37), and Kaartinen et al. (38). However, this modification has to be easy to wear, durable, and adapted to the patient to obtain the best results (35).

Brain et al. (8) included four types of interventions in their review, which were dietary modifications, nutrient intake modifications, use of nutritional supplements, and use of fasting. Comparing our systematic review with that carried out by Brain et al. (8), we found that their team did not include observational studies and interventions that were to add a specific food. In addition, they included non-RCTs, so we could find some bias. On the other hand, if we compare it with Ahmed Ali et al. (39), they conducted a systematic review that specifically focused on clinical trials on chronic pancreatitis, while our team has addressed a broader field.

The main limitation that we found in our study was that there are still a few studies on the relationship between nutrition and pain, maybe because it is a new topic (36). When comparing the 24 documents included in this review, the heterogeneity between them was revealed, which particularly affects the methodology and design of the intervention. It is for this reason that we could not do a meta-analysis. An effort is needed to carry out future research on this topic using validated instruments to assess non-cancer CP and the nutritional variables, with deep described homogeneous interventions on large and well-characterized patient samples.

Conclusion

The results obtained show that there are nutritional interventions, especially diet modification, that can improve and alleviate CNCP. Furthermore, there is a need for future research to study CP as an independent entity and not as a symptom of the disease. If the evidence is strong, interventions could be applied in a clinical setting to improve the quality of life of patients suffering from this problem.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

IX obtained the data, performed the analysis, and contributed to writing the draft. EM performed the literature review, supervised all aspects of its implementation, contributed to the review of the manuscript, and contributed ideas and approved the final version. EG-G and RC-M supervised all aspects of its implementation, contributed to the revision of the manuscript, and contributed ideas and approved the final version. KA edited and corrected the article. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Herrera AS, Rolle PA, Babul KM, Maldonado MA, Zamora HM, Nazar JC. Pain management: Prescription opioid use disorder and its relationship with clinical practice. *Chil J Anesth.* (2017) 45:35–41.

2. World Health Organization [WHO]. *WHO Guideline for the management of chronic pain in children*. Geneva: World Health Organization (2020). p. 1–11.

3. Cáceres-Matos R, Gil-García E, Barrientos-Trigo S, Porcel-Gálvez AM, Cabrera-León A. Consequences of non-oncological chronic pain in adulthood. Scoping review. *Rev Saude Publica.* (2020) 54:39.

4. De Barutell C. Pain units in Spain. SED survey pain day 2007. *Mag Spanish Soc Pain.* (2009) 16:421–8.

5. Reid MC, Williams CS, Gill TM. The relationship between psychological factors and disabling musculoskeletal pain in community-dwelling older persons. *J Am Geriatr Soc.* (2003) 51:1092–8.

6. World Health Organization [WHO]. *WHO world health organization supports global effort to relieve chronic pain*. Geneva: World Health Organization (2004).

7. Piegang BN, Tigoufack IBN, Ngnokam D, Achounna AS, Watcho P, Greffrath W, et al. Cycloartanes from *Oxyanthus pallidus* and derivatives with analgesic activities. *BMC Complement Altern Med.* (2016) 16:97. doi: 10.1186/s12906-016-1075-3

8. Brain K, Burrows TL, Rollo ME, Chai LK, Clarke ED, Hayes C, et al. A systematic review and meta-analysis of nutrition interventions for chronic noncancer pain. *J Hum Nutr Diet.* (2018) 32:198–225. doi: 10.1111/jhn.12601

9. (SIGN) SIGN. *Scottish intercollegiate guidelines network (sign) [internet]. Scottish intercollegiate guidelines network SIGN.* (2020). Available from: https://www.sign.ac.uk/what-we-do/methodology/checklists/ [cited 2020 Jul 11] *

10. Jadad Bechara A. *Jadad scale [Internet]. Journal of controlled clinical trials*. San Francisco, CA: Scribd Inc (1996).

11. Abbasnezhad A, Amani R, Hajiani E, Alavinejad P, Cheraghian B, Ghadiri A. Effect of vitamin D on gastrointestinal symptoms and health-related quality of life in irritable bowel syndrome patients: A randomized double-blind clinical trial. *Neurogastroenterol Motil.* (2016) 28:1533–44. doi: 10.1111/nmo.12851

12. Singh N, Ahuja V, Sachdev V, Upadhyay AD, Goswami R, Ramakrishnan L, et al. Antioxidants for pancreatic functions in chronic pancreatitis: A double-blind randomized placebo-controlled pilot study. *J Clin Gastroenterol.* (2020) 54:284–93. doi: 10.1097/MCG.0000000000001178

13. Dunn-Lewis C, Kraemer WJ, Kupchak BR, Kelly NA, Creighton BA, Luk HY, et al. A multi-nutrient supplement reduced markers of inflammation and improved physical performance in active individuals of middle to older age: A randomized, double-blind, placebo-controlled study. *Nutr J.* (2011) 10:90. doi: 10.1186/1475-2891-10-90

14. Shell WE, Pavlik S, Roth B, Silver M, Breitstein ML, May L, et al. Reduction in pain and inflammation associated with chronic low back pain with the use of the medical food theramine. *Am J Ther.* (2016) 23:e1353–62. doi: 10.1097/MJT.0000000000000068

15. Ghavipour M, Sotoudeh G, Tavakoli E, Mowla K, Hasanzadeh J, Mazloom Z. Pomegranate extract alleviates disease activity and some blood biomarkers of inflammation and oxidative stress in rheumatoid arthritis patients. *Eur J Clin Nutr.* (2017) 71:92–6. doi: 10.1038/ejcn.2016.151

16. Helli B, Shahi MM, Mowla K, Jalali MT, Haghighian HK. A randomized, triple-blind, placebo-controlled clinical trial, evaluating the sesamin supplement effects on proteolytic enzymes, inflammatory markers, and clinical indices in women with rheumatoid arthritis. *Phytother Res.* (2019) 33:2421–8. doi: 10.1002/ptr.6433

17. Fukumitsu S, Villareal MO, Aida K, Hino A, Hori N, Isoda H, et al. Maslinic acid in olive fruit alleviates mild knee joint pain and improves quality of life by promoting weight loss in the elderly. *J Clin Biochem Nutr.* (2016) 59:220–5. doi: 10.3164/jcbn.16-40

18. Malek Mahdavi A, Mahdavi R, Kolahi S. Effects of l-carnitine supplementation on serum inflammatory factors and matrix metalloproteinase enzymes in females with knee osteoarthritis: A randomized, double-blind, placebo-controlled pilot study. *J Am Coll Nutr.* (2016) 35:597–603. doi: 10.1080/07315724.2015.1068139

19. Rondanelli M, Braschi V, Gasparri C, Nichetti M, Faliva MA, Peroni G, et al. Effectiveness of non-animal chondroitin sulfate supplementation in the treatment of moderate knee osteoarthritis in a group of overweight subjects: A randomized, double-blind, placebo-controlled pilot study. *Nutrients.* (2019) 11:2027. doi: 10.3390/nu11092027

20. Cordero MD, Alcocer-Gómez E, De Miguel M, Culic O, Carrión AM, Alvarez-Suarez JM, et al. Can coenzyme Q10 improve clinical and molecular parameters in fibromyalgia? *Antioxid Redox Signal.* (2013) 19:1356–61. doi: 10.1089/ars.2013.5260

21. Sawaddiruk P, Apaijai N, Paiboonworachat S, Kaewchur T, Kasitanon N, Jaiwongkam T, et al. Coenzyme Q10 supplementation alleviates pain in pregabalin-treated fibromyalgia patients via reducing brain activity and mitochondrial dysfunction. *Free Radic Res.* (2019) 53:901–9. doi: 10.1080/10715762.2019.1645955

22. Santanam N, Kavtaradze N, Murphy A, Dominguez C, Parthasarathy S. Antioxidant supplementation reduces endometriosis-related pelvic pain in humans. *Transl Res.* (2013) 161:189–95. doi: 10.1016/j.trsl.2012.05.001

23. Zamani B, Farshbaf S, Golkar HR, Bahmani F, Asemi Z. Synbiotic supplementation and the effects on clinical and metabolic responses in patients with rheumatoid arthritis: A randomised, double-blind, placebo-controlled trial. *Br J Nutr.* (2017) 117:1095–102.

24. Messier SP, Mihalko SL, Legault C, Miller GD, Nicklas BJ, DeVita P, et al. Effects of intensive diet and exercise on knee joint loads, inflammation, and clinical outcomes among overweight and obese adults with knee osteoarthritis: The IDEA randomized clinical trial. *JAMA J Am Med Assoc.* (2013) 310:1263–73. doi: 10.1001/jama.2013.277669

25. Lindqvist HM, Gjertsson I, Eneljung T, Winkvist A. Influence of blue mussel (*Mytilus edulis*) intake on disease activity in female patients with rheumatoid arthritis: The MIRA randomized cross-over dietary intervention. *Nutrients.* (2018) 10:481. doi: 10.3390/nu10040481

26. Pirouzpanah S, Mahboob S, Sanayei M, Hajaliloo M, Safaeiyan A. The effect ofchamomile tea consumption on inflammation among rheumatoid arthritis patients: Randomized clinical trial. *Prog Nutr.* (2017) 19:27–33.

27. Thimóteo NSB, Iryioda TMV, Alfieri DF, Rego BEF, Scavuzzi BM, Fatel E, et al. Cranberry juice decreases disease activity in women with rheumatoid arthritis. *Nutrition.* (2019) 60:112–7. doi: 10.1016/j.nut.2018.10.010

28. Schumacher HR, Pullman-Mooar S, Gupta SR, Dinnella JE, Kim R, McHugh MP. Randomized double-blind crossover study of the efficacy of a tart cherry juice blend in treatment of osteoarthritis (OA) of the knee. *Osteoarthr Cartil.* (2013) 21:1035–41. doi: 10.1016/j.joca.2013.05.009

29. Hashempur MH, Sadrneshin S, Mosavat SH, Ashraf A. Green tea (*Camellia sinensis*) for patients with knee osteoarthritis: A randomized open-label active-controlled clinical trial. *Clin Nutr.* (2018) 37:85–90. doi: 10.1016/j.clnu.2016.12.004

30. Schell J, Hal Scofield R, Barrett JR, Kurien BT, Betts N, Lyons TJ, et al. Strawberries improve pain and inflammation in obese adults with radiographic evidence of knee osteoarthritis. *Nutrients.* (2017) 9:949. doi: 10.3390/nu9090949

31. Di Lorenzo C, Coppola G, Sirianni G, Di Lorenzo G, Bracaglia M, Di Lenola D, et al. Migraine improvement during short lasting ketogenesis: A proof-of-concept study. *Eur J Neurol.* (2015) 22:170–7. doi: 10.1111/ene.12550

32. Veronese N, Stubbs B, Noale M, Solmi M, Luchini C, Maggi S. Adherence to the mediterranean diet is associated with better quality of life: Data from the osteoarthritis initiative. *Am J Clin Nutr.* (2016) 104:1403–9. doi: 10.3945/ajcn.116.136390

33. Shmagel A, Onizuka N, Langsetmo L, Vo T, Foley R, Ensrud K, et al. Low magnesium intake is associated with increased knee pain in subjects with radiographic knee osteoarthritis: Data from the osteoarthritis initiative. *Osteoarthr Cartil.* (2018) 26:651–8. doi: 10.1016/j.joca.2018.02.002

34. Lourdudoss C, Di Giuseppe D, Wolk A, Westerlind H, Klareskog L, Alfredsson L, et al. Dietary intake of polyunsaturated fatty acids and pain in spite of inflammatory control among methotrexate-treated early rheumatoid arthritis patients. *Arthritis Care Res.* (2018) 70:205–12. doi: 10.1002/acr.23245

35. Bimbela Pedrola JL, Gorrotxategi Larrea M. *Tools to improve patient adherence*. Granada: Andalusian School of Public Health (2006).

36. Brain K, Burrows TL, Bruggink L, Malfliet A, Hayes C, Hodson FJ, et al. Diet and chronic non-cancer pain: The state of the art and future directions. *J Clin Med.* (2021) 10:5023. doi: 10.3390/jcm10215203

37. Clinton CM, O'Brien S, Law J, Renier CM, Wendt MR. Whole-foods, plant-based diet alleviates the symptoms of osteoarthritis. *Arthritis.* (2015) 2015:708152. doi: 10.1155/2015/708152

38. Kaartinen K, Lammi K, Hypen M, Nenonen M, Hänninen O. Vegan diet alleviates fibromyalgia symptoms. *Scand J Rheumatol.* (2000) 29:308–13. doi: 10.1080/030097400447697

39. Ahmed Ali U, Jens S, Busch ORC, Keus F, van Goor H, Gooszen HG, et al. Antioxidants for pain in chronic pancreatitis. *Cochrane Database Syst Rev.* (2014) 2014:CD008945.

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Efficacy of nutrition education for the increase of symbiotic intake on nutritional and metabolic status in schizophrenic spectrum disorders: A two-arm protocol

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Background/Objectives: The microbiota plays a vital role in the two-way communication between the gastrointestinal tract and numerous neuropsychiatric disorders, such as schizophrenia. Besides, the microbiota modulation through the use of psychobiotics (prebiotics and probiotics with nutraceutical action) is related to the improvement of the physical and psychopathological health. The objective to this study was to test the efficacy of prebiotic/probiotic dietary modulation in patients diagnosed with schizophrenia, attending to the nutritional and cardio-metabolic impact.

Methods: Two-arms, double-blind, randomized in balanced blocks clinical trial of 6 months of intervention, will be developed in a group of 50 individuals. The control group will receive conventional dietary advice individually from specialized mental health nurses. In the intervention group, an individual dietetic-nutritional education program with high prebiotic and probiotic content (dairy and fermented foods, green leafy vegetables, high-fiber fruit, whole grains, etc.) will be developed by these nurses. Data will be collected on the psychopathological state, and blood test (at the beginning, at 3 and 6 months). The estimation of intestinal microbiota and the usual nutritional pattern will also be assessed at the beginning and 6 months, using a stool test. To evaluate the degree of adherence, the intervention group will fill a specific weekly record of the main dishes/food consumed. Anthropometric parameters will also be analyzed monthly.

Discussion: The study is anticipated to establish feasibility an adequate dietary modulation with a high simbiotic content, leads to a significant improvement in the nutritional status and cardio-metabolic. Furthermore, it is presumed to reach a degree of evidence that allows establishing nutritional management

as an effective therapeutic intervention in the psychopathological treatment of patients with schizophrenia spectrum disorders.

Clinical Trial Registration: [www.ClinicalTrials.gov], identifier [NCT04366401].

KEYWORDS

prebiotic, probiotic, schizophrenia spectrum and other psychotic disorders, diet therapy, mental health

Background

Schizophrenia is a chronic mental illness characterized by significant clinical heterogeneity and a long evolution over time, determined by periods of psychotic exacerbation and phases of stabilization (1–3). The semiology of this nosological entity is established in positive and negative symptoms, with variable levels of dysfunction and clinical presentation, and having an essential impact on the patient's quality of life (2, 4). Similarly, surrounding the schizophrenic spectrum, the existence of the associated neurocognitive impairment stands out, prevailing the disorders of social and occupational functioning, as well as a significant degree of disorganization (2, 3, 5, 6).

Many theories have tried to elucidate the origin of schizophrenia, where the complexity of its etiopathogenesis is a determining factor in establishing an appropriate, specific, and effective therapeutic approach (1, 7). In this sense, despite the numerous etiological premises, the glutamatergic (glutamate/GABA) and dopaminergic hypotheses have acquired greater strength in the development of schizophrenia (8, 9). In addition, however, recent studies have highlighted the theory of vagus nerve dysregulation as a possible etiological factor in the origin and aggravation of mental disorders (1, 5, 10), with numerous associated pathogenic mechanisms, particularly low-grade systemic inflammation and oxidative stress (7, 10, 11).

Undoubtedly, the traditional therapeutic approach has perceived the role of nutrition as a minor intervention in psychiatry, especially in psychotic disorders such as schizophrenia (12). However, the advances established in the last decade, mainly associated with the development of the holobionte theory and the evolution of metagenomics (11, 13), as well as the presence of new dietary patterns of low nutritional quality in different western societies (1, 3), have contributed significantly to the global understanding of the role of nutritional patterns on the functioning of the Central Nervous System (CNS), as well as on the possible mechanisms or etiological pathways of psychiatric disorders (1, 11, 12, 14).

In this regard, it is necessary to highlight the role of the intestinal microbiota (IM) and the intimate relationship it exerts on the numerous functions of the body, such as the development and maturation of the CNS, nutrition, immune response or systemic inflammation (7, 11, 12, 15). This effect is carried out through various established communication pathways: vagal nerve (primary), intestinal hormones, cytokines, exosomes, and microRNAs (10, 14–16). Thus, the existence of possible modifications in the concentration of this biota (considering average concentrations around 1,013 CFU/g) (17, 18), may trigger homeostatic alterations or aggravate pathogenic conditions, a fact commonly called dysbiosis (11–13, 17, 19). This concentration of microbiota is fundamentally determined by dietary patterns, genetic factors, iatrogenic antibiotherapy [highlighting the broad-spectrum ones, reducing the potential for small intestinal bacterial overgrowth (SIBO)], type of breastfeeding (maternal or formula), age, exercise, and continuous stress, among others (10, 18–20).

As a consequence of these discoveries, the concept of the "*Microbiota-Intestine-Brain Axis*" emerges. This term refers to the two-way communication pathway established between the CNS, the gastrointestinal tract, and the IM (1, 13–15, 18), mediated by the microbial metabolites of dietary products such as dietary fiber, tryptophan or arginine, as well as by endocrine and neuronal mechanisms (19, 21). The close relationship established between IM and the CNS lies in the production of a multitude of neurotransmitters essential for normal neuronal functioning, such as serotonin, GABA, dopamine or noradrenaline, among others (11–13, 19, 22). Similarly, IM exerts essential trophic, metabolic, and protective functions, which are a determining factor in the normal neuropsychiatric function (17, 22).

Thus, according to the theory of low-grade systemic inflammation, when a state of dysbiosis occurs in the symbiote IM, it generates a cascade of pro-inflammatory agents, such as lipopolysaccharide (10, 11, 23), a bacterial endotoxin, capable of modifying both the integrity and the permeability of enterocytes (12). This alteration triggers the release of pro-inflammatory cytokines [tumor necrosis factor $\acute{\alpha}$ (TNF-$\acute{\alpha}$) or interleukins type 6 or 1β (IL-6, IL-1β) (7, 11), both capable of altering intestinal tissue integrity], originating synergies between inflammation, increased oxidative stress and imbalance of energetic homeostasis. This cascade of reactions

causes an increase in neurodegeneration and excitotoxicity, mediated by the vagus nerve (7, 12, 15). Thus, it has been shown that the activation of a state of low inflammation is related to a worse prognosis of schizophrenia concerning positive and negative symptoms, cognitive performance, and loss of brain volume (7, 21, 24). Similarly, alterations in specific pro-inflammatory cytokines or state markers have been described, especially in psychotic relapses or prodromal phases (IL-6, TGF-β, among others) (7, 10), as well as a decrease in their concentration after the introduction of antipsychotic treatment, with consequent clinical improvement (21).

Justification

Existing scientific production shows a high rate of disability and morbimortality in people suffering from some psychiatric disorder concerning the rest of the general clinical population, especially in those patients with a severe and long-term mental disorder (LTMD) (1, 12, 14, 24–28), highlighting dysfunctions of the psychotic and affective spectrum: schizophrenia and bipolar disorder, (respectively) (24, 27). This morbidity and mortality rate in the psychiatric population is up to 20% higher and, quantitatively, represents an average of 25 years of life lost (24–27, 29). Besides, patients with LTMD have a life expectancy of less than 20% (57 years in men and 65 years in women) (14, 25). It is estimated that the relative risk of this disease is 2.41 higher for mortality from any causes (24), these being mainly comprised of cardiovascular, infectious, respiratory, and endocrine diseases (60% of premature deaths in this clinical population) (14, 25, 30). Also, the leading established causes of mortality are closely linked to the development of the Metabolic Syndrome (MS) (1, 3, 25–28, 31–33), also called insulin resistance syndrome (24, 33). The MS is considered a determining factor in the physical health of the patient, tripling the incidence of cardio-metabolic diseases (27–29).

The main etiopathogenic determinants of this fact are the factors inherent to the disease itself, as well as genetic factors (3, 24–26, 34) and resistance to adequate care in terms of physical health (27, 33, 35). However, the main modifiable risk factor in the LTMD population lies in the acquisition of unhealthy lifestyles, characterized by high-energy dietary patterns, with high consumption of ultra-processed foods and low fruit and vegetable intake (36). In addition, the psychiatric population has low levels of physical activity, with increased rates of smoking and associated substance abuse (3, 27, 37, 38).

Despite the magnitude and severity of the problem, interventions aimed to modify lifestyles do not play an essential role in therapy and are not part of the usual clinical practice with the psychiatric population (1, 27, 31, 33). This fact could be explained by the lack of understanding of the multiple mechanisms and etiological factors involved in the neurogenesis of schizophrenia (2), and leads to a multidisciplinary approach, but essentially psychopharmacological and psychotherapeutic (33, 39). It is, therefore, vital to address modifiable factors such as dietary patterns, which have evidenced to be an efficient therapeutic intervention to improve both the psychopathological dysfunction and the physical health of the subjects and can be considered as an addition to the conventional therapeutic approach (1, 3, 7, 17, 40).

In this sense, some dietary interventions carried out to modulate intestinal microbiota in psychotic disorders through the use of so-called "psychobiotics" (1, 17–22). This term refers to the set of substances that include probiotics and/or prebiotics and whose administration causes health benefits in psychiatric patients (20–22). Probiotics include microorganisms of the intestinal biota, which, provided in adequate quantities, offer a benefit for the host (highlighting the genera Lactobacillus and Bifidobacterium, among others) (1, 7, 8, 12, 16–21). On the other hand, prebiotics are non-digestible dietary fiber (mainly fructooligosaccharides and oligosaccharides, inulin or pectins) (1, 17), which are substances that promote optimal growth and development of probiotics in the gastrointestinal tract, reducing pathogenic microbiota (7, 12, 15, 19), through the production of short-chain fatty acids (17, 21).

It is worth noting the growing effort to highlight the role played by prebiotics and or probiotics in the microbiota-intestine-brain axis, which is currently a relevant object of study (1, 22, 41).

In this regard, according to Patra (19) and Teasdale et al. (37), adequate dietary planning in psychiatric patients with psychopathological dysfunction and at risk of iatrogenic metabolic syndrome, could be considered as a therapy of choice in these subjects, improving altered clinical patterns and difficulties in the patient's vital and functional performance. Similarly, adequate nutritional management could be used as an adjunct to antipsychotic pharmacotherapy and the cardio-metabolic approach, reducing the number of homeostatic drugs or even replacing them in cases of intolerance in the target population (14, 39).

In short, the future of the development of Mental Health is determined by the need for a multimodal approach, where nutritional factors represent the cornerstone in achieving optimal results in health, level of functionality, and, therefore, quality of life of patients (35, 42).

Likewise, dietary advice on modulation with high prebiotic and probiotic content has the added value of improving the morbidity and mortality associated with schizophrenia, with optimal levels in terms of cost-effectiveness, better than those shown by the approaches currently used.

Methods/design

Study aims

Main objective

Determination of the nutritional and cardio-metabolic efficacy of a prebiotic and probiotic dietary intervention in patients with schizophrenia spectrum disorders.

Specific objectives

- To determine the baseline nutritional status of the target population.
- To identify the usual dietary patterns in this population, clarifying the nutritional value of the main dishes consumed, as well as their link with the physical health status of individuals.
- To know the existing scientific evidence regarding the construction of determinants (explicit and implicit) that influence the microbiota-intestine-brain axis.
- To evaluate the psychopathological impact of the incorporation of prebiotics and probiotics in the habitual dietetic-nutritional pattern in patients diagnosed with the spectrum of schizophrenia.
- To evaluate the cardio-metabolic impact of a standardized dietary planning with high prebiotic and probiotic content, adapted to the inherent characteristics of the psychiatric population.
- To develop and validate a program that allows for the detection of areas of improvement, establishing assessment strategies, and an appropriate action plan in Mental Health, which allows for adequate dietary care through the use of psychobiotics.

Study design

A two-arms, double-blind, randomized in balanced blocks clinical trial of 6 months of intervention, will be carried out in psychiatric patients diagnosed with schizophrenic spectrum disorders (without distinction by type). The control group (CG) will be made up of those participants who will receive conventional dietary advice (43) on an individual basis. In the intervention group (IG), this advice will be established individually through intensive nutritional guidance (44) offering a food pattern with a high prebiotic and probiotic content. In both intervention groups, educational material of visual support will be used during the sessions. The dietary intervention will be designed and supervised by qualified personnel with recognized competencies for this intervention (nurses and dietitians), carried out by specialized mental health nurses, and will be agreed upon through serial interviews and focus groups. In this sense, these focus groups will be applied to improve the established dietetic-nutritional intervention, guaranteeing its correct adaptation, according to the study population.

The study will begin with a group session for the presentation of the research project in the health center and/or psychiatric service consultation. During the development of the study, data will be collected on the psychopathological state [Positive and Negative Syndrome Scale (PANSS) (45) and Personal and Social Functioning Scale (PSP) (46) scales; **Supplementary Tables 1, 2**], and blood test (hemogram, lipid profile, etc.). Measures will be taken at the beginning (basal), at 3 and 6 months. The estimation of intestinal microbiota and the usual nutritional pattern will also be assessed at the beginning and 6 months, using a stool test and a validated Food Frequency Questionnaire (FFQ) (47), respectively. The use of the FFQ will allow us to know both the average intake of grams of fiber, fat, etc. and the frequency of consumption by food groups (with particular attention to fermented foods). However, the main reason for using this tool is that it will allow us to assess changes in dietary patterns over the medium term (39). To evaluate the degree of adherence, participants in the IG will fill a specific weekly record of the main dishes/food consumed with a high prebiotic and/or probiotic content will be measured by the weekly completion (during the 6 months of intervention) of a record that includes the main foods consumed with a high symbiotic value (fermented foods, whole grains, green leafy vegetables, fruit, etc.) (**Supplementary Figure 1**). This record will be completed by the patients themselves or, in cases of incapacity or lack of autonomy in preparing the dishes consumed by family members or the primary caregiver. At least, anthropometric parameters will also be analyzed monthly (BMI, blood pressure, heart rate, abdominal perimeter) (**Supplementary Table 3**).

Selection of participants

For the assessment of the method's effectiveness, a sample size of 22 individuals has been estimated (11 for the IG and 11 for the CG, with a power of 80% and a confidence of 95%, expecting a risk/prevalence difference of 63% post-intervention (48). The final size of 50 individuals has been established (25 for the IG and 25 for the GC) to minimize the effect of possible losses and the low study completion rates, especially in participants with significant negative symptoms. Participants who express a clear wish to participate voluntarily in the study will be assigned, through randomization in balanced blocks, to the IG or CG (**Figure 1**). Randomization will be conducted according to the results found in stool culture analysis (balancing the prevalence of dysbiosis in both groups).

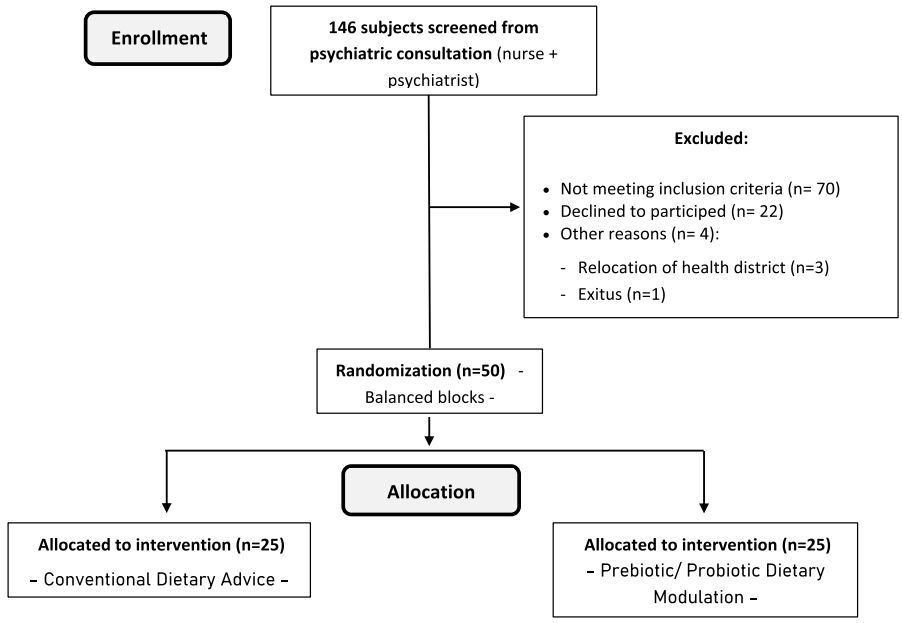

FIGURE 1
Flow chart of the participants (CONSORT diagram).

Concerning the established inclusion/exclusion criteria, these will be:

Inclusion criteria

- Patients diagnosed on the spectrum of schizophrenia (without distinction by type), according to criteria DSM-5 and/or ICD-11.
- Age between 18 and 65 years.
- Absence of gastrointestinal comorbidity that contraindicates the use of prebiotics and/or probiotics (intolerance, explosive diarrhea, acute abdominal pain, etc.).
- To show clinical stability for 6 months before the start of the study (absence of psychiatric hospitalization, maintenance of the level of functionality, and lack of social and occupational absenteeism).
- To manifest agreement to participate in the study and to sign of informed consent (**Supplementary Appendices I–III**).

Exclusion criteria

- To suffer from somatic or neurocognitive situation that prevents participation and collaboration in the fulfillment of the protocol.
- To follow standardized dietary planning not modulated by the population under study (catering, institutional or collective feeding, etc.).
- Concomitant administration of antibiotherapy during the intervention phase.
- Refusal to participate in the study.

Study variables

Result variables

Clinical efficacy of prebiotic/probiotic dietary modulation

- Scale for Positive and Negative Schizophrenia Syndrome (45) (categorized PANSS): discrete.
- Personal and Social Functioning Scale (46) (categorized PSP) outcome: discrete.

Tolerability and modulation of the nutritional dietary pattern

- Food Frequency Questionnaire (FFQ) (47) result: continuous
- Culinary knowledge and responsibility for feeding: nominal.

Anthropometric variables and physical health

Weight (kg, continuous), height (cm, continuous), BMI (kg/m^2, continuous) abdominal circumference (cm, continuous), systolic blood pressure (mmHg, continuous),

TABLE 1 Blood test variables: Abbreviations.

	Abbreviation	Meaning
Hematological profile	M.C.V	Mean corpuscular volume
	H.C.M	Mean corpuscular hemoglobin
	M.C.H.C	Mean corpuscular hemoglobin concentration
	R.D.W	Red cell distribution width
	M.P.V	Mean platelet volume
Biochemical profile	ALT	Alanine transaminase
	GPT	Glutamic pyruvic transaminase enzyme
	G-GT	Gamma-glutamyl transferase
	ALP	Alkaline phosphatase
	Na^+	Sodium
	K^+	Potassium
	Cl^-	Chlorine
	Ca^{2+}	Calcium
	HbA1c	Glycosylated hemoglobin
	IFCC	International federation of clinical chemistry and laboratory medicine
	Fe^{2+}	Iron
	FRT	Ferritin
	LDH	Lactate dehydrogenase
	C-HDL	High-density lipoprotein
	C-LDL	Low-density lipoprotein
	TSH	Thyroid stimulating hormone
	PRL	Prolactin
	LUES	Syphilis
	HCV	Hepatitis C virus
	HAV	Hepatitis A virus
	HIV	Human immunodeficiency virus

diastolic blood pressure (mmHg, continuous), heart rate (ppm, discrete).

Blood test variables

- *Hematological profile*: Red blood cells (x10e^6/mm^3, continuous), hemoglobin (g/dL, continuous), hematocrit (%, continuous), M.C.V. (fL, discrete), H.C.M. (pg, discrete), C.H.C.M. (g/dL, discrete), R.D.W (%, continuous), leukocytes (×10e^3/mm^3, discrete), neutrophils (×10e^3/m, continuous), lymphocytes (×10e^3/m, continuous), monocytes (×10e^3/m, continuous), eosinophils (×10e^3/m, continuous), basophils (×10e^3/m, continuous), platelets (×0e^3/mm^3, discrete), M.P.V. (fL, discrete). See **Table 1** for abbreviations corresponding to the blood test variables.
- *Biochemical profile.* ALT/GPT (IU/L, discrete), G-GT (IU/L, discrete), FAL (IU/L, discrete), Na^+/K^+ (mEq/L, discrete/continuous, respectively), Cl^- (mEq/L, continuous), Ca^{2+} (mEq/L, continuous), urate (mg/dL, continuous), glucose (mg/dL, discrete), HbA1c (%, continuous), HbA1c IFCC (mmol/mol, continuous), fructosamine (mcmol/L, discrete), creatinine (mg/dL, continuous), Fe^{2+} (mcg/dL, discrete), FRT (mcg/dL, discrete), folate (mcg/dL, continuous), vit.B12 (ng/mL, discrete), vit.D total (D2 + D3) 25-OH (ng/mL, discrete) cholesterol (mg/dL, discrete), triglycerides (mg/dL, discrete), LDH (IU/L, discrete), C-HDL (mg/dL, discrete), C-LDL (mg/dL, discrete), total cholesterol/C-HDL (mg/dL, discrete), glomerular filtrate estimation (mL/m/173, discrete), TSH (mU/L, continuous), PRL (ng/dL, continuous), LUES (IU/L, nominal/discrete), a-HAV-M (IU/L, nominal/discrete), a-HCV (IU/L, nominal/discrete), HBsAg (UI/L, nominal/discrete), a-HBC-IgG (UI/L, nominal/discrete), a-HBs (UI/L, continuous), a-HIV (mcL, nominal/discrete).

Stool variables

Stool culture

General bacteriology. Usual mixed flora: Lactobacillus (CFU/g, continuous), Bifidobacterium (CFU/g, continuous)//Disbiosis: *Salmonella* spp. (nominal, Presence/absence), *Shigella* spp. (nominal, presence/absence), *Yersinia* spp. (nominal, presence/absence), Hafnia alvei (nominal, presence/absence), *Aermonas* spp. (nominal, presence/absence), *Campylobacter* spp. (nominal, presence/absence).

Independent variables

Sociodemographic variables

Age (continuous), gender (nominal), legal representative (nominal), household composition (nominal), economic level (ordinal), level of studies (ordinal), area of residence (nominal).

Therapeutic variables

Previous antipsychotic (nominal), the dose of antipsychotic (mg, continuous), the reason for a change in antipsychotic treatment (nominal).

Clinical variables

Type of psychotic disorder (nominal), duration of illness (continuous), age of first hospitalization (continuous), number of previous hospitalizations (discrete), number of previous relapses (discrete), number of previous suicidal behaviors (discrete), number of subsequent hospitalizations (discrete), number of subsequent relapses (discrete), number of subsequent suicidal behaviors (discrete), number of subsequent unscheduled consultations (discrete), substance abuse (nominal), type of substance (nominal), associated cardio-metabolic diagnosis (nominal).

Anthropometric measurements will be collected following the recommendations of the Manual of Standardized

Anthropometry (49). Weight, height, and BMI shall be measured with an SECA® 703s stadiometer and scale, with an accuracy of 0.1 kg and 0.1 cm, respectively. The abdominal perimeter shall be determined at the midpoint between the last rib and the iliac crest at the end of a normal expiration. The WelchAllyn® ProBP 2,400 digital sphingomanometer shall be used for the study of blood pressure and heart rate.

Statistical analysis

Quantitative variables will be presented with mean and standard deviation, and qualitative variables will be shown in frequencies and percentages.

The Kolmogorov-Smirnov test will be used to compare the goodness of fit to a normal distribution of data from quantitative variables. For the contrast of bivariate hypotheses, the Student *t*-test will be performed for two means, while for qualitative variables, the Chi-Square and Fisher's exact test will be used, when necessary. Likewise, for the analysis of three or more means, the ANOVA of repeated means will be used. On the other hand, the correlation between quantitative variables will be verified through the Pearson's coefficient r. When the normality or homoscedasticity criteria are not met, non-parametric versions of the above tests will be performed.

Logistic regressions will be carried out to determine which variables can determine the improvement of the nutritional pattern and physical health through the FFQ (47), as well as blood and stool analytical values. Similarly, this analysis will be established concerning the psychopathological status through the PSP (46) and PANSS (45) scales, both of which have a discrete quantitative and nominal result values, according to established cut-off points and clinical interpretation. Raw and adjusted odds ratios will be calculated. Log-likelihood, the goodness of fit statistic, Cox and Snell R2, Nagelkerke R2, and Hosmer-Lemeshow tests should be used to assess the overall model fit. The exponentiation will be used to calculate the beta coefficients.

These multivariate tests will allow us to identify and adjust for the possible confounding effect of the independent variables on the outcome variables.

For all statistical analyses, an alpha error probability of less than 5% ($p < 0.05$) will be accepted, and the confidence interval will be calculated at 95%. The software SPSS (version 25.0) and EPIDAT (version 4.2) shall be used for the statistical analysis.

Work plan

An intervention schedule has been established, with a total duration of 6 months, which is divided into three blocks:

Block 1

This first block focuses on the selection of the target population according to inclusion criteria. Firstly, a group session to present the program and the methodology of the study will be carried out. During the first 15 days of the study, a focus group with professionals to reach a consensus on the intervention will be held. Subsequently, the appropriate modifications will be made to improve and adapt to the dietary and nutritional intervention.

Consequently, the recruitment and initial psychopathological and nutritional assessment of the participants will be carried out, using the PANSS (45) and PSP (46) scales. For the nutritional evaluation, the analytical and anthropometric basal determination of the participating patients will be carried out, as well as the evaluation of the habitual dietary pattern through a validated FFQ (47) and weekly record of the main dishes and foods consumed. Similarly, an estimate of the intestinal bacterial flora is required employing stool culture.

Block 2

The second block includes the implementation of the 6-month individual nutrition education program (associated with 2 months of educational reinforcement, according to block 3). It will consist of eight sessions, the first four being biweekly, followed by 4 monthly, to which four sessions of educational reinforcement will be added to the 3 and 5 months of study. The minimum duration of each session has been established for 30 min. However, this length could be different, considering the characteristics of the participants. The control group will be made up of those participants who will receive standardized dietary advice (35). In this sense, the education content in the intervention group will be based on general principles of conventional dietary advice in an intensified manner (36), centered on the acquisition of specific knowledge about: (I) Underlying mental pathology, lifestyles and associated comorbidities; (II) Immediate principles: Carbohydrates, lipids, proteins, fiber, vitamins, and minerals; energy needs; consumption requirements; (III) Water requirements; (IV) Foodstuff; (V) Description and justification of prescribed prebiotic and probiotic diet; (VI) Culinary techniques: conservation of properties of the prebiotic and probiotic diet; (VII) Optimal distribution and interchange of foods with high prebiotic and probiotic content; (VIII) Feeding in particular situations.

In both the IG and the CG, visual support resources will be used during the development of the established sessions.

Block 3

Finally, to evaluate the effectiveness of the intervention, the modification in the nutritional, the cardio-metabolic, and the psychopathological area will be assessed. For doing this, researchers will carry out anthropometric determination, clinical evaluation, the performance of stool culture, and the study of the dietary pattern, as we commented above.

Likewise, in this block, an educational reinforcement (both in IG and CG) of what was treated in Block 2 will be offered, 3 and 5 months after the beginning of the block, every 15 days for the IG and monthly for the CG.

Once the intervention is concluded, the analysis of the collected data will be performed, culminating in the development of the scientific production and the writing of the research report.

Discussion

Firstly, it is expected to obtain the necessary information for the determination of the optimal dietary pattern for those participants in the study, thus allowing the development of a nutritional intervention with high prebiotic and probiotic content, appropriate for the population under study.

Likewise, the aim is to ensure that all participants improve their health status through the adaptation of the feeding pattern, developing adherence to healthier lifestyles adapted to the conditions of each patient.

Finally, it is expected to demonstrate that an adequate dietary modulation with a high prebiotic and probiotic content, leads to a significant improvement in the nutritional status and, therefore, the cardio-metabolic, of the participants, mediated by the microbiota-intestine-brain axis. Furthermore, it is presumed to reach a degree of evidence that allows establishing nutritional management as an effective therapeutic intervention in the psychopathological treatment of patients with schizophrenia spectrum disorders, in any of its variants.

Limitations

Potential limitations lie in the sample size of subjects included during the recruitment phase, the possible loss or non-cooperation of participants, and the loss or non-cooperation of participants in the intervention phase (especially those subjects with a predominance of negative schizophrenia symptomatology). This fact can lead to a possible lack of representativeness of the target population. Thus, to reverse this situation, the recruitment of the target population will be increased (more than doubled).

Likewise, the lack of capacity to prescribe symbiotic formulas and placebo management by mental health nurses, according to the legislative context in which the study will be carried out, prevents us from knowing the accurate range that an intervention with a high symbiotic content could have on these patients. Nevertheless, it is essential to emphasize that the main objective of the intervention is to assess the efficacy of a strategy based on health education. This tool is very cost-effective and is available for nursing professionals. Furthermore, from our perspective, this intervention can significantly impact the lifestyle habits of these patients in a sphere that has been forgotten as part of the traditional treatments.

Finally, the available evidence on the object of study makes it difficult to contrast the results obtained in different contexts of application.

Ethics statement

The study will be carried out respecting the fundamental principles established in the Declaration of Helsinki (1964)50, the Council of Europe Convention on Human Rights and Biomedicine (1997)51, the UNESCO Universal Declaration on the Human Genome and Human Rights (1997)52. The research will also follow the requirements established by Spanish legislation (Organic Law 3/2018 of 5 December and Law 41/2002 of 14 November). This study protocol has been registered on the platform clinicaltrials.gov (NCT04366401). This research also has the permission of the Zamora Health Area Drug Research Ethics Committee at the Regional Government of Castile and León, Spain (No. reg. 468). All the information analyzed by the principal investigator of this study is subject to the maintenance of professional secrecy. In any case, each participant will be assigned a code as a registry, where all the comparative data will be mechanised in an anonymous way, delimiting the access to the database only to the personnel linked to the development of the study, previous authorisation of the investigator in charge of it.

Author contributions

AS-J, GM-R, MG-R, and MR-S contributed to the conception and design of the study. AS-J, GM-R, JAG-M, RM-L, and MR-S contributed to the acquisition, analysis, and interpretation of results. AS-J and GM-R drafted the manuscript. AS-J, GM-R, and MR-S critically revised the manuscript. All authors contributed and approved the submitted version and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Balanzá V. Nutritional supplements in psychotic disorders. *Actas Esp Psiquiatr.* (2017) 45(Suppl. 1):16–25.

2. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Madrid: Editorial Médica Panamericana (2014).

3. Gómez AE. Nutrition and mental illness. Schizophrenia and omega 3 fatty acids. *Farmacia Profesional.* (2007) 21:60–3.

4. Bernardo M, Cañas F, Herrera B, García M. Adherence predicts symptomatic and psychosocial remission in schizophrenia: naturalistic study of patient integration in the community. *Rev Psiquiatr Salud Ment.* (2017) 10:149–59. doi: 10.1016/j.rpsm.2016.04.001

5. Tandon R, Nasrallah HA, Keshavan MS. Schizophrenia, "just the facts" 4. Clinical features and conceptualization. *Schizophr Res.* (2009) 110:1–23.

6. Godoy JF, Caballero M, Godoy-Izquierdo D, Vázquez ML, Muela JA. Relapse prevention in schizophrenia: proposal for an intervention programme during the prodromal phase. *Rei Do Crea.* (2016) 5:56–8.

7. Soria V, Uribe J, Salvat N, Palao D, Menchón JM, Labad J. Psychoneuroimmunology of mental disorders. *Rev Psiquiatr Salud Ment (Barc).* (2018) 11:115–24. doi: 10.1016/j.rpsm.2017.07.006

8. Egerton A, Grace AA, Stone J, Bossong MG, Sand M, McGuire P. Glutamate in schizophrenia: neurodevelopmental perspectives and drug development. *Schizophr Res.* (2020) 223:59–70. doi: 10.1016/j.schres.2020.09.013

9. Stahl SM. Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: dopamine, serotonin, and glutamate. *CNS Spectr.* (2018) 23:187–91. doi: 10.1017/S1092852918001013

10. Cepeda V, Mondragón A, Lamas A, Miranda JM, Cepeda A. Use of prebiotics and probiotics in the management of anxiety. *Farmaceut Comunit.* (2019) 11:30–40. doi: 10.5672/FC.2173-9218

11. Icaza ME. Gut microbiota in health and disease. *Rev Gastroenterol Mex.* (2013) 78:240–8. doi: 10.1016/j.rgmx.2013.04.004

12. Castillo F, Marzo ME. Role of the gut microbiota in the development of various neurological diseases. *Neurol Sci.* (2019) S0213-4853(19)30082-9. doi: 10.1016/j.nrl.2019.03.017

13. Rodríguez A, Solano M. Nutrition and mental health: a literature review. *Rev Post Psiquiat UNAH.* (2008) 1:1–5.

14. Salagre E, Vieta E, Grande I. The visceral brain: bipolar disorder and microbiota. *Rev Psiquiatr Salud Ment.* (2017) 10:67–9. doi: 10.1016/j.rpsm.2017.02.001

15. Wang HX, Wang YP. Gut microbiota-brain axis. *Chin Med J.* (2016) 129:2373–80. doi: 10.4103/0366-6999.190667

16. Mariño A, Núñez M, Barreto JM. Microbiota, probiotics, probiotics, prebiotics and symbiotics. *Pediatr Integral.* (2015) 19:337–54.

17. Andreo P, García N, Sánchez EP. The gut microbiota and its relation to mental illnesses through the microbiota-gut-brain axis. *Rev Dis Cli Neuro.* (2017) 4:52–8.

18. Kim YK, Shin C. The microbiota-gut-brain axis in neuropsychiatric disorders: pathophysiological mechanisms and novel treatments. *Curr Neuropharmacol.* (2018) 16:559–73. doi: 10.2174/1570159X15666170915141036

19. Patra S. Psychobiotics: a paradigm shift in psychopharmacology. *Indian J Pharmacol.* (2016) 48:469–70.

20. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet P. Psychobiotics and the manipulation of bacteria–gut–brain signals. *Trends Neurosci.* (2016) 39:763–81. doi: 10.1016/j.tins.2016.09.002

21. Kali A. Psychobiotics: an emerging probiotic in psychiatric practice. *Biomed J.* (2016) 3:223–4. doi: 10.1016/j.bj.2015.11.004

22. Forsythe P, Kunze W, Bienestock J. Moody microbes or fecal phrenology: what do we know about the microbiota-gutbrain axis? *BMC Med.* (2016) 14:58. doi: 10.1186/s12916-016-0604-8

23. Galletero JM. Nutrition and mental illness. Biochemical markers in bipolar disorder. *Zainak.* (2011) 34:323–34.

24. Franch CM, Molina V, Franch JI. Determinants of metabolic risk in atypical antipsychotic treatment. *Rev Psiquiatr Salud Ment.* (2016) 23:87–130. doi: 10.1016/j.psiq.2016.08.001

25. Franch CM, Molina V, Franch JI. Metabolic syndrome and atypical antipsychotics: possibility of prediction and control. *Rev Psiquiatr Salud Ment.* (2017) 10:38–44. doi: 10.1016/j.rpsm.2016.09.003

26. Sánchez ML, González J, Martínez MC. Metabolic control and prolactin in severe mental illness. Nursing interventions. *Rev Enferm Salud Ment.* (2018) 9:24–8. doi: 10.5538/2385-703X.2018.9.24

27. Pringsheim T, Kelly M, Urness D, Teehan M, Ismail Z, Gardner D. Physical health and drug safety in individuals with schizophrenia. *Can J Psychiatry.* (2017) 62:673–83. doi: 10.1177/0706743717719898

28. Pina L, Díaz MC, Saiz PA, Bobes J, Corripio I, Grasa E, et al. Pharmacogenetic study of second-generation antipsychotic long-term treatment metabolic side effects (the SLiM Study): rationale, objectives, design and sample description. *Rev Psiquiatr Salud Ment.* (2014) 7:166–78. doi: 10.1016/j.rpsm.2014.05.004

29. Severi E, Ferrara M, Tedeschini E, Vacca F, Mungai F, Amendolara R, et al. Assessment of cardiovascular risk in an Italian psychiatric outpatient sample: a chart review of patients treated with second-generation antipsychotics. *Int J Ment Health Nurs.* (2018) 27:1002–8. doi: 10.1111/inm.12407

30. Ocando L, Roa A, León M, González R. Atypical antipsychotics and their role in the development of metabolic disease. *Rev Iberoam Hipert.* (2018) 13:44–51.

31. Gurusamy J, Gandhi S, Damodharan D, Ganesan V, Palaniappan M. Exercise, diet and educational interventions for metabolic syndrome in persons with schizophrenia: a systematic review. *Asian J Psychiatr.* (2018) 36:73–85. doi: 10.1016/j.ajp.2018.06.018

32. Cortés B. Metabolic syndrome and second generation antipsychotic agents. *Rev Asoc Esp Neuropsiq.* (2011) 31:303–20. doi: 10.4321/S0211-57352011000200009

33. González E. Obesity: etiologic and pathophysiological analysis. *Endocrinol Nutr.* (2013) 60:17–24. doi: 10.1016/j.endonu.2012.03.006

34. Chee GL, Wynaden D, Heslop K. Improving metabolic monitoring rate for young people aged 35 and younger taking antipsychotic medications to treat a psychosis: a literature review. *Arch Psychiatr Nurs.* (2017) 31:624–33. doi: 10.1016/j.apnu.2017.09.002

35. Firth J, Siddiqi N, Koyanagi A, Siskind D, Rosenbaum S, Galletly C, et al. The lancet psychiatry commission: a blueprint for protecting physical health in people with mental illness. *Lancet Psychiatry.* (2019) 6:675–712. doi: 10.1016/S2215-0366(19)30132-4

36. Firth J, Teasdale SB, Allott K, Siskind D, Marx W, Cotter J, et al. The efficacy and safety of nutrient supplements in the treatment of mental disorders: a meta-review of meta-analyses of randomized controlled trials. *World Psychiatry.* (2019) 18:308–24. doi: 10.1002/wps.20672

37. Teasdale SB, Ward PB, Rosenbaum S, Samaras K, Stubbs B. Solving a weighty problem: systematic review and meta-analysis of nutrition interventions in severe mental illness. *Br J Psychiatry.* (2017) 210:110–8. doi: 10.1192/bjp.bp.115.177139

38. Teasdale SB, Ward PB, Samaras K, Firth J, Stubbs B, Tripodi E, et al. Dietary intake of people with severe mental illness: systematic review and meta-analysis. *Br J Psychiatry.* (2019) 214:251–9. doi: 10.1192/bjp.2019.20

39. Sánchez V, Romero D, Abad MJ, Descalzo MA, Alonso S, Salazar J, et al. Metabolic syndrome and cardiovascular risk in people treated with long-acting injectable antipsychotics. *Endocr Metab Immune Disord Drug Targets.* (2018) 18:379–87. doi: 10.2174/1871530317666171120151201

40. Firth J, Solmi M, Wootton RE, Vancampfort D, Schuch FB, Hoare E, et al. A meta-review of "lifestyle psychiatry": the role of exercise, smoking, diet and sleep in the prevention and treatment of mental disorders. *World Psychiatry.* (2020) 19:360–80. doi: 10.1002/wps.20773

41. Pérez BY, Jasso JA, López MM. Assessment of nutritional status in patients with psychiatric disorders in a hospital unit. *Nutr Clín Diet Hosp.* (2017) 37:24–33. doi: 10.12873/371brendayadira

42. Curtis J, Watkins A, Rosenbaum S, Teasdale S, Kalucy M, Samaras K, et al. Evaluating an individualized lifestyle and life skills intervention to prevent antipsychotic-induced weight gain in first-episode psychosis. *Early Interv Psychiatry.* (2016) 10:267–76. doi: 10.1111/eip.12230

43. Andalusian Regional Ministry of Health. Dietary advice in Primary Care. Plan for the Promotion of Physical Health and Balanced Diet 2004-2008. Andalusian Health Service. Andalusian Regional Ministry of Health. (2010). Available online at: https://www.juntadeandalucia.es/export/drupaljda/Guia_Consejo_Dietetico_AP_2005_imp2010.pdf (accessed November 20, 2019).

44. Andalusian Regional Ministry of Health. Guide to Intensive Dietetic Counselling in Primary Health Care. Plan for the Promotion of Physical Health and Balanced Diet 2004-2008. Andalusian Health Service. Andalusian Regional Ministry of Health. (2007). Available online at: https://www.repositoriosalud.es/handle/10668/1220 (accessed November 23, 2019).

45. Andalusian Health Service, Andalusian Regional Ministry of Health. Assessment Instrument No. 8: Early Detection and Intervention in Psychosis. Positive and Negative Schizophrenia Syndrome Scale (PANSS). Andalusian Health Service. (2010). Available online at: https://www.sspa.juntadeandalucia.es/servicioandaluzdesalud/publicaciones/deteccion-e-intervencion-temprana-en-laspsicosis-documentos-e-instrumentos-de-evaluacion (accessed November 5, 2019).

46. García MP, Alejandra P, Bousoño M, Bascarán MT, Guzmán C, Bobes J. Validation of the Spanish personal and social performance scale (PSP) in outpatients with stable and unstable schizophrenia. *Rev Psiquiatr Salud Ment (Barc).* (2011) 4:9–18. doi: 10.1016/j.rpsm.2010.11.003

47. Martín JM, Boyle P, Gorgojo L, Maisonneuve P, Fernández JC, Salvini S, et al. Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol.* (1993) 22:512–9. doi: 10.1093/ije/22.3.512

48. Sugawara N, Sagae T, Yasui-Furukori N, Yamakazi M, Shimoda K, Mori T, et al. Effects of nutritional education on weight change and metabolic abnormalities among patients with schizophrenia in Japan: a randomized controlled trial. *J Psychiatr Res.* (2018) 97:77–83. doi: 10.1016/j.jpsychires.2017.12.002

49. Carmenate L, Moncada FE, Borjas WE. *Manual of Anthropometric Measurements.* 1st ed. Costa Rica: SALTRA (2014).

50. Shrestha B, Dunn L. The declaration of helsinki on medical research involving human subjects: a review of seventh revision. *J Nepal Health Res Counc.* (2020) 17:548–552. doi: 10.33314/jnhrc.v17i4.1042

51. Dommel FW, Alexander D. The convention on human rights and biomedicine of the council of europe. *Kennedy Inst Ethics J.* (1997) 7:259–76. doi: 10.1353/ken.1997.0023

52. UNESCO: universal declaration on the human genome and human rights. *J Med Philos.* (1998) 23:334–41. doi: 10.1076/jmep.23.3.334.2578

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Functional characterization of nutraceuticals using spectral clustering: Centrality of caveolae-mediated endocytosis for management of nitric oxide and vitamin D deficiencies and atherosclerosis

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It is well recognized that redox imbalance, nitric oxide (NO), and vitamin D deficiencies increase risk of cardiovascular, metabolic, and infectious diseases. However, clinical studies assessing efficacy of NO and vitamin D supplementation have failed to produce unambiguous efficacy outcomes suggesting that the understanding of the pharmacologies involved is incomplete. This raises the need for using systems pharmacology tools to better understand cause-effect relationships at biological systems levels. We describe the use of spectral clustering methodology to analyze protein network interactions affected by a complex nutraceutical, Cardio Miracle (CM), that contains arginine, citrulline, vitamin D, and antioxidants. This examination revealed that *interactions between protein networks affected by these substances modulate functions of a network of protein complexes regulating* caveolae-mediated endocytosis (CME), TGF beta activity, vitamin D efficacy and host defense systems. Identification of this regulatory scheme and the working of embedded reciprocal feedback loops has significant implications for treatment of vitamin D deficiencies, atherosclerosis, metabolic and infectious diseases such as COVID-19.

KEYWORDS

atherosclerosis, cardio miracle, caveolae-mediated endocytosis, endothelial cells dysfunction, nitric oxide, TGF beta degradation, spectral clustering of protein swarms, vitamin D efficacy

Introduction

The term "nutraceuticals" includes dietary supplements, functional foods, vitamins, and nutritional products. These products are generally mixtures of natural products, vitamins, minerals and/or herbal ingredients. For the most part, clinical evidence is generally limited at the ingredient level since in the United States it is optional to make claims of clinical benefit to bring a nutraceutical to market. Further, unlike pharmaceuticals, nutraceuticals offer narrow profit margins; this in-conjunction with the non-stringent global regulatory environment allows manufacturers to avoid running expensive and time-consuming clinical trials for demonstrating health benefits for gaining marketing approval. However, with a plethora of products in the marketplace, it is becoming more and more important to competitively position the nutraceutical in terms of its health and wellness benefits.

Critical for predicting health effects of the nutraceuticals is the understanding of how complex mixtures of substances (ingredients, probes) influence the propagation of information in biological networks (1). While the structures and functions of cellular components are relatively well understood, very little is known on how system components work or cease to work together in case of injuries, medications or diets (1). This knowledge gap can largely be attributed to the complexity of network-network interactions giving rise to system plasticity and emergent properties (2). Thus, system perturbations affecting behavior frequently display "modularity" and "interdependence" wherein modularity refers to effects on system components that by working together deliver well defined outputs, and interdependence refers to effects of the perturbations on the organization of components necessary for delivering optimal end results (3). In the framework of network biology, interdependence results from interactions between networks of tissues, cells and proteins (4). However, predicting properties regulated by interacting network systems in real-world settings requires large amounts of data and, if absent, escapes the reach of even the most sophisticated statistical methodologies (3). For addressing this gap, applications of various spectral clustering methodologies have been explored (6, 7).

We have developed a novel spectral clustering methodology for advancing these efforts. It allows tracking of perturbation-induced information flows through multiple interacting network systems and facilitates determination of cause-effect relationships for even complex mixtures (see section "Materials and methods").

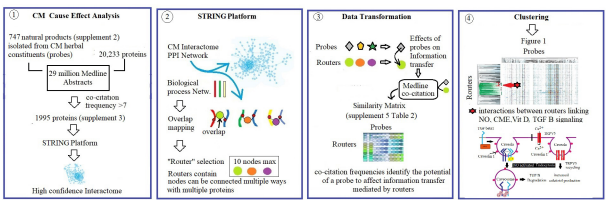
The aim of this study was to use spectral clustering for determining cause-effect relationships of the nutraceutical, Cardio Miracle (CM) marketed as a nitric oxide booster (see **Supplementary Data 1**), containing amongst its 50 + ingredients arginine, citrulline, vitamin D and antioxidants, which have recently been shown to increase the bioavailability of NO and decrease oxidative stress *in vitro* (8). Previous studies have linked vitamin D and NO deficiencies to nutrient-sensing (10, 11). It has also been shown that the addition of antioxidants to a combination of arginine, citrulline and vitamin D synergistically increases the ratio between NO and peroxynitrite production in endothelial cells (9). Critical for endothelial cell function; these signaling systems are important for health: endothelial cells dysfunction (ED) plays a key role in development of cardiovascular diseases, diabetes, obesity, and inflammatory conditions (12).

We describe the use of spectral clustering for identifying how CM affects the propagation of signals and impacts biological processes. We present evidence that interaction(s) between arginine, citrulline, vitamin D3 and antioxidants not only synergistically balance NO and peroxynitrate generation, but also affect functions of a protein network that regulates caveolae mediated endocytosis (CME), TGF beta activity, and vitamin D efficacy. Identification of this regulatory scheme and the working of embedded reciprocal feedback loops advances our understanding of how the various signaling systems/biological processes interact at a body-wide scale and generates meaningful hypotheses for bridging the gap between preclinical and clinical studies.

Materials and methods

Cardio miracle, marketed by Evolution Nutraceuticals (12), is a NO supplement. It is a mixture of arginine, citrulline (13), cholecalciferol vitamin D, various vitamins, quercetin, minerals and over 700 natural products that can be isolated from herbal and vegetable product constituents (see **Supplementary Material** Section) (13–15).[1]

Spectral Clustering Methodology, specifically designed to discover emergent properties resulting from network-network interactions, has been described in detail in PCT/US2016/06379. Its application for analysis of CM is detailed in the work-flow below.



Specifically,

(1) Protein network components affected by CM ingredients were identified by determining co-occurrence frequencies of 700 natural products isolated from its herbal constituents with 20,233 proteins in over 17 million Medline abstracts. This data-gathering step resulted in the selection of 1,995 proteins with a co-occurrence frequency count of more than seven.

1 https://www.cardiomiracle.com/

(2) Use of the STRING platform's highest confidence in network connectivity level for protein network construction delineated a high confidence CM interactome (see **Supplementary Material** Section) (2). STRING's gene enrichment analysis using biological process networks as background was used to divide the 1,995 protein-containing CM interaction network into smaller network fragments. This step resulted in > 4,000 protein network fragments that overlap with biological process networks regulating functions throughout the body. Selection of network fragments with < 10 network nodes (associated with a strength of > 0.9 and a p value of < 0.0001) produced 1,373 biological structure-function constraint network fragments.

(3) These 1,373 network fragments were used as topological descriptors for determining information densities of 747 CM ingredients associated with these 1,373 fragments in the Medline database resulting in the generation of a similarity matrix containing 1,373 × 747 information density measurements (**Supplementary Material** Section: **Supplementary Table 2**).

(4) Clustering of this similarity matrix using the TIBCO Spotfire platform (15)[2], and cosine correlation as similarity measure provided **Figure 1**.

Results

Identification of molecular underpinnings of the observation that interaction(s) between arginine, citrulline, vitamin D3 and antioxidants synergistically balance NO and peroxynitrate generation, using hierarchical clustering of the 1,373 × 747 similarity matrix in SPOTFIRE revealed that 747 CM ingredients induce network-network interactions involving a core group of 416 network fragments (**Figure 1**).

Using STRING's highest confidence level for investigating the connectivity between these 416 network fragments identified

2 https://www.tibco.com/products/tibco-spotfire

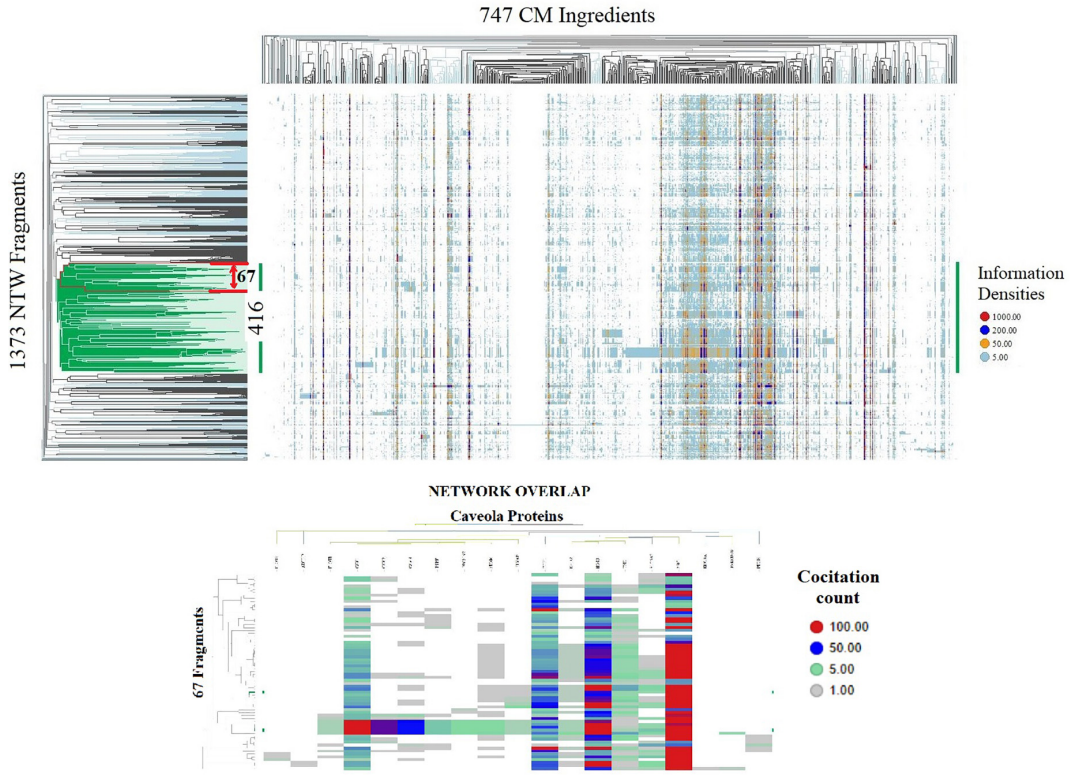


FIGURE 1
Spotfire generated heatmaps. TOP shows on the vertical dendrogram axis the organization of 1,373 CM interactome fragments overlapping with biological process network and identifies network-network interactions; the horizontal dendrogram axis identifies phenotypes of 747 natural products and CM ingredients containing substance groups inducing similar interactions in network fragment phenotypes; the vertical dendrogram section highlighted in green identifies network-network connectivity of core group of 416 network fragments induced by all 747 CM ingredients. BOTTOM identifies that a phenotype of 67 Fragment within the red boundary of the top heatmap containing biological process regulating vitamin D metabolism, Nitric oxide production and redox balance overlap with a network regulating caveola functions. Shown are the information densities of proteins CAV1, CAV3, PTRF, CAV2, FLOT1, FLOT2, NOS3, SRC, STOM, PRKCDBP, EHD2, SLC6A3, KIF18A, PACSIN2, CDH1, ADTRP, TFPI, PTGIS, PLVA with 67 network fragments.

networks of protein complexes containing 1,320 proteins overlapping with networks involved in the regulation of caveolae-associated functions, redox stress (17), and nutrient sensing. Of relevance to the regulation of endothelial functions is that the flattening of the curvature of caveolae under mechanical pressure (e.g., shear stress, blood pressure) that functions as a stress sensor (18) and modulates CME (18, 19). CME, by connecting mechanical input signals to the nucleus, regulates system-wide responses to ED associated with NO and vitamin D deficiency (20–24).

Determination of the connectivity between CM interactome fragments overlapping with biological process networks involved in NO and vitamin D signaling (**Figures 1**, **2**) identified caveolin 1 (caveolin 1), a major structural component of caveolae, as a key regulator of endothelial nitric oxide synthetase (NOS3) activity, vitamin D activation (VDR/CYP27B1), TGF beta activity and CME. Evidence discussed below supports the premise that this functional relationship represents a novel mechanism for regulating vitamin D efficacy.

Discussion

Spectral clustering based functional analysis of CM provides valuable insights regarding supplement-mediated regulation of vitamin D in cardiovascular diseases and for exploring ED. CM delivers vitamin D in form of pure cholecalciferol and as a mixture of cholecalciferol and 25-hydroxycholecalciferol (25(OH)D3) in the form of shitake and maitake mushroom powders. Cholecalciferol and 25-hydroxycholecalciferol are precursors of the hormone 1,25 dihydroxyvitamin D3 (calcitriol) and reach the bloodstream via intestinal absorption. The oral bioavailability of vitamin D precursors is limited by the ABCA1 transport protein which functions as an intestinal absorption barrier (25–27). Determination of co-investigation frequencies of 747 CM ingredients and ABCA1 transport protein was used to establish that the natural product, quercetin (28) and catechins (found in abundance in CM's antioxidant ingredient group) can inhibit ABCA1 transport and thereby, increase the oral bioavailability of vitamin D (29). Clinical observations provide strong evidence that co-administration of quercetin with vitamin D increases the oral bioavailability and efficacy of vitamin D and support the premise that CM ingredients enhance the oral bioavailability of cholecalciferol vitamin D (30, 31).

As shown in **Figure 3**, cytochrome CYP2R1 and CYP27A1 convert cholecalciferol into 25-hydroxyvitamin D (32) and which, upon binding to vitamin D binding protein and albumin (33, 34) circulates in the bloodstream. Vitamin D binding protein regulates circulating free and total levels of vitamin D metabolites, where ∼0.03% of 25(OH)D is free and 99.97% is bound to the vitamin D binding protein and to albumin. Cytochrome CYP24A1 transforms 25 (OH)D3 into an inactive metabolite 24,25-di-hydroxyvitamin D. Activation of 25 (OH)D3 bound to vitamin D binding protein requires active transport via megalin-mediated endocytosis into kidney proximal tubule cells and conversion by CYP27B1 into calcitriol. Calcitriol is metabolized by cytochrome CYP24A1 into its inactive form, 1,24,25 (OH)3 vitamin D (35) and has a half-life of ∼ 6 h vs. the half-life of the inactive form (25 (OH)D3) which is up to 3 weeks (36). Considering the plethora of vitamin D effects, the balance between vitamin D inactivating and activating metabolic enzymes and the speed with which calcitriol and its precursors can enter cells determines overall efficacy profile of vitamin D – and is therefore, dynamically regulated (37).

Amongst the many vitamin D efficacy regulators is calcitriol itself; it adjusts the expressions of CYP27B1, CYP24A1 and the vitamin D receptor (VDR) (38, 39). Calcitriol upon binding to VDR (enriched in caveolae) (40) is transported via CME across cell membranes (41). This caveolae-mediated active transport of receptor bound vitamin D3 is activated by NO (42) and inhibited by peroxynitrite (43, 44). Since CM has been documented to increase levels of bioavailable NO and lower peroxynitrate concentrations, it has therefore the capacity to activate and stabilize of CME leading to increased cellular uptake and genomic activity of vitamin D3 (41, 45–47).

The premise that CME activation increases calcitriol production is grounded in observations that CME regulates activity of a vanillin-type selective calcium channel TRPV5, present on the apical membrane of distal kidney tubule epithelial cells (48, 49), and that the loss of TRPV5 channel activity causes calcitriol overproduction and vitamin D hypervitaminosis (50, 51). Hence CME activation, by removing TRPV5 from the cell surface, decreases TRPV5 activity and increases calcitriol production (52, 53). This fine-tuning of CME-mediated vitamin D activation involves protein kinase C (PKC) and phospholipase D (PLD) wherein the activation of PKC inhibits CME and the inhibition of PKC activates CME (54). Since vitamin D3 (55) in combination with PKC inhibitors quercetin (56), oleanolic acid (57, 58), and curcumin (59) in CM's herbal constituents reinforces CME activation, this ingredient combination is projected to increase calcitriol production. The vitamin D efficacy of CM is further enhanced by oleanic acid, a natural product isolated from CM's hawthorn and mango extracts, which decreases the expression and protein levels of the calcitriol inactivator CYP24A1 (60).

Activation of CME also increases the degradation of TGF beta (61), an immunosuppressive cytokine that upregulates ROS production (62), arginases expression, and decreases NO production (63, 64). Thus, CM-mediated activation of CME is expected to reduce TGF beta signaling; this inhibitory effect is enhanced by hesperidin, a natural product isolated from CM's citrus extracts, which downregulates TGF beta expression (65). CME-mediated decrease in TGF beta activity adds to CM's projected capacity to increase vitamin D efficacy (66). This is

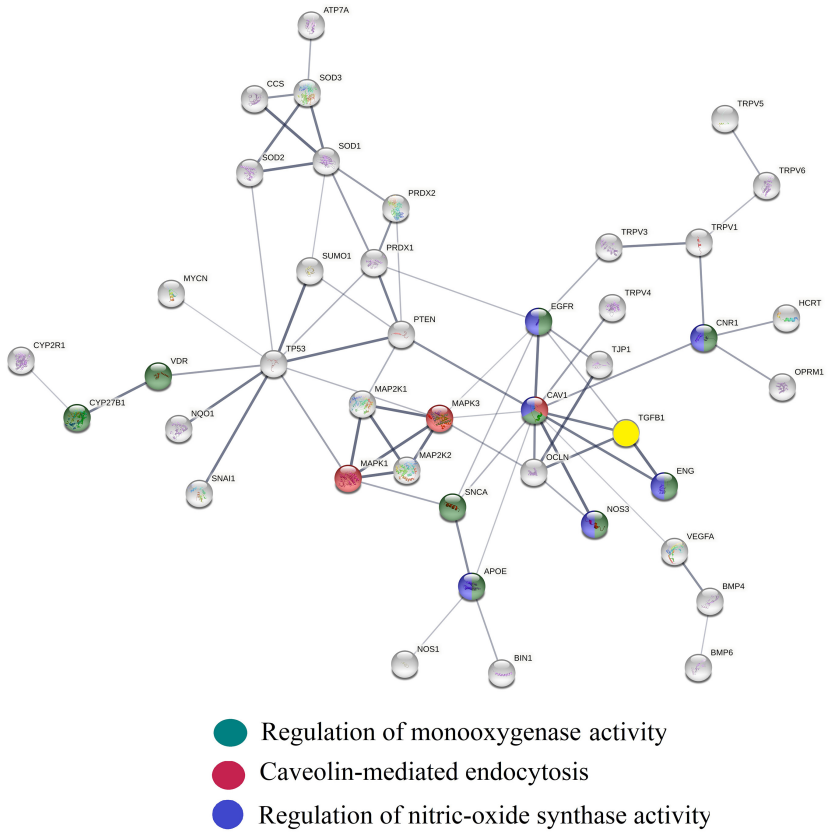


FIGURE 2
Protein interaction network generated using the String platform's highest confidence level (0.9) and CM interactome proteins: ADCYAP1, ADRB1, ADRBK1, AKAP5, AKT1, APOE, ATF4, ATP7A, BIN1, BMP2, BMP4, BMP6, CACNA1C, CACNA1D, CASQ2, caveolin1, CCK, CCS, CD320, CD9, CEACAM1, CNR1, CUBN, CYBA, CYP19A1, CYP1A1, CYP1A2, CYP1B1, CYP24A1, CYP26A1, CYP27B1, CYP2R1, CYP3A4, CYP3A4, CYP7A1, ENG, ENO1, EREG, F2, FGF19, FGF23, FGFR1, FOXO1, FOXO3, HCRT, HMOX1, IFNG, IL1B, IL4, ISCU, JAG1, KLF4, LEP, LMNA, MAPK3, MPO, MTOR, NF1, NFATC1, NFKB1, NOS1, NOS3, NOTCH1, NPY, NQO1, OCLN, OPRM1, ORAI1, PARK7, PDGFRA, PGR, PIAS4, PINK1, PLTP, POR, PPARA, PPARGC1A, PRDX1, PRDX2, PRKCD, PTEN, PTGS2, RBPJ, RORA, SCARB1, SIRT1, SMAD3, SNAI1, SNCA, SOD1, SOD2, SOD3, SPP1, STAT3, STIM1, STRA6, SUMO1, TGFB1, TJP1, TLR2, TNF, TNFRSF1A, TREM2, TRPV4, UBIAD1, UCN, UGT1A1, UGT1A8, VDR, VEGFA. Edges show physical interactions between proteins. Biological processes overlapping with this network fragment regulate caveola mediated endocytosis (yellow), NO biosynthesis (green), vitamin D activation (red) and TGF beta activity (light blue). Caveolin 1 (caveolin 1) serves as a hub protein connecting these biological process networks.

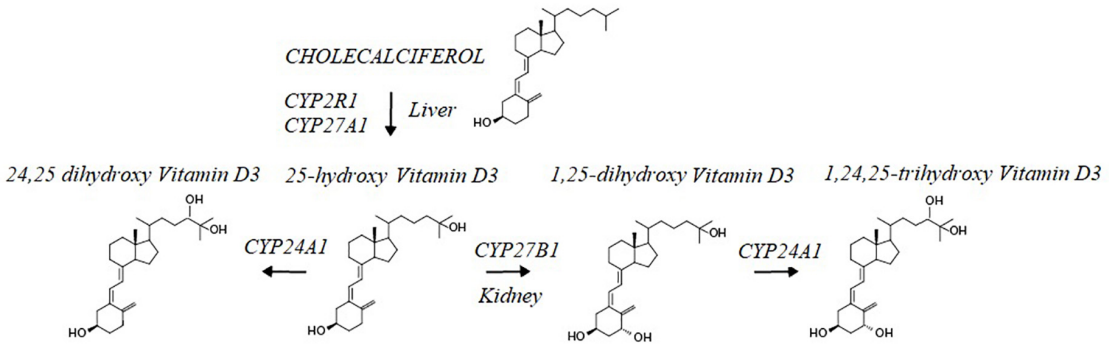


FIGURE 3
Cholecalciferol Vit D is metabolized in the liver by CYP2R1 and CYP27a1 into the Vitamin D3 precursor, 25 hydroxy Vitamin D3. This intermediate is further metabolized by CYP24A1 into a hormonal inactive form of Vit D3; 24,25 dihydroxy vitamin D3 and in the kidney by CYP27B1 into the active from of Vit D3, 1,25 dihydroxy-Vitamin D3. Levels of the hormonal active form of Vit D3 are decreased through the action of CYP24A1 converting the hormone into 1,25,25-trihydroxy Vitamin D3.

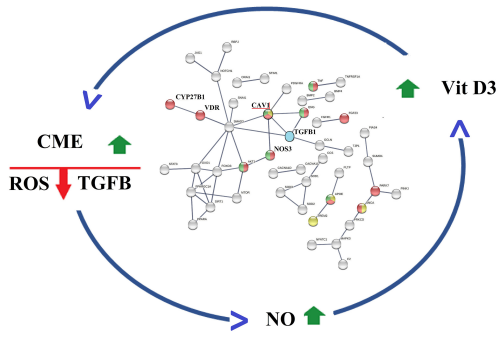


FIGURE 4
Caveola mediated endocytosis (CME) modulates activities of a reciprocal feedback loops that finetunes ROS production, TGF beta activity, Nitric oxide levels O and Calcitriol production.

because TGF beta has been shown to inhibit the expression of megalin, the intracellular protein essential for uptake of 25 hydroxyvitamin D by kidney proximal tubule cells and its subsequent conversion to calcitriol by CYP27B1 (67, 68). Accordingly, the experimental observations summarized in Figure 4, provides strong support for the existence of a CME-based regulatory scheme that upon activation controls functions of reciprocal feedback loops that decrease ROS, decrease TGF beta activity, and increase NO and calcitriol production (45).

Inhibition of TGF beta signaling has been shown to impede progression of atherosclerosis and results in regression of established disease (69). Thus, CM-mediated activation of CME in combination with hesperidin's capacity to downregulate TGF beta expression is projected to have beneficial effects in atherosclerosis. Secondly, CM-mediated increase in calcitriol production via CME-activation is projected to lower caveolin 1 level. This is therefore expected to also intercept development of atherosclerosis since increased caveolin 1 expression is linked to disease progression (70–73). The mechanistic rationale is that caveolin 1 levels are regulated by autophagy and prevent its own degradation since it is itself an autophagy inhibitor (64, 74, 75). Caveolin 1-mediated inhibition of autophagy is reduced by calcitriol which activates tyrosine kinase activity of pp60src and results in the phosphorylation of caveolin 1 at tyrosine 14 and increased autophagy (76–78).

Phosphorylation of caveolin 1 at tyrosine 14 also increases endothelial nitric oxide synthase activity and increases NO production to further reinforce the CM feedback loop. Thus, the capacity of CM to enhance TGF beta and caveolin 1 degradation is projected to enhance the anti-atherosclerotic efficacy of this nutraceutical (79–82).

It is also important to note that CM's projected pharmacology to lower caveolin 1 level by increasing autophagic degradation is enhanced by other autophagy-activating CM ingredients: palmitic acid (83), resveratrol (84), pterostilbene (85), quercetin (86), piceatannol (87), delphinidin (88), cyanidin-3-o-glucoside (89), and sulforaphane (89).

Summary

Identification of network-network interactions regulating reciprocal feedback loops advances our understanding of how ingredients/natural products of nutraceuticals interact within a system and provide guidance for product optimization and improving preclinical and clinical outcomes. Spectral clustering of protein interaction information associated with ingredients of a complex nutraceutical supplement, CM, uncovered several biological functions supported by its ability to correct cellular redox imbalance. These include its ability to increase oral bioavailability of cholecalciferol vitamin D3 and to activate and stabilize caveolin-mediated endocytosis. The combination of these functionalities infers involvement of reciprocal cellular feedback loops that increase NO production and vitamin D3 efficacy, decrease TGF beta signaling and oxidative stress, and activate autophagy. Since down-regulation of TGF beta activity and activation of autophagy is anticipated to intercept/reverse endothelial dysfunction associated diseases including atherosclerosis (90–93), diabetic kidney disease (94–96), and COVID-19 (97–99), supplementation with CM-like functionalities is projected to benefit treatment of these diseases. For validating these effect predictions, clinical trials are warranted.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

AF developed the spectral clustering methodology and performed the data analysis of this study. SK contributed to the interpretation of analysis outcomes. Both authors contributed to the manuscript and agreed to be accountable for the content of the work.

Acknowledgments

John Hewlett contributed valuable information on CM ingredients and suggestions to the study.

Conflict of interest

AF and SK are the founders of Emergent System Analytics LLC.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.885364/full#supplementary-material

References

1. Santolini M, Barabási AL. Predicting perturbation patterns from the topology of biological networks. *Proc Natl Acad Sci U.S.A.* (2018) 115:E6375–83. doi: 10.1073/pnas.1720589115

2. Liu W, Suzumura T, Ji H, Hu G. Finding overlapping communities in multilayer networks. *PLoS One.* (2018) 13:e0188747. doi: 10.1371/journal.pone.0188747

3. Noell G, Faner R, Agustí A. From systems biology to P4 medicine: applications in respiratory medicine. *Eur Respir Rev.* (2018) 27:170110. doi: 10.1183/16000617.0110-2017

4. Sun PG, Quan Y, Miao Q. Interdependent patterns in protein-protein interaction networks. *IEEE Trans Network Sci Eng.* (2020) 7:3257–65. doi: 10.1109/TNSE.2020.3022170

5. Ahn YY, Bagrow JP, Lehmann S. Link communities reveal multiscale complexity in networks. *Nature.* (2010) 466:761–4. doi: 10.1038/nature09182

6. Liu W, Chang Z, Jia C, Zheng Y. A generative node-attribute network model for detecting generalized structure and semantics. *Phys A Statist Mech Appl.* (2022) 588:126557. doi: 10.1016/j.physa.2021.126557

7. Sadikaj Y, Velaj Y, Behzadi S, Plant C. "Spectral clustering of attributed multi-relational graphs," in *Proceedings of the 27th ACM SIGKDD Conference on Knowledge Discovery & Data Mining*, Singapore (2021). doi: 10.1145/3447548.3467381

8. Zhang H, Wang CD, Lai JH. Modularity in complex multilayer networks with multiple aspects: a static perspective. *Appl Inform.* (2017) 4:7. doi: 10.1186/s40535-017-0035-4

9. Dawoud H, Malinski T. Vitamin D3, L-Arginine, L-Citrulline, and antioxidant supplementation enhances nitric oxide bioavailability and reduces oxidative stress in the vascular endothelium–Clinical implications for cardiovascular system. *Pharmacogn Res.* (2020) 12:17–23. doi: 10.4103/pr.pr_79_19

10. Hsu CN, Tain YL. Regulation of nitric oxide production in the developmental programming of hypertension and kidney disease. *Int J Mol Sci.* (2019) 20:681. doi: 10.3390/ijms20030681

11. El Maaty MAA, Gad MZ. Vitamin D deficiency and cardiovascular disease: potential mechanisms and novel perspectives. *J Nutr Sci vitaminol.* (2013) 59:479–88. doi: 10.3177/jnsv.59.479

12. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev.* (2007) 87:315–424. doi: 10.1152/physrev.00029.2006

13. Cieri-Hutcherson NE, Jaenecke A, Bahia A, Lucas D, Oluloro A, Stimmel L, et al. Systematic review of L-arginine for the treatment of hypoactive sexual desire disorder and related conditions in women. *Pharmacy.* (2021) 9:71. doi: 10.3390/pharmacy9020071

14. Rashid J, Kumar SS, Job KM, Liu X, Fike CD, Sherwin CM. Therapeutic potential of citrulline as an arginine supplement: a clinical pharmacology review. *Pediatr Drugs.* (2020) 22:279–93. doi: 10.1007/s40272-020-00384-5

15. Afendi FM, Okada T, Yamazaki M, Hirai-Morita A, Nakamura Y, Nakamura K, Ikeda S, et al. KNApSAcK family databases: integrated metabolite-plant species databases for multifaceted plant research. *Plant Cell Physiol.* (2012) 53:1–12. doi: 10.1093/pcp/pcr165

16. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* (2019) 47:D607–13. doi: 10.1093/nar/gky1131

17. Tebay LE, Robertson H, Durant ST, Vitale SR, Penning TM, Dinkova-Kostova AT, et al. Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radical Biol Med.* (2015) 88:108–46. doi: 10.1016/j.freeradbiomed.2015.06.021

18. Joseph JG, Liu AP. Mechanical regulation of endocytosis: new insights and recent advances. *Adv Biosyst.* (2020) 4:1900278. doi: 10.1002/adbi.201900278

19. Del Pozo MA, Lolo FN, Echarri A. Caveolae: mechanosensing and mechanotransduction devices linking membrane trafficking to mechanoadaptation. *Curr Opin Cell Biol.* (2021) 68:113–23. doi: 10.1016/j.ceb.2020.10.008

20. Xu Y, Buikema H, van Gilst WH, Henning RH. Caveolae and endothelial dysfunction: filling the caves in cardiovascular disease. *Eur J Pharmacol.* (2008) 585:256–60. doi: 10.1016/j.ejphar.2008.02.086

21. Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S. Endothelial dysfunction as a target for prevention of cardiovascular disease. *Diabetes Care.* (2009) 32(suppl. 2):S314–21. doi: 10.2337/dc09-S330

22. Majkova Z, Toborek M, Hennig B. The role of caveolae in endothelial cell dysfunction with a focus on nutrition and environmental toxicants. *J Cell Mol Med.* (2010) 14:2359–70. doi: 10.1111/j.1582-4934.2010.01064.x

23. Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. Caveolin regulation of endothelial function. *Am J Physiol Lung Cell Mol Physiol.* (2003) 285:L1179–83. doi: 10.1152/ajplung.00242.2003

24. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension.* (2011) 57:63–9. doi: 10.1161/HYPERTENSIONAHA.110.160929

25. Margier M, Collet X, Le May C, Desmarchelier C, André F, Lebrun C, et al. ABCB1 (P-glycoprotein) regulates vitamin D absorption and contributes to its transintestinal efflux. *FASEB J.* (2019) 33:2084–94. doi: 10.1096/fj.201800956R

26. Shekhawat PB, Pokharkar VB. Understanding peroral absorption: regulatory aspects and contemporary approaches to tackling solubility and permeability hurdles. *Acta Pharm Sin B.* (2017) 7:260–80. doi: 10.1016/j.apsb.2016.09.005

27. Maurya VK, Aggarwal M. Factors influencing the absorption of vitamin D in GIT: an overview. *J Food Sci. Technol.* (2017) 54:3753–65. doi: 10.1007/s13197-017-2840-0

28. Borska S, Chmielewska M, Wysocka T, Drag-Zalesinska M, Zabel M, Dziegiel P. In vitro effect of quercetin on human gastric carcinoma: targeting cancer cells death and MDR. *Food Chem. Toxicol.* (2012) 50:3375–83. doi: 10.1016/j.fct.2012.06.035

29. Kitagawa S, Nabekura T, Kamiyama S. Inhibition of P-glycoprotein function by tea catechins in KB-C2 cells. *J Pharm Pharmacol.* (2004) 56:1001–5. doi: 10.1211/0022357044003

30. Paller CJ, Kanaan YM, Beyene DA, Naab TJ, Copeland RL, Tsai HL, et al. Risk of prostate cancer in African-American men: evidence of mixed effects of dietary

quercetin by serum vitamin D status. *Prostate.* (2015):1376–83. doi: 10.1002/pros.23018

31. Hassan JK, Sharrad AK, Sheri FH. Effect of quercetin supplement on some bone mineralization biomarkers in diabetic type 2 patients. *Adv Pharmacol Pharm.* (2018) 6:43–9. doi: 10.13189/app.2018.060202

32. Bikle DD. Vitamin D: newer concepts of its metabolism and function at the basic and clinical level. *J Endocr Soc.* (2020) 4:bvz038. doi: 10.1210/jendso/bvz038

33. Daiger SP, Schanfield MS, Cavalli-Sforza LL. Group-specific component (Gc) proteins bind vitamin D and 25-hydroxyvitamin D. *Proc Natl Acad Sci U.S.A.* (1975) 72:2076–80. doi: 10.1073/pnas.72.6.2076

34. Bikle D. Vitamin D: production, metabolism, and mechanisms of action and clinical applications. *Chem Biol.* (2015) 21:319–29. doi: 10.1016/j.chembiol.2013.12.016

35. Meyer MB, Lee SM, Carlson AH, Benkusky NA, Kaufmann M, Jones G, et al. A chromatin-based mechanism controls differential regulation of the cytochrome P450 gene Cyp24a1 in renal and non-renal tissues. *J Biol Chem.* (2019) 294:14467–81. doi: 10.1074/jbc.RA119.010173

36. Mosekilde L. Vitamin D and the elderly. *Clin Endocrinol.* (2005) 62:265–81. doi: 10.1111/j.1365-2265.2005.02226.x

37. Meyer MB, Pike JW. Mechanistic homeostasis of vitamin D metabolism in the kidney through reciprocal modulation of Cyp27b1 and Cyp24a1 expression. *J Steroid Biochem Mol Biol.* (2020) 196:105500. doi: 10.1016/j.jsbmb.2019.105500

38. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *J Lipid Res.* (2014) 55:13–31. doi: 10.1194/jlr.R031534

39. Lu X, Chen Z, Watsky MA. Effects of 1, 25 and 24, 25 vitamin D on corneal fibroblast VDR and vitamin D metabolizing and catabolizing enzymes. *Current Eye Res.* (2021) 46:1271–82. doi: 10.1080/02713683.2021.1884726

40. Donati S, Palmini G, Romagnoli C, Aurilia C, Miglietta F, Falsetti I, et al. In vitro non-genomic effects of calcifediol on human preosteoblastic cells. *Nutrients.* (2021) 13:4227. doi: 10.3390/nu13124227

41. Schaefer R. *Calcitriol Conjugated Quantum Dots, An Innovative Tool As Both Probe And Treatment*. Doctoral dissertation, University of Delaware, Newark, DE (2012).

42. Chen Z, Ds Oliveira S, Zimnicka AM, Jiang Y, Sharma T, Chen S, et al. Reciprocal regulation of eNOS and caveolin-1 functions in endothelial cells. *Mol Biole Cell.* (2018) 29:1190–202. doi: 10.1091/mbc.E17-01-0049

43. Mougeolle A, Poussard S, Decossas M, Lamaze C, Lambert O, Dargelos E. Oxidative stress induces caveolin 1 degradation and impairs caveolae functions in skeletal muscle cells. *PLoS One.* (2015) 10:e0122654. doi: 10.1371/journal.pone.0122654

44. Shamsaldeen YA, Lione LA, Benham CD. Dysregulation of TRPV4, eNOS and caveolin-1 contribute to endothelial dysfunction in the streptozotocin rat model of diabetes. *Eur J Pharmacol.* (2020) 888:173441. doi: 10.1016/j.ejphar.2020.173441

45. Martínez-Miguel P, Valdivielso JM, Medrano-Andrés D, Román-García P, Cano-Peñalver JL, Rodríguez-Puyol M, et al. The active form of vitamin D, calcitriol, induces a complex dual upregulation of endothelin and nitric oxide in cultured endothelial cells. *Am J Physiol Endocrinol Metab.* (2014) 307:E1085–96. doi: 10.1152/ajpendo.00156.2014

46. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. *Endocrinol Metab Clin.* (2017) 46:815–43.

47. Christakos S. In search of regulatory circuits that control the biological activity of vitamin D. *J Biol Chem* (2017) 292:17559–60. doi: 10.1074/jbc.H117.806901

48. Cha SK. Wu T, Huang CL. Protein kinase C inhibits caveolae-mediated endocytosis of TRPV5. *Am J Physiol Renal Physiol.* (2008) 294:F1212–21. doi: 10.1152/ajprenal.00007.2008

49. Gkika M, Hoenderop JG, Bindels RJ. The epithelial Ca2+ channel TRPV5 in health and disease. *Naunyn Schmiedebergs Arch Pharmacol.* (2004) 371:295–306.

50. Jacquillet G, Unwin RJ. Physiological regulation of phosphate by vitamin D, parathyroid hormone (PTH) and phosphate (Pi). *Pflügers Arch.* (2019) 471:83–98.

51. Renkema KY, Nijenhuis T, van der Eerden BC, van der Kemp AW, Weinans H, van Leeuwen JP, et al. Hypervitaminosis D mediates compensatory Ca2+ hyperabsorption in TRPV5 knockout mice. *J Am Soc Nephrol.* (2005) 16:3188–95. doi: 10.1681/ASN.2005060632

52. Estadella I, Pedrós-Gámez O, Colomer-Molera M, Bosch M, Sorkin A, Felipe A. Endocytosis: a turnover mechanism controlling ion channel function. *Cells.* (2020) 9:1833.

53. De Groot T, Bindels RJ, Hoenderop JG. TRPV5: an ingeniously controlled calcium channel. *Kidney Int.* (2008) 74:1241–6. doi: 10.1038/ki.2008.320

54. Kim JH, Han JM, Lee S, Kim Y, Lee TG, Park JB, et al. Phospholipase D1 in caveolae: regulation by protein kinase Cα and caveolin-1. *Biochemistry.* (1999) 38:3763–9. doi: 10.1021/bi982478+

55. Lin LM, Peng F, Liu YP, Chai DJ, Ning RB, Xu CS, et al. Coadministration of VDR and RXR agonists synergistically alleviates atherosclerosis through inhibition of oxidative stress: an in vivo and in vitro study. *Atherosclerosis.* (2016) 251:273–81. doi: 10.1016/j.atherosclerosis.2016.06.005

56. Kang TB, Liang NC. Effect of quercetin on activities of protein kinase C and tyrosine protein kinase from HL-60 cells. *Acta Pharmacol Sin.* (1997) 18:374–6.

57. Mooi LY, Yew WT, Hsum YW, Soo KK, Hoon LS, Chieng YC. Suppressive effect of maslinic acid on PMA-induced protein kinase C in human B-lymphoblastoid cells. *Asian Pac J Cancer Prev.* (2012) 13:1177–82. doi: 10.7314/apjcp.2012.13.4.1177

58. Li S, Wang YN, Wan ZL, Zhang XW, Cong PJ. Study on extraction and purification of ursolic acid and oleanolic acid from hawthorn fruits. *Food Sci.* (2007) 28:141–4.

59. Mahmmoud YA. Modulation of protein kinase C by curcumin; inhibition and activation switched by calcium ions. *Br J Pharmacol.* (2007) 150:200–8. doi: 10.1038/sj.bjp.0706970

60. Cao S, Dong XL, Ho MX, Yu WX, Wong KC, Yao XS, et al. Oleanolic acid exerts osteoprotective effects and modulates vitamin D metabolism. *Nutrients.* (2018) 10:247.

61. Chen YG. Endocytic regulation of TGF-β signaling. *Cell Res.* (2009) 19:58–70.

62. Krstić J, Trivanović D, Mojsilović S, Santibanez JF. Transforming growth factor-beta and oxidative stress interplay: implications in tumorigenesis and cancer progression. *Oxid Med Cell Longev.* (2015) 2015:654594. doi: 10.1155/2015/654594

63. Salminen A. Immunosuppressive network promotes immunosenescence associated with aging and chronic inflammatory conditions. *J Mol Med.* (2021) 99:1553–69.

64. Zhang X, Ramírez CM, Aryal B, Madrigal-Matute J, Liu X, Diaz A, et al. Cav-1 (Caveolin-1) deficiency increases autophagy in the endothelium and attenuates vascular inflammation and atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2020) 40:1510–22. doi: 10.1161/ATVBAHA.120.314291

65. Maneesai P, Bunbupha S, Potue P, Berkban T, Kukongviriyapan U, Kukongviriyapan V, et al. Hesperidin prevents nitric oxide deficiency-induced cardiovascular remodeling in rats via suppressing TGF-β1 and MMPs protein expression. *Nutrients.* (2018) 10:1549. doi: 10.3390/nu10101549

66. Gliozzi ML, Rbaibi Y, Long KR, Vitturi DA, Weisz OA. Metabolism, oxidative stress and cell signaling: hemoglobin alters vitamin carrier uptake and vitamin D metabolism in proximal tubule cells: implications for sickle cell disease. *Am J Physiol Cell Physiol.* (2019) 317:C993. doi: 10.1152/ajpcell.00287.2019

67. Cabezas F, Farfán P, Marzolo MP. Participation of the SMAD2/3 signalling pathway in the down regulation of megalin/LRP2 by transforming growth factor beta (TGF-ß1). *PLoS One.* (2019) 14:e0213127. doi: 10.1371/journal.pone.0213127

68. Shankar AS, van den Berg SA, Tejeda Mora H, Du Z, Lin H, Korevaar SS, et al. Vitamin D metabolism in human kidney organoids. *Nephrol Dial Transpl.* (2022) 37:190–3. doi: 10.1093/ndt/gfab264

69. Chen PY, Qin L, Li G, Wang Z, Dahlman JE, Malagon-Lopez J, et al. Endothelial TGF-β signalling drives vascular inflammation and atherosclerosis. *Nat Metab.* (2019) 1:912–26. doi: 10.1038/s42255-019-0102-3

70. Zhang X, Fernández-Hernando C. Transport of LDLs into the arterial wall: impact in atherosclerosis. *Curr Opin Lipidol.* (2020) 31:279–85.

71. Deng L, Vrieling F, Stienstra R, Hooiveld G, Feitsma AL, Kersten S. Caveolae mediated endocytosis of VLDL particles in macrophages requires NPC1 and STARD3 for further lysosomal processing. *bioRxiv* [Preprint] (2021):doi: <PMID<PMID:NOPMID</PMID<

72. Shiroto T, Romero N, Sugiyama T, Sartoretto JL, Kalwa H, Yan Z, et al. Caveolin-1 is a critical determinant of autophagy, metabolic switching, and oxidative stress in vascular endothelium. *PLoS One.* (2014) 9:e87871. doi: 10.1371/journal.pone.0087871

73. Pavlides S, Gutierrez-Pajares JL, Iturrieta J, Lisanti MP, Frank PG. Endothelial caveolin-1 plays a major role in the development of atherosclerosis. *Cell Tissue Res.* (2014) 356:147–57. doi: 10.1007/s00441-013-1767-7

74. Poredos P, Poredos AV, Gregoric I. Endothelial dysfunction and its clinical implications. *Angiology.* (2021) 72:604–15.

75. Münzel T, Templin C, Cammann VL, Hahad O. Takotsubo syndrome: impact of endothelial dysfunction and oxidative stress. *Free Radical Biol Med.* (2021) 169:216–23. doi: 10.1016/j.freeradbiomed.2021.03.033

76. Nah J, Yoo SM, Jung S, Jeong EI, Park M, Kaang BK, et al. Phosphorylated caveolin1 activates autophagy through an interaction with BECN1 under oxidative stress. *Cell Death Dis.* (2017) 8:e2822. doi: 10.1038/cddis.2017.71

77. Buitrago C, Boland R. Caveolae and caveolin-1 are implicated in 1α, 25 (OH) 2-vitamin D3-dependent modulation of Src. MAPK cascades and VDR localization in skeletal muscle cells. *J Steroid Biochem Mol Biol.* (2010) 121:169–75. doi: 10.1016/j.jsbmb.2010.03.002

78. Wang RC, Levine B. Calcipotriol induces autophagy in HeLa cells and keratinocytes. *J Invest Dermatol.* (2011) 131:990. doi: 10.1038/jid.2010.423

79. Yokoyama M. Oxidant stress and atherosclerosis. *Current Opin Pharmacol.* (2004) 4:110–5.

80. Bennett AL, Lavie CJ. Vitamin D metabolism and the implications for atherosclerosis. *Adv Exp Med Biol.* (2017) 996:185–92.

81. Miao J, Zang X, Cui X, Zhang J. Autophagy, hyperlipidemia, and atherosclerosis. *Adv Exp Med Biol.* (2020) 1207:237–64.

82. Layne J, Majkova Z, Smart EJ, Toborek M, Hennig B. Caveolae: a regulatory platform for nutritional modulation of inflammatory diseases. *J Nutr Biochem.* (2011) 22:807–11.

83. Tan SH, Shui G, Zhou J, Li JJE, Bay BH, Wenk MR, et al. Induction of autophagy by palmitic acid via protein kinase C-mediated signaling pathway independent of mTOR (mammalian target of rapamycin). *J Biol Chem.* (2012) 287:14364–76. doi: 10.1074/jbc.M111.294157

84. Tian Y, Song W, Li D, Cai L, Zhao Y. Resveratrol as a natural regulator of autophagy for prevention and treatment of cancer. *OncoTargets Ther.* (2019) 12:8601.

85. Chen RJ, Lee YH, Yeh YL, Wu WS, Ho CT, Li CY, et al. Autophagy-inducing effect of pterostilbene: a prospective therapeutic/preventive option for skin diseases. *J Food Drug Anal.* (2017) 25:125–33. doi: 10.1016/j.jfda.2016.10.022

86. Ashrafizadeh M, Ahmadi Z, Farkhondeh T, Samarghandian S. Autophagy as a molecular target of quercetin underlying its protective effects in human diseases. *Arch Physiol Biochem.* (2019) 128:200–8.

87. Siedlecka-Kroplewska K, Ślebioda T, Kmieć Z. Induction of autophagy, apoptosis and aquisition of resistance in response to piceatannol toxicity in MOLT-4 human leukemia cells. *Toxicol Vitro.* (2019) 59:12–25. doi: 10.1016/j.tiv.2019.03.040

88. Lee DY, Park YJ, Song MG, Kim DR, Zada S, Kim DH. Cytoprotective effects of delphinidin for human chondrocytes against oxidative stress through activation of autophagy. *Antioxidants.* (2020) 9:83. doi: 10.3390/antiox9010083

89. Wang S, Huang Y, Luo G, Yang X, Huang W. Cyanidin-3-O-glucoside attenuates high glucose–induced podocyte dysfunction by inhibiting apoptosis and promoting autophagy via activation of SIRT1/AMPK pathway. *Can J Physiol Pharmacol.* (2021) 99:589–98. doi: 10.1139/cjpp-2020-0341

90. Matthaeus C. Caveolae mediated lipid uptake and trafficking in health and disease. *FASEB J.* (2021) 35. doi: 10.1096/fasebj.2021.35.S1.01983

91. Martelli A, Citi V, Calderone V. Recent efforts in drug discovery on vascular inflammation and consequent atherosclerosis. *Expert Opin Drug Discov.* (2021) 16:411–27. doi: 10.1080/17460441.2021.1850688

92. Pillai SC, Borah A, Jacob EM, Kumar DS. Nanotechnological approach to delivering nutraceuticals as promising drug candidates for the treatment of atherosclerosis. *Drug Deliv.* (2021) 28:550–68. doi: 10.1080/10717544.2021.1892241

93. Poznyak AV, Nikiforov NG, Wu WK, Kirichenko TV, Orekhov AN. Autophagy and mitophagy as essential components of atherosclerosis. *Cells.* (2021) 10:443. doi: 10.3390/cells10020443

94. Zhao L, Zou Y, Liu F. Transforming growth factor-beta1 in diabetic kidney disease. *Front Cell Dev Biol.* (2020) 8:187. doi: 10.3389/fcell.2020.00187

95. Shi GJ, Shi GR, Zhou JY, Zhang WJ, Gao CY, Jiang YP, et al. Involvement of growth factors in diabetes mellitus and its complications: a general review. *Biomed Pharmacother.* (2018) 101:510–27. doi: 10.1016/j.biopha.2018.02.105

96. Wu M, Zhang M, Zhang Y, Li Z, Li X, Liu Z, et al. Relationship between lysosomal dyshomeostasis and progression of diabetic kidney disease. *Cell Death Dis.* (2021) 12:1–10. doi: 10.1038/s41419-021-04271-w

97. Chen W. A potential treatment of COVID-19 with TGF-β blockade. *Int J Biol Sci.* (2020) 16:1954. doi: 10.7150/ijbs.46891

98. Shen WX, Luo RC, Wang JQ, Chen ZS. Features of cytokine storm identified by distinguishing clinical manifestations in COVID-19. *Front Public Health.* (2021) 9:614. doi: 10.3389/fpubh.2021.671788

99. Maity S, Saha A. Therapeutic potential of exploiting autophagy cascade against coronavirus infection. *Front Microbiol.* (2021) 12:675419. doi: 10.3389/fmicb.2021.675419

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U-shaped association between serum albumin and pediatric intensive care unit mortality in critically ill children

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Introduction: The detailed association between albumin levels and mortality has not been studied in critically ill children. The aim of this study was to reveal an association between albumin levels in detail and mortality in critically ill children.

Materials and methods: We retrospectively collected data from children admitted to four pediatric intensive care units (PICUs) in China between January 2015 and October 2020. Restricted cubic spline curves based on logistic regression models were generated to evaluate the detailed associations between serum albumin levels and PICU mortality. Threshold effect analysis was performed using two piecewise regression models.

Results: The study included 9,123 children. The overall mortality was 5.3%. The detailed association between serum albumin levels and the risk of mortality followed a U-shape. The risk of mortality decreased with increasing serum albumin levels (OR = 0.919; 95% CI: 0.886, 0.954) in children with serum albumin levels $<$ 43.2 g/L and increased with increasing serum albumin levels (OR = 1.174; 95% CI: 1.044, 1.316) in children with serum albumin levels $\geq$ 43.2 g/L.

Conclusion: There was a U-shaped association between serum albumin levels and mortality in critically ill children in the PICU.

KEYWORDS

children, albumin, critical care, restricted cubic spline, mortality

A serum albumin level of less than 35 g/L is considered hypoalbuminemia, which is frequent in hospitalized patients, especially critically ill patients. It has been reported that more than 60% of pediatric patients have hypoalbuminemia in intensive care units. Many studies have revealed that hypoalbuminemia is associated with poor clinical outcomes. However, the detailed association between albumin levels and outcomes has not been well elucidated. In this study, we found a U-shaped association between serum albumin levels and mortality in critically ill children in the pediatric intensive care unit (PICU). Low albumin levels and high albumin levels were associated with mortality. The findings in this study suggest that maintaining the optimal serum albumin levels is important in critically ill patients in the PICU.

Introduction

Serum albumin is the main contributor to intravascular colloid osmotic pressure. The normal range of serum albumin concentrations in healthy subjects is approximately 35–50 g/L. A serum albumin level of less than 35 g/L is considered hypoalbuminemia, which is frequent in hospitalized patients, especially critically ill patients. It has been reported that more than 60% of pediatric patients and nearly 50% of adult patients have hypoalbuminemia in intensive care units (ICUs) (1, 2). Albumin is frequently administered in the ICU to combat low albumin production. However, many albumin prescriptions are thought to be inappropriate, which may result in albumin administration being less cost-effective (3, 4). Determining the detailed association between albumin levels and outcomes could be clinically meaningful. On the one hand, it is impossible to maintain a serum albumin level above 35 g/L throughout the ICU stay. More importantly, this information could help intensivists appropriately administer albumin and reduce albumin abuse.

Serum albumin levels have long been recognized as an indicator of outcomes in hospitalized patients (5–8). A large number of studies have revealed that hypoalbuminemia is associated with adverse outcomes, including increased mortality, readmission, ICU admission, and prolonged hospital stay (1, 2, 8–10). However, the detailed association between albumin levels and outcomes has not been well elucidated. In this study, we aimed to quantify the association between serum albumin levels and mortality in critically ill children in the pediatric intensive care unit (PICU) in detail.

Materials and methods

Study population and setting

This was a retrospective study of critically ill children admitted to four PICUs in three tertiary referral hospitals in China. None of the four PICUs were a neonatal intensive care unit (NICU) and only received very few neonates. All children admitted to the PICUs between January 2015 and October 2020 were included in this study except those discharged from the PICU with uncertain outcomes, those without documented albumin values during the ICU stay, and those older than 18 years. The study was approved by the Ethics Committee of the Central Processing Center (West China Hospital of Sichuan University) (20201113-202022402017). The requirement for informed consent was waived due to the retrospective nature of the study and because the study did not divulge the patients' private information.

The patients' demographics, clinical characteristic data, laboratory values, vital signs upon admission to the ICU, including respiratory rate, blood pressure, heart rate and oxygen saturation, and PICU outcome were extracted from the Electronic Medical Record Systems of the hospitals. Albumin levels on admission, the lowest albumin level during the PICU stay, and the duration with an albumin level below the normal range were collected for analysis. The duration with an albumin level below the normal range was defined as the total number of days with an albumin level below 35 g/L during the PICU stay. It was 0 days for patients with no days with an albumin level below 35 g/L and 1 day for patients with a single day with albumin levels below 35 g/L. For patients with more than 1 day with an albumin level below 35 g/L, the duration was calculated by summing the number of days with an albumin level below 35 g/L. If there were days with albumin levels higher than 35 g/L between two albumin levels below 35 g/L, we separately calculated the durations and then added them together. Data management was performed by PostgreSQL version 11.11 (PostgreSQL Global Development Group).

Statistics

The detailed associations between serum albumin levels and PICU mortality were evaluated with restricted cubic spline curves based on logistic regression models with adjustment for other covariates, including Pediatric Risk of Mortality (PRISM) score, age, race, sex, admission from emergency department, cardiac surgery, oxygen partial pressure (PO_2), partial pressure of carbon dioxide (PCO_2), shock index, respiratory rate (RR), bilirubin, estimated glomerular filtration rate (eGFR) being calculated according to Schwartz equation (11), mechanical ventilation, inotropes, and acute kidney injury (AKI) development (The baseline creatinine levels were the creatinine values on admission). The number of knots was set as 4 (0.05, 0.35, 0.65, 0.95) because 4 knots not only provide sufficient fit of the model but are a good compromise between flexibility and overfitting (12). Stratified analyses also used the same number of knots for comparison of overall and stratified analyses. The albumin levels not associated with mortality were defined as the albumin concentrations at which

the 95% confidence interval (CI) of the odds ratio (OR) for mortality included a value of 1.0 in the cubic spline curves. Threshold effect analysis was performed using two piecewise regression models.

Continuous variables with normal distributions are presented as the means ± standard deviations (SDs). Non-normally distributed variables were described as medians [interquartile ranges (IQRs)]. Categorical variables were expressed as counts (percentages). $P < 0.05$ was considered statistically significant. All statistical analyses were performed in R version 3.6.1.

Results

Patient characteristics

A total of 9,671 pediatric patients were admitted to the PICU during the study period. Five hundred forty-eight patients were excluded for the following reasons: 235 patients had no albumin values during the ICU stay; 131 children had unknown outcomes; and 192 patients were older than 18 years. Finally, 9,123 patients were included in the analysis. Table 1 lists the characteristics of the patients by albumin centile subgroups according to the knots. In the overall population, the median age of the pediatric population was 1.0 (IQR, 0.0–4.0) years. The ratio of males was 54.7%. The mean albumin level was 38.2 ± 5.5 g/L. The most frequent reasons for PICU admission were respiratory disorders, followed by cardiovascular and digestive disorders. The median length of ICU stay was 3.0 (IQR, 1.0–6.0) days. The overall hospital mortality rate was 5.3%. The mortality of children with the lowest albumin level was 18.5%, which was the highest among the five categories.

Association between serum albumin levels and mortality

The association between serum albumin levels and the risk of mortality was U-shaped (Figure 1). Low and high albumin levels were both associated with the risk of mortality. In the unadjusted cubic spline, albumin levels between 35.9 g/L (OR = 1.101; 95% CI: 0.998–1.215) and 43.2 g/L (OR = 1.139; 95% CI: 0.993–41.306) were not associated with mortality (Figure 1A). In multivariable adjusted analyses, the association between serum albumin levels and the risk of mortality still followed a U-shape (Figure 1B). Albumin levels between 35.8 g/L (OR = 1.196; 95% CI: 0.993–1.440) and 50.1 g/L (OR = 1.731; 95% CI: 0.999–3.0) were not associated with mortality in the adjusted cubic spline (Figure 1B). The albumin level associated with the lowest risk of mortality in multivariable analyses was 40.6 g/L. The lowest albumin levels also had a U-shaped association with the risk of mortality in the unadjusted model (Figure 2A) but not in the adjusted model (Figure 2B). In addition, the risk of mortality increased with increasing duration of albumin levels below 35 g/L (Figure 3).

Threshold effect analysis

In the threshold effect analysis using two piecewise regression models, the turning points were 43.2 g/L in multivariable analyses and 35.8 g/L in univariable analysis (Table 2). In multivariable analyses, the risk of mortality was significantly decreased with the increment of albumin level (OR = 0.919; 95% CI: 0.886–0.954) in children with a serum albumin level < 43.2 g/L and increased with the increment of serum albumin level (OR = 1.174; 95% CI: 1.044–1.316) in children with a serum albumin level ≥ 43.2 g/L. In the univariable analysis, albumin level also showed a negative association with the risk of mortality (OR = 0.841; 95% CI: 0.815–0.867) in children with a serum albumin level < 35.8 g/L and a positive association with risk of mortality (OR = 1.038; 95% CI: 1.038–1.069) in children with a serum albumin level ≥ 35.8 g/L.

Subgroup analysis

Serum albumin concentrations had a U-shaped association with the risk of mortality in male patients and a J-shaped association with the risk of mortality in female patients (Figure 4). In the children who received cardiac surgery, the 95% CI included an OR of 1.0 at any concentration of albumin (Figure 5A). In the patients who underwent non-cardiac surgery, the association between serum albumin and the risk of mortality followed a U-shape (Figure 5B).

Discussion

In this study of 9,123 critically ill patients admitted to the PICU, we evaluated the association in detail between serum albumin concentrations and mortality by using restricted cubic spline curves based on a logistic regression model. We found a U-shaped association between serum albumin levels and the risk of mortality in critically ill children. Low and high albumin levels were both associated with a higher risk of mortality.

Serum albumin has been found to be a predictor of clinical outcomes in the last century (7, 13). A lower serum albumin concentration is inversely related to the risk of mortality. For each 25 g/L decrement in serum albumin concentration, the risk of mortality was estimated to increase as high as 56% (13). In addition to mortality, albumin levels have also been revealed to be associated with morbidities, such as sepsis and major infections (7). In a large prospective study, a decrease in albumin levels of 25 g/L was associated with an increase in morbidity

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TABLE 1 Baseline characteristics of the children.

	Albumin (g/L)					
	1st–5th <29.5 g/L *N* = 448	**6th–35th 29.5–36.3 g/L *N* = 2,745**	**36th–65th 36.3–40.0 g/L *N* = 2,697**	**66th–95th 40.0–47.2 g/L *N* = 2,763**	**96th–100th ≥47.2 g/L *N* = 470**	**All *N* = 9,123**
Albumin, g/L	26.0 (3.6)	33.6 (1.8)	38.1 (1.0)	42.6 (1.9)	49.9 (2.4)	38.2 (5.5)
Age, years	1 (0,6)	1 (0,4)	1 (0,4)	1 (0,4)	0 (0,2)	1 (0,4)
Albumin infusion, *n* (%)	146 (32.5%)	654 (23.8%)	467 (17.3%)	675 (24.4%)	96 (20.4%)	2,038 (22.3%)
Male, *n* (%)	250 (55.8%)	1,500 (54.6%)	1,476 (54.7%)	1,492 (54%)	271 (57.6%)	4,989 (54.7%)
Race, *n* (%)						
Han	335 (74.8%)	2,342 (85.3%)	2,375 (88.1%)	2,433 (88.1%)	400 (85.1%)	7,885 (86.4%)
Tibet	53 (11.8%)	199 (7.2%)	145 (5.4%)	158 (5.7%)	32 (6.8%)	587 (6.4%)
Yi	48 (10.7%)	130 (4.7%)	97 (3.6%)	114 (4.1%)	23 (4.9%)	412 (4.5%)
Other	12 (2.7%)	74 (2.7%)	80 (3.0%)	58 (2.1%)	15 (3.2%)	239 (2.6%)
Primary reason for PICU admission, *n* (%)						
Respiratory	54 (12.0%)	909 (33.1%)	1,253 (46.5%)	1,143 (41.4%)	157 (33.4%)	3,516 (38.5%)
Cardiovascular	87 (19.4%)	581 (21.2%)	449 (16.6%)	527 (19.1%)	108 (23.1%)	1,752 (19.2%)
Digestive	112 (24.9%)	423 (15.4%)	372 (13.7%)	421 (15.3%)	96 (20.5%)	1,424 (15.6%)
Neurological	26 (5.8%)	196 (7.1%)	181 (6.7%)	178 (6.4%)	26 (5.5%)	607 (6.7%)
Malignant	60 (13.4%)	280 (10.2%)	195 (7.2%)	223 (8.1%)	23 (4.9%)	781 (8.6%)
Injury and poisoning	24 (5.3%)	78 (2.8%)	52 (1.9%)	49 (1.8%)	15 (3.2%)	218 (2.4%)
Other	86 (19.1%)	278 (10.1%)	195 (7.2%)	221 (7.9%)	45 (9.6%)	825 (9.1%)
Admission from emergency department	233 (52.0%)	784 (28.6%)	602 (22.3%)	736 (26.7%)	166 (35.3%)	2,521 (27.6%)
Surgical type, *n* (%)						
Non-cardiac surgery	190 (42.4%)	1,104 (40.2%)	1,014 (37.6%)	1,030 (37.3%)	176 (37.4%)	3,512 (38.5%)
Cardiac surgery	31 (6.9%)	471 (17.2%)	619 (22.9%)	588 (21.3%)	107 (22.8%)	1,816 (19.9%)
Non-surgical	227 (50.7%)	1,170 (42.6%)	1,064 (39.5%)	1,145 (41.4%)	187 (39.8%)	3,795 (41.6%)
Shock index	1.2 (0.9, 1.6)	1.2 (1.0, 1.5)	1.2 (1.0, 1.4)	1.2 (0.9, 1.4)	1.4 (1.1, 1.6)	1.2 (1.0, 1.5)
RR,/min	26 (20, 32)	26 (20, 30)	26 (20, 30)	28 (20, 31)	30 (23, 32)	26 (20, 30)
PO_2, mmHg	85.9 (62.0, 100.0)	84.0 (65.4, 100.0)	89.7 (70.6, 102.6)	89.2 (70.0, 100.0)	87.0 (63.4, 100.0)	87.4 (68.2, 100.0)
PCO_2, mmHg	40.1 (13.9)	39.7 (9.8)	40.2 (11.4)	39.6 (8.7)	42.7 (16.7)	40.1 (11.0)
eGFR, ml/min/1.73 m^2	143.2 (82.8)	158.6 (65.0)	162.9 (57.0)	149.6 (58.6)	117.6 (47.4)	154.3 (61.2)
BUN, mmol/L	3.4 (2.3, 5.1)	3.3 (2.3, 4.7)	3.7 (2.7, 5.1)	4.0 (2.9, 5.4)	4.9 (3.6, 7.1)	3.7 (2.6, 5.1)
AKI, *n* (%)	164 (36.5%)	1,006 (36.6%)	1,008 (37.4%)	1,207 (43.7%)	267 (56.8%)	3,652 (40.0%)
Sodium, mmol/L	139.6 (6.6)	139.8 (4.8)	139.8 (4.5)	140.1 (6.1)	140.6 (4.8)	139.9 (4.8)
Hematocrit, %	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.4 (0.1)	0.4 (0.1)	0.3 (0.1)
Bilirubin, μmol/L	7.3 (3.2, 21.6)	8.0 (3.6, 16.2)	8.7 (4.3, 15.7)	9.7 (4.4, 18.1)	8.6 (4.3, 17.7)	8.6 (4.1, 16.9)
Inotropes, *n* (%)	18 (4.0%)	122 (4.4%)	110 (4.1%)	146 (5.3%)	44 (9.4%)	440 (4.8%)
Mechanical ventilation, *n* (%)	120 (26.7%)	713 (26.0%)	653 (24.2%)	662 (24.0%)	133 (28.3%)	2,281 (25.0%)
PRISM score	5.3 (3.5)	4.5 (2.4)	4.5 (2.4)	4.6 (2.6)	4.9 (3.2)	4.6 (2.6)
Mortality, *n* (%)	83 (18.5%)	145 (5.3%)	113 (4.2%)	106 (3.8%)	38 (8.1%)	485 (5.3%)
LOS, days	2 (1, 3)	3 (1, 5)	3 (1, 6)	4 (2, 8)	7 (4, 12)	3 (1, 6)

Values are presented as the mean (standard deviation), median (interquartile range) or number (%). PICU, Pediatric Intensive Care Unit; RR, Respiratory Rate; PO_2, Oxygen Partial Pressure; PCO_2, Partial Pressure of Carbon Dioxide; eGFR, estimated Glomerular Filtration Rate; BUN, Blood Urea Nitrogen; AKI, Acute Kidney Injury; PRISM Pediatric Risk of Mortality Score; LOS, Length Of PICU Stay.

of 55%. Consistently, albumin concentrations have also been shown to be associated with poor clinical outcomes in critically ill children (2, 14). Each increase of 10 g/L in serum albumin in critically ill children is associated with a 73% reduction in the risk of 60-day mortality (hazard ratio = 0.27; 95% CI: 0.14–0.51) (2). In a recent study, albumin levels still showed a negative association with mortality in patients admitted to the PICU (OR = 0.289; 95% CI: 0.136–0.615) (14).

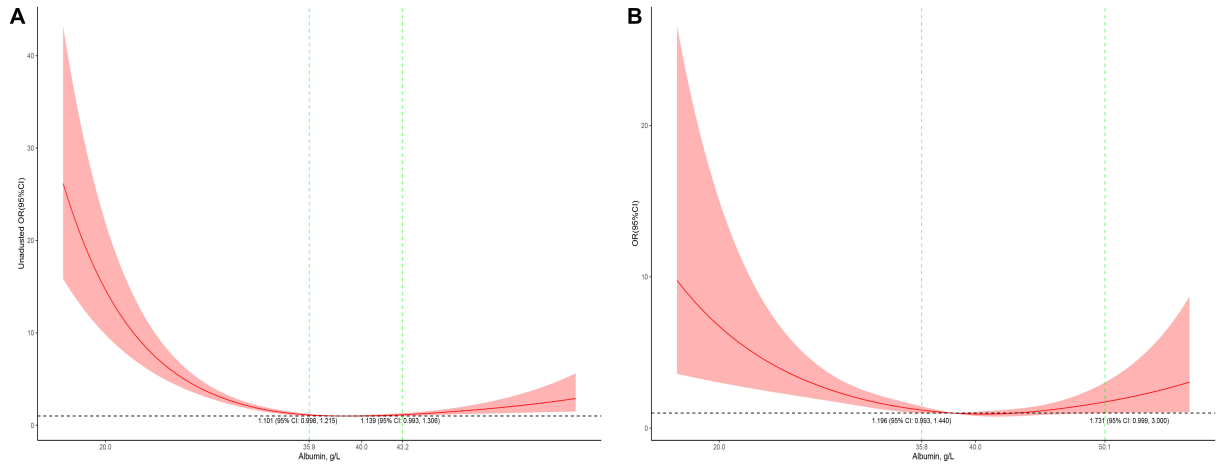

FIGURE 1
Association between serum albumin levels and mortality in all patients (**A**, the crude model; **B,** the model adjusted for age, race, sex, admission form emergency department, cardiac surgery, PO_2, PCO_2, shock index, RR, bilirubin, eGFR, mechanical ventilation, inotropes, AKI, and PRISM). Y-axis present the odds ratio, and the X-axis present albumin values. The 95% CI included an OR of 1.0 at albumin concentrations between 35.9 and 43.2 g/L in the crude model and between 35.9 and 50.1 g/L in the adjusted model.

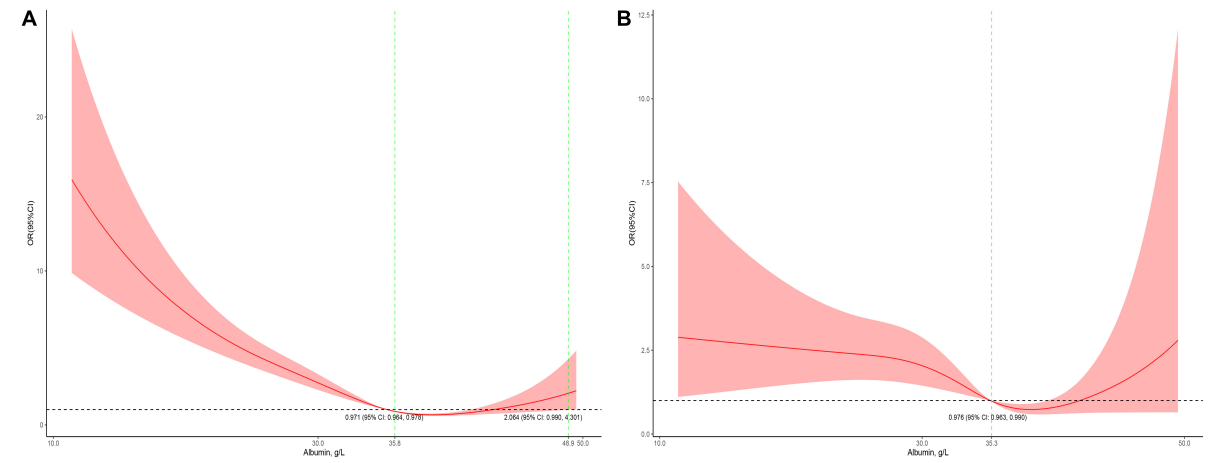

FIGURE 2
Association between the lowest serum albumin levels during the PICU stay and mortality in all patients (**A,** the crude model; **B,** the model adjusted for age, race, sex, admission form emergency department, cardiac surgery, PO_2, PCO_2, shock index, RR, bilirubin, eGFR, mechanical ventilation, inotropes, AKI, and PRISM). The 95% CI included an OR of 1.0 at albumin concentrations between 35.8 and 48.9 g/L in the crude model and > 35.3 in the adjusted model.

In the current study, as expected, we also found an association between low serum albumin levels and a higher risk of mortality in critically ill children. In patients with serum albumin levels < 43.2 g/L, each increase of 1 g/L in serum albumin was associated with an 8.1% reduction in the risk of PICU mortality. This finding was similar to that of a previous study in critically ill children, in which a 7.3% reduction in the risk of mortality was associated with an increase of 1 g/L in serum albumin (hazard ratio = 0.27; 95% CI: 0.14–0.51) (2). In critically ill adult patients, each 1 g/L increase in serum albumin also resulted in a reduction in mortality of more than 60% (OR = 0.383; 95% CI: 0.198–0.740) (15).

What was new in this study was that high serum albumin also showed an association with mortality. There was a U-shaped association between serum albumin levels and the risk of mortality in critically ill children. To the best of our knowledge, there is no evidence that a high serum albumin level is associated with mortality in critically ill children. However, there was a study revealing a U-shaped association between clinical outcomes and children with end-stage renal

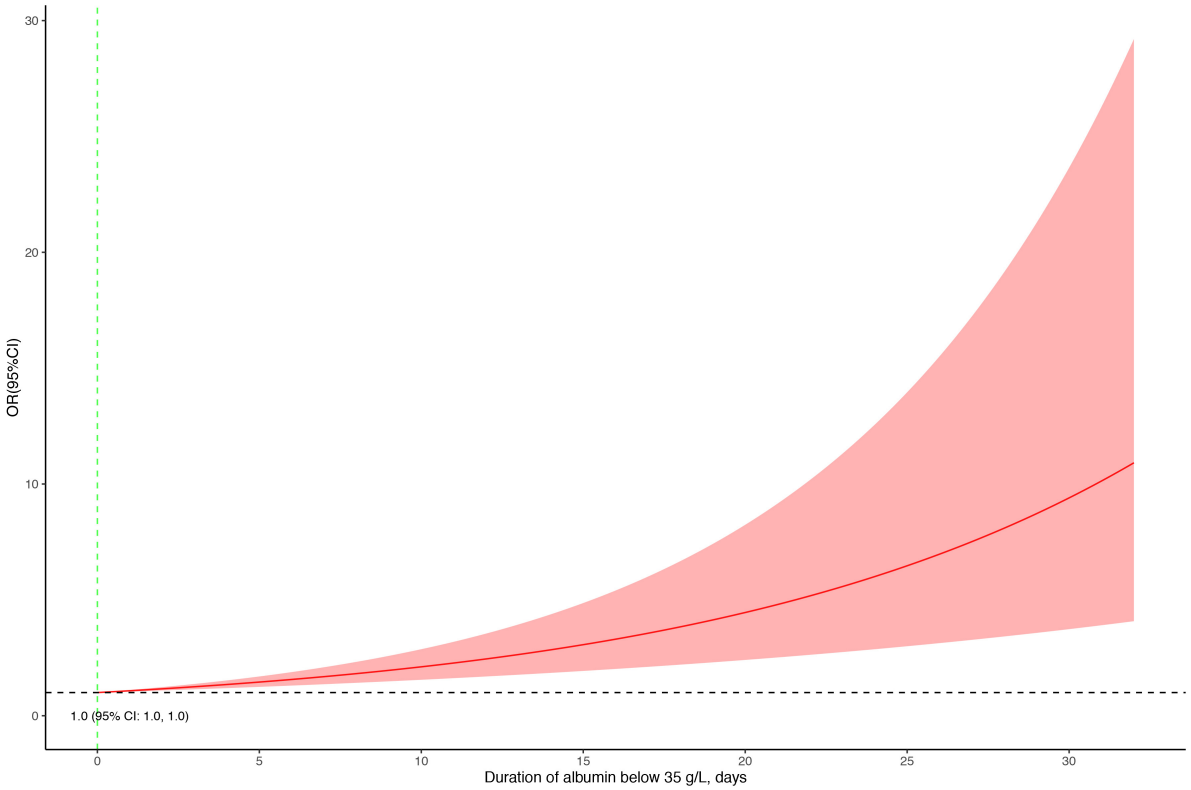

FIGURE 3
Association between the duration with an albumin level below 35 g/L and the risk of mortality.

TABLE 2 Threshold effect analyses of serum albumin levels on mortality using two piecewise regression models.

	Adjusted			Crude	
Turnpoint	**OR (95% CI)**	**P**	**Turnpoint**	**OR (95% CI)**	***P***
<43.2 g/L	0.919 (0.886, 0.954)	<0.001	<35.8 g/L	0.841 (0.815, 0.867)	<0.001
≥43.2 g/L	1.174 (1.044, 1.316)	<0.01	≥35.8 g/L	1.038 (1.007, 1.069)	<0.05

OR, odds ratio; CI, confidence interval.

disease (16). In the study, serum albumin levels < 35 g/L and ≥ 45 g/L were both associated with hospitalization frequency and hospitalization days. Similar findings were also found in adult patients. In a retrospective study, by stratifying patients into groups according to serum albumin levels, the investigators revealed that the risk of hospital-acquired AKI was related to a low albumin level (≤ 24 g/L) (OR = 1.52; 95% CI: 1.18–1.94) and a high albumin level (≥ 45 g/L) (OR = 2.16; 95% CI: 1.74–2.96) (17). In a recent study among adult patients with hypertension, a U-shaped association between serum albumin levels and chronic kidney disease was also revealed by using cubic spline curves (18). In the study, albumin levels < 51.4 g/L and ≥ 51.4 showed opposite associations with outcomes in patients (OR = 0.92; 95% CI: 0.88–0.96 vs. OR = 1.06; 95% CI: 1.01–1.11). The underlying mechanism of the association between high albumin levels and poor clinical outcomes is not fully understood. One of the potential mechanisms might be dehydration, which is common in sick young children. Studies have reported that high serum albumin levels are mostly caused by dehydration (19, 20). In the current study, the patients with the highest serum albumin levels had relatively higher blood urea nitrogen (BUN), hematocrit (HCT%) and serum sodium levels as well as a higher shock index but a lower estimated glomerular filtration rate (eGFR) (**Table 1**), which is a sign of dehydration. However, further studies are needed to understand the potential mechanism because high albumin levels are generally regarded as normal.

In children receiving cardiac surgery, hypoalbuminemia is associated with an increased length of hospital stay and mortality (21, 22). However, in this study, the serum albumin

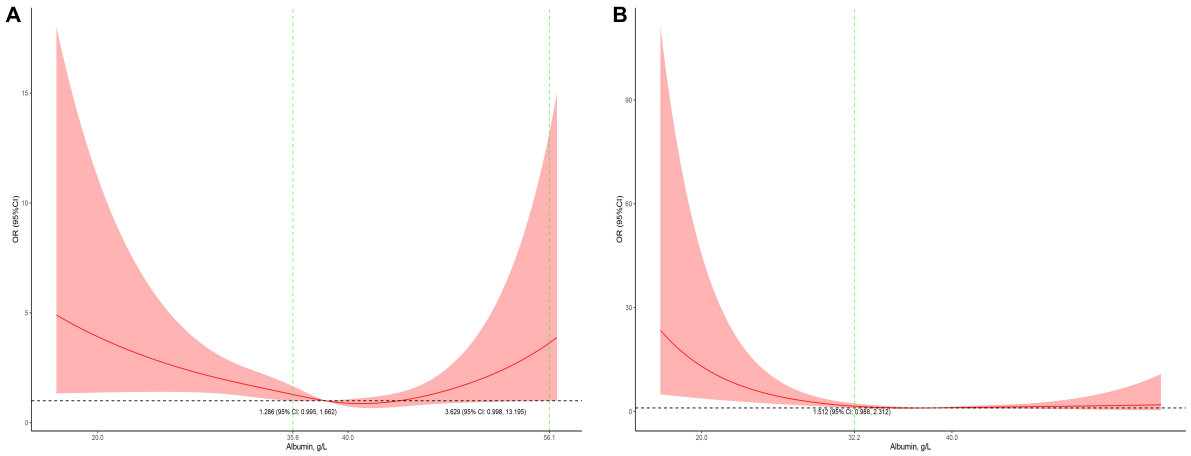

FIGURE 4
Stratified analysis for the association between serum albumin levels and mortality (**A,** subgroup analysis in male patients; **B**, subgroup analysis in female patients). The models were adjusted for age, race, admission form emergency department, cardiac surgery, PO_2, PCO_2, shock index, RR, bilirubin, eGFR, mechanical ventilation, inotropes, AKI, and PRISM.

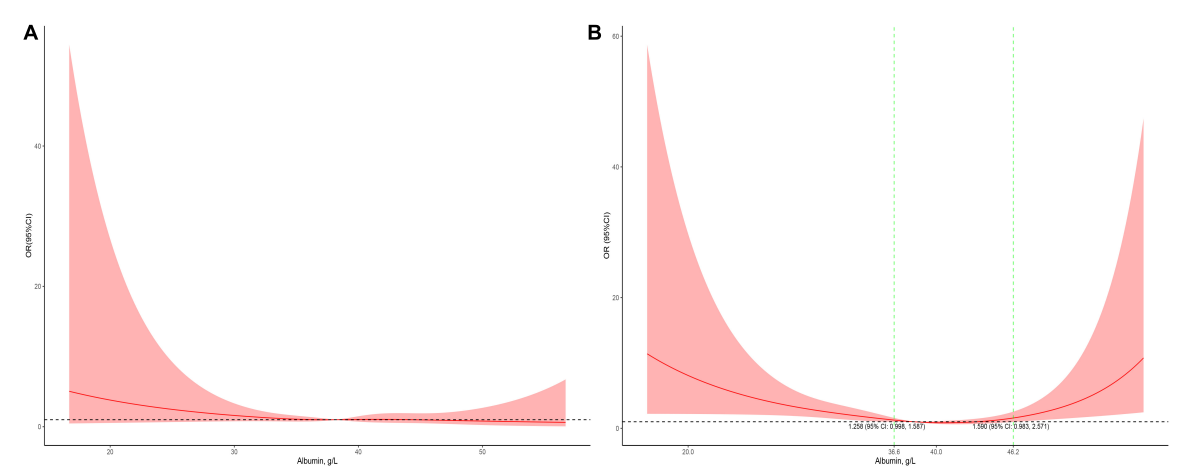

FIGURE 5
Stratified analysis for the association between serum albumin levels and mortality in surgical patients (**A,** subgroup analysis in cardiac surgery patients; **B,** subgroup analysis in non-cardiac surgery patients). The models were adjusted for age, race, sex, admission form emergency department, PO_2, PCO_2, shock index, RR, bilirubin, eGFR, mechanical ventilation, inotropes, AKI, and PRISM.

levels did not appear to be not associated with mortality in children receiving cardiac surgery. The most likely explanation may be that the number of patients with an albumin level < 29.5 g/L was too small. Only 31 patients undergoing cardiac surgery had albumin levels < 29.5 g/L, which may be too small to reveal statistical significance. Unlike the subgroup analysis in cardiac surgical patients, serum albumin levels showed the same U-shaped association with mortality in non-cardiac surgical patients.

In the adjusted analysis in the overall population, albumin levels between 35.8 and 50.1 g/L were not associated with mortality. This range might be clinically meaningful because maintaining the optimal serum albumin level is important in critically ill children in the PICU. Male patients had an approximate albumin level range between 35.6 and 56.1 g/L. The range in non-cardiac surgical patients was 36.6–46.2 g/L, narrower than those in the overall population.

This study has several limitations. First, although we included nearly all patients with documented albumin test results in this study, incomplete data were inevitable due to the inherent limitations of retrospective studies. Second, the dataset used for analysis came from large tertiary referral centers. The study design may limit the generalizability of our results to other non-tertiary centers and may introduce referral bias because of increased disease severity. Furthermore, we failed to reveal an association between serum albumin levels and

mortality in children receiving cardiac surgery. This result should be cautiously treated because of the small sample of cardiac surgery patients with low albumin levels.

Conclusion

In this study, we found a U-shaped association between serum albumin levels and mortality in critically ill children in the PICU. Both low and high albumin levels were associated with mortality. Serum albumin levels between 35.8 and 50.1 g/L were identified as the range not related to mortality in critically ill children. The range may suggest that maintaining optimal serum albumin levels is important in critically ill patients in the PICU.

Data availability statement

The datasets used for the analysis in the current study are not in a public repository. However, the datasets are available from the corresponding author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the West China Hospital of Sichuan University. Written informed consent for participation was not provided by the participants' legal guardians/next of kin because: The requirement for informed consent was not required due to the retrospective nature of the study and containing no individual information.

Author contributions

XPZ, LZ, CW, SC, and YJ: concept and design. XPZ, LZ, CW, LF, GZ, KY, JZ, and RW: acquisition, analysis, and interpretation of data. XPZ, LZ, JY, and YZ: statistical analysis. LF, JY, and XG: administrative, technical, or material support. XPZ, CW, and LZ: drafting of the manuscript. SC and YJ: revision of the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. van Beek DEC, Königs MHH, Kuijpers YAM, van der Horst ICC, Scheeren TWL. Predictive value of serum albumin levels on noradrenaline and fluid requirements in the first 24 h after admission to the intensive care unit – a prospective observational study. *J Crit Care.* (2018) 47:99–103. doi: 10.1016/j.jcrc.2018.06.011

2. Leite HP, Rodrigues da Silva AV, de Oliveira Iglesias SB, Koch Nogueira PC. Serum albumin is an independent predictor of clinical outcomes in critically Ill children. *Pediatr Crit Care.* (2016) 17:e50–7. doi: 10.1097/pcc.0000000000000596

3. Caraceni P, Domenicali M, Tovoli A, Napoli L, Ricci CS, Tufoni M, et al. Clinical indications for the albumin use: still a controversial issue. *Eur J Intern Med.* (2013) 24:721–8. doi: 10.1016/j.ejim.2013.05.015

4. Lyu PF, Hockenberry JM, Gaydos LM, Howard DH, Buchman TG, Murphy DJ. Impact of a sequential intervention on albumin utilization in critical care. *Crit Care Med.* (2016) 44:1307–13. doi: 10.1097/ccm.0000000000001638

5. Reinhardt GF, Myscofski JW, Wilkens DB, Dobrin PB, Mangan JE Jr, Stannard RT. Incidence and mortality of hypoalbuminemic patients in hospitalized veterans. *JPEN J Parenter Enteral Nutr.* (1980) 4:357–9. doi: 10.1177/0148607180004 00404

6. Ferguson RP, O'Connor P, Crabtree B, Batchelor A, Mitchell J, Coppola D. Serum albumin and prealbumin as predictors of clinical outcomes of hospitalized elderly nursing home residents. *J Am Geriatr Soc.* (1993) 41:545–9. doi: 10.1111/j. 1532-5415.1993.tb01893.x

7. Gibbs J, Cull W, Henderson W, Daley J, Hur K, Khuri SF. Preoperative serum albumin level as a predictor of operative mortality and morbidity: results from the national VA surgical risk study. *Arch Surg.* (1999) 134:36–42. doi: 10.1001/archsurg. 134.1.36

8. Viasus D, Garcia-Vidal C, Simonetti A, Manresa F, Dorca J, Gudiol F, et al. Prognostic value of serum albumin levels in hospitalized adults with community-acquired pneumonia. *J Infect.* (2013) 66:415–23. doi: 10.1016/j.jinf.2012.12.007

9. Herrmann FR, Safran C, Levkoff SE, Minaker KL. Serum albumin level on admission as a predictor of death, length of stay, and readmission. *Arch Intern Med.* (1992) 152:125–30.

10. Rich MW, Keller AJ, Schechtman KB, Marshall WG Jr, Kouchoukos NT. Increased complications and prolonged hospital stay in elderly cardiac surgical patients with low serum albumin. *Am J Cardiol.* (1989) 63:714–8. doi: 10.1016/ 0002-9149(89)90257-9

11. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics.* (1976) 58:259–63.

12. Harrell FE. *Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis*. Berlin: Springer (2015).

13. Goldwasser P, Feldman J. Association of serum albumin and mortality risk. *J Clin Epidemiol.* (1997) 50:693–703. doi: 10.1016/s0895-4356(97)00015-2

14. Bekhit OE, Yousef RM, Abdelrasol HA, Mohammed MA. Serum albumin level as a predictor of outcome in patients admitted to pediatric intensive care units. *Pediatr Emerg Care.* (2021) 37:e855–60. doi: 10.1097/pec.00000000000 02567

15. Ramesh VJ, Umamaheswara Rao GS, Kandavel T, Kumaraswamy SD, Iyyamanda UB, Chandramouli BA. Predictive model for survival among neurosurgical intensive care patients. *J Neurosurg Anesthesiol.* (2011) 23:183–7. doi: 10.1097/ANA.0b013e31821cb9ec

16. Okuda Y, Obi Y, Streja E, Laster M, Rhee C, Langman CB, et al. Serum albumin and hospitalization among pediatric patients with end-stage renal disease who started dialysis therapy. *Pediatr Nephrol.* (2019) 34:1799–809. doi: 10.1007/ s00467-019-04270-2

17. Thongprayoon C, Cheungpasitporn W, Mao MA, Sakhuja A, Kashani K. U-shape association of serum albumin level and acute kidney injury risk in hospitalized patients. *PLoS One.* (2018) 13:e0199153. doi: 10.1371/journal.pone. 0199153

18. Jiang C, Wang B, Li Y, Xie L, Zhang X, Wang J, et al. U-shaped association between serum albumin and development of chronic kidney disease in general hypertensive patients. *Clin Nutr.* (2020) 39:258–64. doi: 10.1016/j.clnu.2019.02.002

19. Wegman DH, Apelqvist J, Bottai M, Ekström U, García-Trabanino R, Glaser J, et al. Intervention to diminish dehydration and kidney damage among sugarcane workers. *Scand J Work Environ Health.* (2018) 44:16–24. doi: 10.5271/sjweh.3659

20. Tanriverdi O. A discussion of serum albumin level in advanced-stage hepatocellular carcinoma: a medical oncologist's perspective. *Med Oncol.* (2014) 31:282. doi: 10.1007/s12032-014-0282-3

21. Henry BM, Borasino S, Ortmann L, Figueroa M, Rahman A, Hock KM, et al. Perioperative serum albumin and its influence on clinical outcomes in neonates and infants undergoing cardiac surgery with cardiopulmonary bypass: a multi-centre retrospective study. *Cardiol Young.* (2019) 29:761–7. doi: 10.1017/ s1047951119000738

22. Leite HP, Fisberg M, de Carvalho WB, de Camargo Carvalho AC. Serum albumin and clinical outcome in pediatric cardiac surgery. *Nutrition.* (2005) 21:553–8. doi: 10.1016/j.nut.2004. 08.026

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Effect of preoperative predigested formula vs. polymeric formula on bowel function recovery after definitive surgery for small intestinal entero-atmospheric fistula in patients with chyme reinfusion

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Purpose: The purpose of this study is to compare the effect of preoperative predigested formula vs. polymeric formula on bowel function recovery following definitive surgery (DS) for small intestinal enteroatmospheric fistula (EAF).

Methods: In this retrospective study, from January 2005 to December 2019, the patients with small intestinal EAF and receiving a DS were enrolled. During the preoperative treatment, each patient received enteral nutrition *via* nasojejunal feeding and chyme reinfusion. The enrolled subjects were classified into two groups, based on their formula type: polymeric formula and predigested formula. Then, propensity scores matching (PSM) was used to further divide these patients into PSM polymeric formula group or PSM predigested formula group. The clinical characteristics of the groups were analyzed.

Result: A total of 137 patients were finally enrolled, with 72 patients in the polymeric formula group and 65 patients in predigested formula group. The postoperative ileus was manifested in a total of 61 (44.5%) cases, with 27 (37.5%) in the polymeric formula group and 34 (52.3%) in the predigested formula group ($P = 0.04$). It was predicted that the polymeric formula could result in a reduction in postoperative ileus (OR = 0.47; 95% CI: 0.21–0.95; $P = 0.04$). After 1:1 PSM, there were 110 patients included. The postoperative ileus was observed in 47 patients, with 18 (32.7%) in the polymeric formula group and 29 (52.7%) in the predigested formula group ($P = 0.03$). After PSM, the polymeric formula demonstrated a reduction in the incidence of postoperative ileus (OR = 0.42; 95% CI: 0.19–0.92; $P = 0.03$).

Conclusion: Compared with predigested formula, the preoperative polymeric formula appears to be associated with earlier recovery of bowel function after DS for EAF.

KEYWORDS

outcomes, surgery, postoperative ileus, polymeric formula, predigested formula

Introduction

Enteroatmospheric fistula (EAF), a particular subset of enterocutaneous fistula (ECF), is defined as communication between the gastrointestinal tract and the atmosphere, without skin or soft tissue surrounding or overlying the opening in the bowel (1). EAF is almost impossible to achieve spontaneous closure (2), and a definitive surgery (DS) with high morbidity is essential (3), in which the incidence of postoperative ileus might be up to 50% (4–6). A typical nutritional strategy consists of enteral nutrition (EN) in conjunction with chyme reinfusion (CR) (5–8).

The predigested formula is more readily absorbed than the polymeric formula, making it easier to achieve nutrition goals when gastrointestinal continuity is established with CR. However, it is reasonable that predigested formula is more fully absorbed in the jejunum, so fewer unabsorbed predigested preparations will reach the ileum than the polymeric formula (9). The essential nutrition for gastrointestinal mucosa stimulates chyme rich in nutrients (10). Consequently, this difference in the absorption rate makes it plausible to hypothesize that this may affect the number of nutrients in the distal chyme, thereby affecting the appearance and function of the terminal small intestinal mucosa, thus impacting the healing time of bowel function after DS.

Methods

Study design

This was a retrospective study performed at two tertiary hospitals with more than 2,500 beds. The institutional review board approved the study. All procedures were performed in compliance with relevant guidelines and regulations. Informed consent was obtained from all individuals.

Grouping

From January 2005 to December 2019, the characteristics of patients receiving a DS for small intestinal EAF were reviewed. Before the DS, EN was given *via* nasojejunal tube during the entire treatment process, for which the energy was calculated according to 30 kcal/kg.

Before 2012, CR was not extensively employed. As a result, predigested formula (Peptison Liquid, Nutricia, Wuxi, China) was used until DS due to the characteristics of easy absorption. After 2012, because of the widespread use of CR, polymeric formula (Nutrison Fiber, Nutricia, Wuxi, China) was widely used.

According to the type of formula used, patients were divided into polymeric formula group and predigested formula group (Figure 1). Then, propensity scores matching (PSM) was used to further divide these patients into PSM polymeric formula group or PSM predigested formula group. There was a thorough examination of the groups' clinical features (Figure 1).

Excluded criterion

The excluded criterion were as follows: (1) patients ≤17-year-old; (2) patients with concurrent upper gastrointestinal fistula, colon fistula, pancreatic fistula, or pancreatitis, which may influence the difficulty of the operation; (3) patients with EN providing <60% of the nutritional needs; (4) patients with inflammatory bowel disease (IBD); and (5) patients without complete data.

Patients were followed up to discharge. The primary outcome was postoperative ileus. The secondary outcomes were as follows: (1) defecation time and (2) duration from initial postoperative resume of EN to total EN.

Treatment of EAF and DS

The treatment of EAF followed the SOWATS treatment guidelines, consisting of the following components: sepsis, optimization of nutritional state, wound care, anatomy (of the leakage), the timing of surgery, and surgical strategy (11). Additionally, the temporary abdominal closure technique (TAC) was used to protect the exposed intestine until a frozen abdomen formed. After forming the frozen abdomen, a planned abdominal hernia was designed *via* stamp skin grafting from the patient's head.

At least 3 months after grafting, DS for EAF was performed. The following are the conclusive surgical criteria. First, C-reactive protein (CRP), white blood cell (WBC), and procalcitonin (PCT) are kept regular for more than 1 month. Second, BMI ≥ 18.0 and normal physical strength are maintained. Third, hemoglobin ≥ 110 g/L. Finally, the interval exceeds 4 months after the first time getting discharged from our institution (5).

Our chief surgeon, Dr. Yunzhao Zhao, MD and Ph.D., performed the DS. During the DS, a lateral-lateral end anastomosis was performed in each fistula using a linear stapler (Pride Medical Inc., Jingjiang, Taizhou, Jiangsu, China). In addition, serosa and muscularis injuries were sutured using a 4–0 absorbable band (Vicryl Plus, Ethicon, Inc., TX, USA). Before anastomosis, the digestive tract was gradually dissociated. In all cases, intra-intestinal splinting was carried out before abdominal closure. In addition to the closure of the fistula(s), hernia repair was also performed for each patient during the DS. Besides, component separation technology was applied and onlay mesh repair was carried out. A Cook Biodesign advanced tissue repair device (Cook

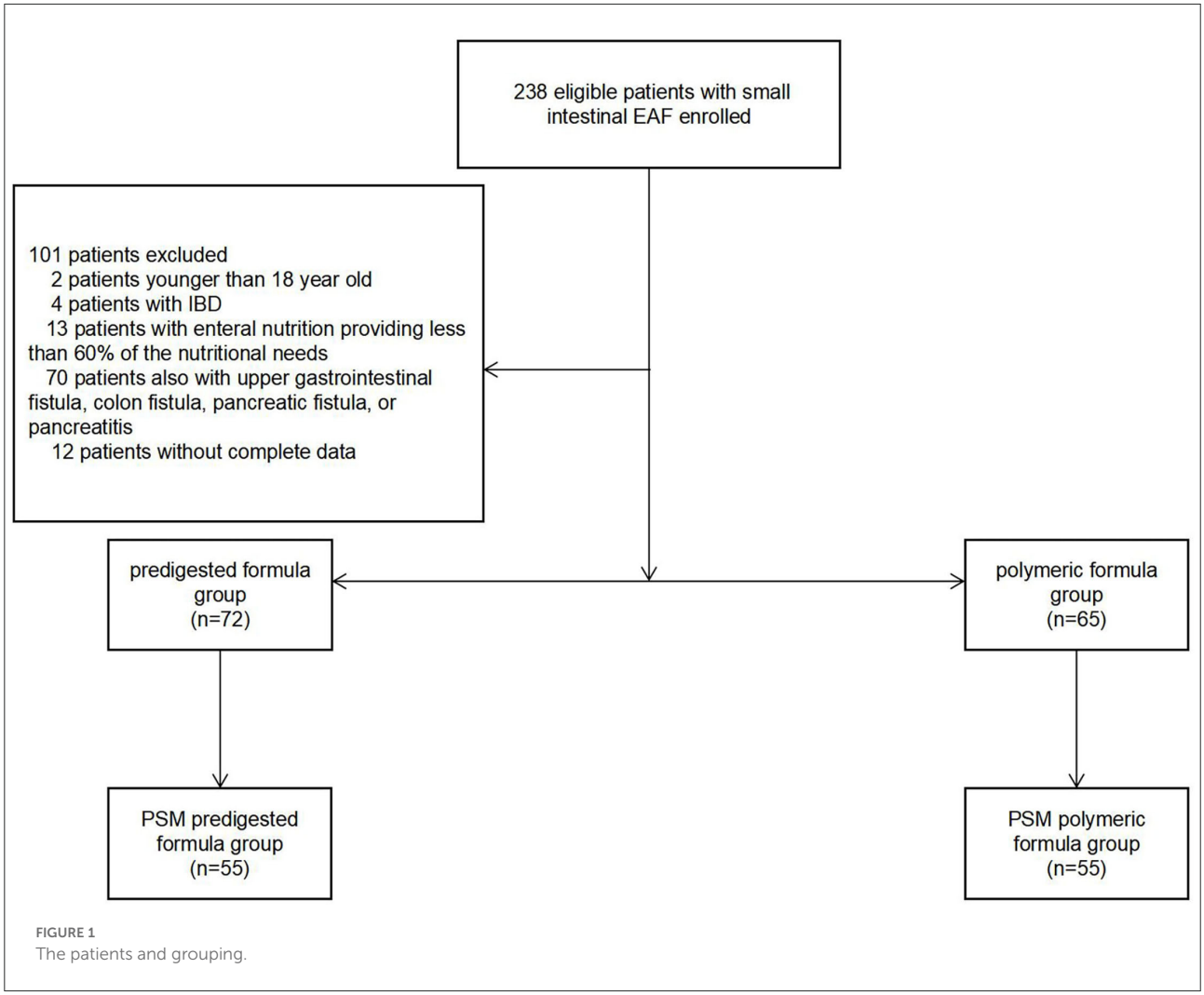

FIGURE 1
The patients and grouping.

Medical Inc., Bloomington, IN, USA) was employed in this process. Negative pressure drainage took place under all incisions. During the postoperative treatment, RBC and human serum Alb were used to maintain patients with hemoglobin (Hb) >100 g/L and/or albumin (Alb) >30 g/L, respectively.

Perioperative treatment

The preoperative discussion focused on the possible difficulties that may arise during the operation and potential solutions were performed 2 days before each operation. Because the adhesions might be severe and cause excessive bleeding during the operation, at 6 p.m. the day before DS, EN was stopped, and preoperative bowel preparation with sodium phosphate was performed. An additional 1,500 ml of intravenous crystalloid was infused overnight to prevent dehydration. At 6 a.m. on the day of DS, a nasogastric tube for decompression was placed. Second-generation cephalosporin was used within 30 min of the DS as a preoperative prophylactic antimicrobial.

After postoperative defecation, the EN with predigested formula began to resume. During the process, minimal EN with a speed of 20 ml/h was initially used. Provided the patient had no gastrointestinal symptoms, such as abdominal distension and diarrhea, the speed of EN was progressively raised from 20 to 80 ml/h over 6 days at a rate of 10 ml/h per day. If gastrointestinal symptoms existed, the increase of EN was slowed down to accommodate the patient's circumstances. Due to the prolonged absence of a regular oral diet, postoperative continuous and stable EN was performed for about 1 month. The liquid diet was gradually resumed. During this process, the necessary intravenous fluids were used to maintain electrolyte balance.

TABLE 1 Patients characteristics.

Clinical variables	Polymeric formulas group ($n = 72$)	Predigested formula group ($n = 65$)	*P*	PSM polymeric formulas group ($n = 55$)	PSM predigested formula group ($n = 55$)	*P*
Demographic data						
Female, No. (%)	39 (54.2)	32 (49.2)	0.69	30 (54.5)	29 (52.7)	0.85
Age, years; (median,IQR)	45.0 (34.0–54.75)	42.0 (37.0–51.0)	0.98	43.0 (32.0–54.0)	41.0 (37.0–48.0)	0.36
BMI, kg/m^2, (median,IQR)	19.0 (18.3–20.1)	19.2 (18.5–20.2)	0.17	19.0 (18.3–20.1)	19.4 (18.5–20.3)	0.99
Time period, No. (%)			-			-
Year 2005–Year 2008	-	30 (21.9)		-	26 (23.6)	
Year 2008–Year 2011	-	35 (25.5)		-	29 (26.4)	
Year 2012–Year 2015	32 (23.4)	-		25 (22.7)	-	
Year 2016–Year 2019	40 (29.2)	-		30 (27.3)	-	
Fistula characteristics						
Interval from fistula occurred to admission, day, (median,IQR)	15 (12–19)	16 (14–19)	0.26	15 (13–21)	16 (14–19)	0.89
Distance from treitz ligament to fistula, No. (%)			0.78			0.78
<100 cm	25 (34.8)	22 (33.8)		17 (30.9)	18 (32.7)	
From 100 to 200 cm	33 (45.8)	33 (50.8)		25 (45.5)	27 (49.1)	
>200 cm	14 (19.4)	10 (15.4)		13 (23.6)	10 (18.2)	
Length of small intestine, No. (%)			0.58			0.65
<300 cm	17 (23.6)	18 (27.7)		14 (25.5)	12 (21.8)	
≥300 cm	55 (76.4)	47 (72.3)		41 (74.5)	43 (78.2)	
Duration of CR, month (median, IQR)	5 (4–6)	5(4–6)	0.78	5 (4–6)	5(4–6)	0.89
Duration of enteral nutrition, month (median, IQR)	5 (4–6)	5(4–6)	0.78			
Duration of usage of polymeric formulas, month (median, IQR)	4 (3–4)	-	-	4 (3–4)	-	-
High output, No. (%)	69 (95.8)	63 (96.9)	1.00	65 (100)	65 (100)	1.00
The area of planed ventral hernia, No. (%)			0.81			0.0.59
<50 cm^2	7 (9.7)	8 (12.3)		7 (12.7)	5 (9.1)	
≥50 and<100cm^2	48 (66.7)	44 (67.7)		34 (61.8)	39 (70.9)	
≥100 cm^2	17 (23.6)	13 (20.0)		14 (25.5)	11 (20.0)	
Etiology, No. (%)			0.48			0.61
Trauma	49 (68.1)	43 (66.2)		35 (63.6)	37 (67.3)	

(Continued)

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TABLE 1 (Continued)

Clinical variables	Polymeric formulas group ($n = 72$)	Predigested formula group ($n = 65$)	*P*	PSM polymeric formulas group ($n = 55$)	PSM predigested formula group ($n = 55$)	*P*
Unexplained perforation	2 (2.8)	1 (1.5)		2 (3.6)	1 (1.8)	
Adhesive obstruction	14 (19.4)	18 (27.7)		13 (23.6)	15 (27.3)	
Mesenteric thrombosis	7 (9.7)	3 (4.2)		5 (9.1)	2 (3.6)	
Characteristics related to DS						
Hemoglobin before DS, No. (%)			0.81			0.65
<120 g/L	19 (26.4)	16 (24.6)		12 (21.8)	14 (25.5)	
≥120 g/L	53 (73.6)	49 (75.4)		43 (78.2)	41 (74.5)	
Albumin before DS, No. (%)			0.56			0.84
<35 g/L	20 (27.8)	21 (32.3)		17 (30.9)	18 (32.7)	
≥35 g/L	52 (72.2)	44 (67.7)		38 (69.1)	37 (67.3)	
Grade of abdominal adhesion, No. (%)			0.17			0.83
≤IV	28 (38.9)	18 (27.7)		16 (29.1)	15 (27.3)	
V	47 (65.3)	44 (67.7)		39 (79.9)	40 (72.3)	
Duration of DS, No. (%)			0.15			0.84
<4 h	33 (45.8)	22 (33.8)		21 (38.2)	20 (36.4)	
≥4 h	39 (54.2)	43 (66.2)		34 (61.8)	35 (63.6)	
Bleeding loss during DS, No. (%)			0.65			0.57
<1,000 ml	12 (16.7)	9 (13.8)		8 (14.5)	6 (10.9)	
≥1,000 ml	60 (83.3)	56 (86.2)		47 (85.5)	49 (89.1)	
The amount of red blood cell suspension input during DS and 48 h after DS*, No. (%)			0.57			0.69
<10 U	30 (41.7)	24 (36.9)		18 (32.8)	20 (36.4)	
≥10 U	42 (58.3)	41 (63.1)		37 (67.3)	35 (63.6)	
The amount of albumin input during DS and 48 h after DS**, No. (%)			0.15			0.84
<100 g	32 (44.4)	21 (32.3)		20 (36.4)	21 (38.2)	
≥100 g	40 (55.6)	44 (67.9)		35 (63.6)	34 (61.8)	

*In order to maintain the Hemoglobin >100 g/L within 48 h after definitive surgery.
**In order to maintain the Albumin >30 g/L within 48 h after definitive surgery.

10.3389/fnut.2022.923191

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TABLE 2 Logistics regression analysis of the risk factors for defecation before PSM.

Clinical variables	Unadjusted regression			Adjusted regression		
	OR	95% CI	*P*	OR	95% CI	*P*
Female	0.69	0.35–1.38	0.30			
Polymeric formulas	0.49	0.24–0.96	0.04	0.47	0.21–0.95	0.04
Age	1.02	0.99–1.05	0.25			
BMI	0.94	0.73–1.20	0.61			
Time period						
Year 2005–Year 2008	Ref					
Year 2008–Year 2011	0.93	0.35–2.46	0.88			
Year 2012–Year 2015	0.53	0.19–1.45	0.21			
Year 2016–Year 2019	0.53	0.20–1.37	0.19			
Interval from fistula occurred to admission	0.99	0.93–1.07	0.97			
Distance from treitz ligament to fistula						
<100 cm	Ref					
From 100 to 200 cm	0.79	0.38–2.09	0.56			
>200 cm	0.525	0.20–1.28	0.18			
Length of small intestine						
<300 cm	Ref					
≥300 cm	1.93	0.86–4.37	0.11			
Duration of CR	1.11	0.67–2.19	0.21			
Duration of enteral nutrition	1.81	0.72–3.47	0.36			
Hight output	1.25	0.67–2.33	0.49			
The area of planed ventral hernia						
<50 cm^2	Ref					
≥50 and<100 cm^2	1.47	0.46–4.65	0.51			
≥100 cm^2	2.00	0.55–7.27	0.29			
Etiology, No. (%)						
Trauma	Ref					
Unexplained perforation	0.68	0.06–7.76	0.76			
Adhesive obstruction	1.54	0.69–3.46	0.29			
Mesenteric thrombosis	0.34	0.07–1.69	0.19			
Hemoglobin before DS						
<120 g/L	Ref					
≥120 g/L	0.54	0.25–1.18	0.12			
Albumin before DS						
<35 g/L	Ref					
≥35 g/L	0.83	0.39–1.73	0.61			
Grade of abdominal adhesion						
≤IV	Ref			Ref		
V	1.94	0.92–4.06	0.08	0.62	0.44–0.88	0.009
Duration of DS						
<4 h	Ref					
≥4 h	0.66	0.33–1.32	0.244			
Bleeding loss during DS						
<1,000 ml	Ref					
≥1,000 ml	2.10	0.76–5.80	0.15			

(Continued)

TABLE 2 (Continued)

Clinical variables	Unadjusted regression			Adjusted regression		
	OR	95% CI	*P*	OR	95% CI	*P*
The amount of red blood cell suspension input during DS and 48 h after DS*						
<10 U	Ref			Ref		
≥10 U	2.23	1.09–4.57	0.03	1.67	0.82–4.12	0.18
The amount of albumin input during DS and 48 hours after DS**						
<100 g	Ref			Ref		
≥100 g	2.43	1.17–5.01	0.02	1.88	0.75–4.89	0.27

*In order to maintain the Hemoglobin >100 g/L within 48 h after definitive surgery.
**In order to maintain the Albumin >30 g/L within 48 h after definitive surgery.

Definition, data collection, and statistical analysis

A preliminary assessment consisted of calculating the gender, age, and time interval from occurrence to admission of the EAF, as well as recording the etiology at admission. The preoperative Hemoglobin (Hb), Albumin (Alb), body mass index (BMI), the output of fistula, and the area of planned ventral hernia were estimated within 1 week before DS. As part of the surgical procedure, the length from the Treitz ligament to the location of the fistula, length of the small intestine, degree of abdominal adhesion, duration of DS, and intraoperative blood loss were evaluated. The patient's postoperative course record was evaluated concerning red blood cell (RBC) and Alb transfusions within 48 h after DS.

The degree of abdominal adhesion was primarily classified according to Hobson KG (12), which could be assessed simply according to the operation record and was suitable for retrospective study (Degree I = no adhesions; Degree II = minimal adhesions localized to 1 or 2 areas; Degree III = diffuse adhesions, but not extensive; Degree IV = diffuse extensive adhesions, easily lysed; Degree V = diffuse extensive, dense adhesions, difficult to lyse). Adhesions occupying more than half the surgical field in the abdominal cavity were considered "diffuse extensive" in our study. In cases where the intestines did not have a gap at 50% adhesion sites, adhesive lesions were termed "dense adhesions." If the intestinal damage and bleeding during the dissociation were inevitable, the adhesion was defined as "difficult to lyse." Postoperative ileus was defined as a longer defecation time than 7 days after DS (13).

All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 26.0 for Windows (IBM, Analytics, Armonk, NY). Comparing continuous variables between groups was carried out using Student's *t*-test and a Mann–Whitney U-test. Comparing categorical variables was conducted using Fisher's exact test. We investigated confounding variables *via* multivariate Cox regression and logistic regression. The practice of estimating treatment effects with observational data is reduced with the use of a 1:1 PSM. The patients in the PSM groups were matched based on the calculated propensity scores by a regression model with demographic data, fistula characteristics, and characteristics related to DS. A value of 0.05 was chosen as the match tolerance. Statistical significance was defined as a $P < 0.05$.

Results

Baseline characteristics and population

Our study encompassed 238 patients with small intestinal EAF, who underwent DS from January 2005 to December 2019. There were two patients ≤17-year-old, four patients with IBD, 13 patients with EN providing <60% of the postoperative nutritional needs, 70 patients with concurrent upper gastrointestinal fistula, colon fistula, pancreatic fistula, or pancreatitis, and 12 patients without complete data. A total of 137 patients were finally enrolled in our study. Seventy-two patients were assigned to the polymeric formula group and 65 patients were in predigested formula group. Except for the different time period of treatment, the characteristics were comparable between the two groups (Table 1).

A total of 110 patients were further divided into PSM polymeric formula group ($n = 55$) and PSM predigested formula group ($n = 55$). Difference between the groups in the time period was observed after PSM (Table 1).

Duration of usage of polymeric formula

The median duration of usage of polymeric formula was 4 months (IQR: 3–4 months), while the usage of EN was 5 months (IQR: 4–6 months). Before using the polymeric formula, the predigested formula was used for transition in 56 patients, and

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TABLE 3 Logistics regression analysis of the risk factors for postoperative ileus after PSM.

Clinical variables	Unadjusted regression			Adjusted regression		
	OR	95% CI	*P*	OR	95% CI	*P*
Female	0.94	0.67–1.32	0.72			
Polymeric formulas	0.44	0.20–0.95	0.03	0.42	0.19–0.92	0.03
Age	0.99	0.97–1.00	0.13			
BMI	0.97	0.75–1.26	0.82			
Time period						
Year 2005–Year 2008	Ref					
Year 2008–Year 2011	1.23	0.43–3.56	0.70			
Year 2012–Year 2015	0.56	0.18–1.73	0.32			
Year 2016–Year 2019	0.43	0.14–1.28	0.13			
Interval from fistula occurred to admission	0.97	0.94–1.00	0.14			
Distance from treitz ligament to fistula						
<100 cm	Ref					
From 100 to 200 cm	1.27	0.87–1.85	0.23			
>200 cm	1.02	0.78–2.36	0.46			
Length of small intestine						
<300 cm	Ref					
≥300 cm	0.39	0.28–1.65	0.68			
Duration of CR	1.11	0.67–2.19	0.21			
Duration of enteral nutrition	1.58	0.87–3.09	0.31			
Hight output	1.16	0.58–2.33	0.67			
The area of planed ventral hernia						
<50 cm^2	Ref					
≥50 and<100 cm^2	1.39	0.38–5.06	0.61			
≥100 cm^2	2.17	0.51–9.09	0.29			
Etiology, No. (%)						
Trauma	Ref					
Unexplained perforation	0.74	0.06–8.56	0.81			
Adhesive obstruction	1.71	0.71–4.12	0.23			
Mesenteric thrombosis	0.59	0.11–3.27	0.55			
Hemoglobin before DS						
<120 g/L	Ref					
≥120 g/L	1.03	0.71–1.49	0.89			
Albumin before DS						
<35 g/L	Ref					
≥35 g/L	0.84	0.37–1.88	0.67			
Grade of abdominal adhesion						
≤IV	Ref					
V	1.86	0.77–4.43	0.17			
Duration of DS						
<4 h	Ref					
≥4 h	1.41	0.31–1.47	0.61			
Bleeding loss during DS						
<1,000 ml	Ref					
≥1,000 ml	1.66	0.74–3.79	0.22			
The amount of red blood cell suspension input during DS and 48 h after DS*						
<10 U	Ref			Ref		
≥10 U	2.78	1.17–6.55	0.02	1.87	0.62–4.14	0.38

(Continued)

TABLE 3 (Continued)

Clinical variables	Unadjusted regression			Adjusted regression		
	OR	95% CI	*P*	OR	95% CI	*P*
The amount of albumin input during DS and 48 hours after DS**						
<100 g	Ref			Ref		
≥100 g	2.33	1.03–5.31	0.04	1.75	0.77–3.99	0.18

*In order to maintain the Hemoglobin >100 g/L within 48 h after definitive surgery.
**In order to maintain the Albumin >30 g/L within 48 h after definitive surgery.

the duration of using predigested formula was 1 month (IQR: 1–2 months). There were other 16 patients without transition, and the polymeric formula was used directly.

Postoperative ileus

Before PSM, postoperative ileus occurred in a total of 61 patients (44.5%) with 27 (37.5%) in the polymeric formula group and 34 (52.3%) in the predigested formula group ($P = 0.04$). The adjusted logistics regression indicated that the polymeric formula could result in a reduction in postoperative ileus (OR = 0.47; 95% CI: 0.21–0.95; $P = 0.04$; Table 2). After PSM, there were a total of 47 of the 110 patients with postoperative ileus, 18 (32.7%) in the polymeric formula group and 29 (52.7%) in the predigested formula group ($P = 0.03$). The polymeric formula reduced the incidence of postoperative ileus (OR = 0.42; 95% CI: 0.19–0.92; $P = 0.03$; Table 3).

Defecation time

Before PSM, the defecation time was 7 days (IQR: 6–11 days) in the polymeric formula group and 8 days (IQR: 7–13 days) in the predigested formula group ($P = 0.02$). In the predigested formula group, after DS, there were 27 (41.5%) patients with defecation time more than 10 days, and 5 (7.7%) patients with defecation time more than 20 days. There were 14 (19.4%) patients with defecation time more than 10 days and 4 (5.6%) patients with defecation time more than 20 days (Figure 2A) in the polymeric formula group. The preoperative usage of polymeric formula accelerated postoperative defecation (adjusted HR = 1.72; 95%CI: 1.17–2.26; $P = 0.03$, Figures 3A,B).

After PSM, the defecation time was 7 days (IQR: 6–11 days) in the PSM polymeric formula group and 8 days (IQR: 7–13 days) in the PSM predigested formula group ($P = 0.02$). In the PSM predigested formula group, after DS, there were 23 (41.8%) patients with defecation time more than 10 days and 5 (9.0%) patients with defecation time more than 20 days. There were 11 (20.0%) patients with defecation time more than 10 days and 4 (7.3%) patients with defecation time more than 20 days (Figure 2B) in the PSM polymeric formula group. The preoperative usage of polymeric formula accelerated postoperative defecation (adjusted HR = 1.68; 95%CI: 1.13–2.49; $P = 0.01$, Figures 4A,B).

The resumption from minimal EN to total EN

Before PSM, the median duration of resumption from minimal EN to total EN in the polymeric formula group was 6 days (IQR: 6–6 days), and it was 6 days (IQR: 6–7 days) in the predigested formula group (Figure 5A). Polymeric formula accelerated the resumption process (adjusted HR = 1.32 95%CI: 1.03–3.49; $P = 0.02$). The median duration of resumption from minimal EN to total EN in the PSM polymeric formula group was 6 days (IQR: 6–6 days), and it was 6 days (IQR: 6–7 days) in the PSM predigested formula group (Figure 5B). Polymeric formula also accelerated the resumption process (adjusted HR = 1.44 95%CI: 1.02–3.17.; $P = 0.02$) after PSM.

Discussion

In the present study, we found that postoperative ileus occurred in 45% of patients after DS for EAF, and the preoperative EN with polymeric formula appears to shorten the postoperative defecation time and reduce the incidence of postoperative ileus, although this issue has not been reported by other researchers.

The high incidence of postoperative ileus in patients with EAF was not a particularly innovative discovery. In a previous study including 159 subjects, it was similarly demonstrated that the overall incidence of postoperative ileus after DS for EAF was more than 50% (6). During the DS, severe abdominal adhesion could lead to extensive bowel and substantial blood loss. Those adverse intraoperative events not only exacerbate the postoperative inflammation response (14, 15) but also inhibit adrenergic neural pathways (15), leading to a high incidence of postoperative ileus.

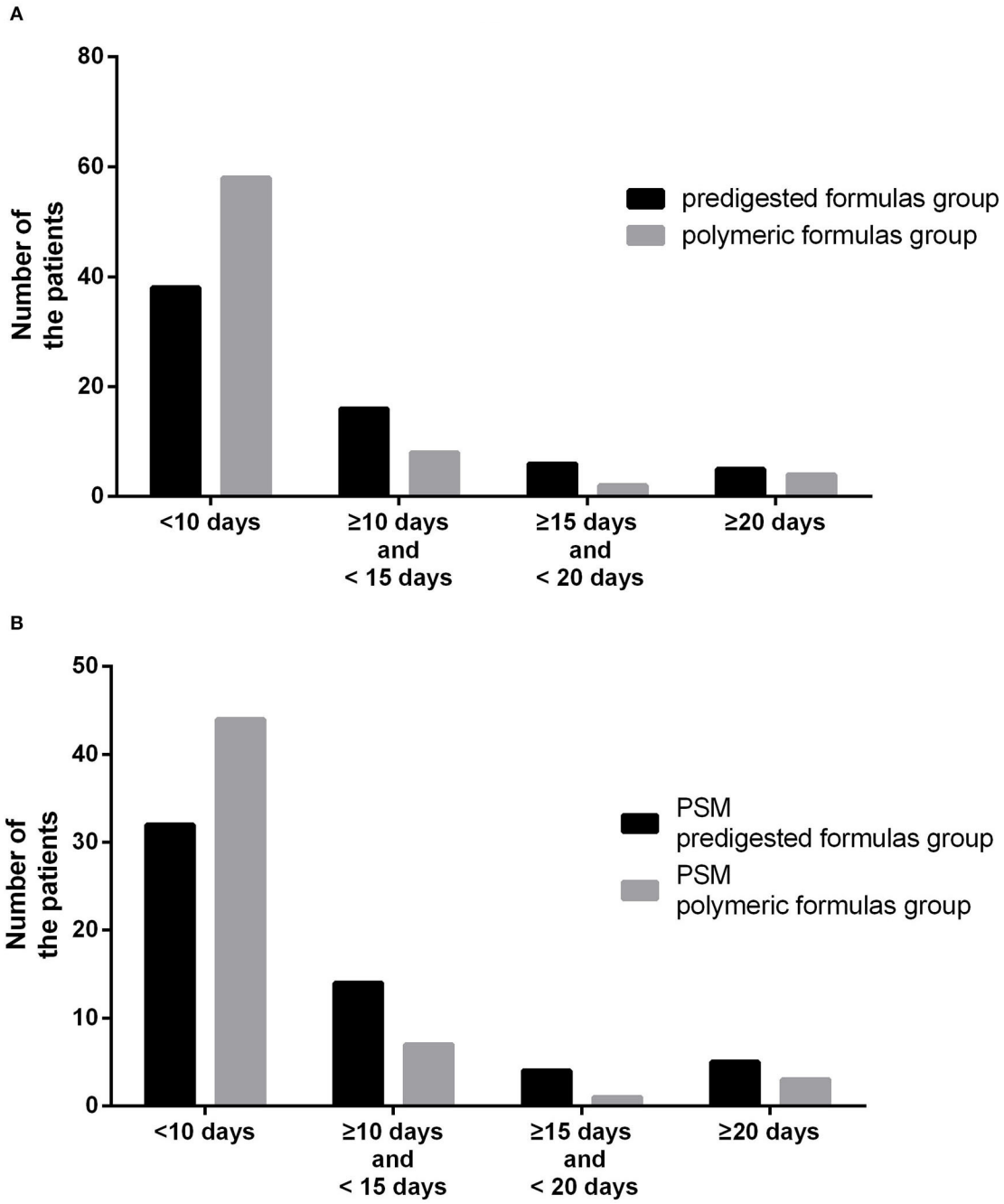

FIGURE 2
(A) Number of patients with different defecation times between the predigested formula group and polymeric formula group. **(B)** Number of patients with different defecation times between the PSM predigested formula group and PSM polymeric formula group.

While ensuring effective CR, the present study has identified that the polymeric formula may minimize the possibility of postoperative ileus compared with predigested formula. Predigested formula is more straightforward and readier assimilated and has enhanced the tolerance. As a result, it has been reported to present nutritional and clinical benefits in nutritionally high-risk non-ICU patients suffering from intestinal failure (16). In patients with EAF, most intestinal juice leaks from the fistula and cannot flow into the distal small intestine of the fistula, resulting in excessive output (5, 17–19). Accordingly, as the predigested formula needs little further intraluminal digestion and is

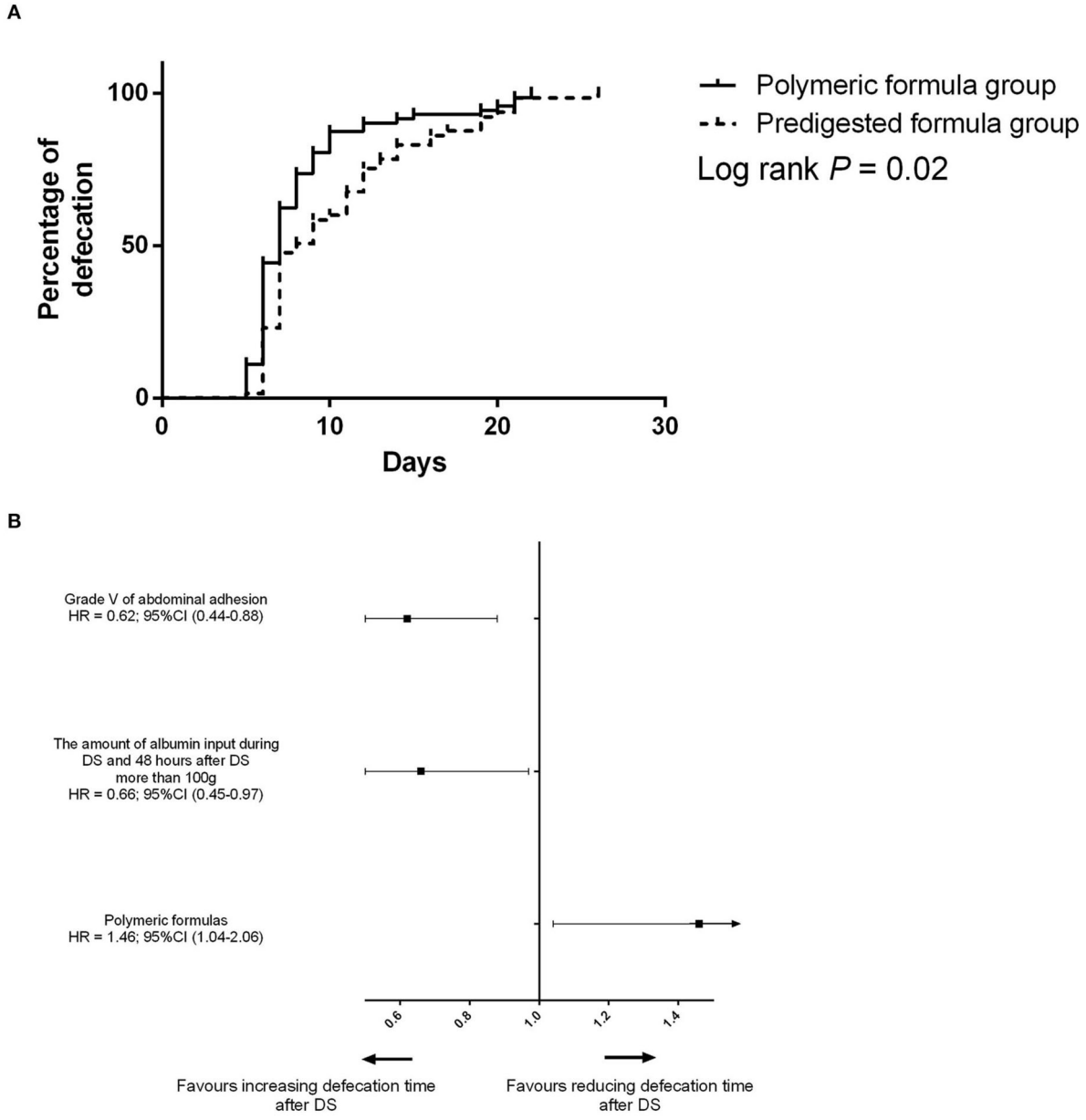

FIGURE 3
(A) Difference of the defecation time between the predigested formula group and polymeric formula group. **(B)** Risk factors for defecation before PSM.

easily absorbed (9), it has been extensively utilized in EAF patients. However, with the same energy supplement, it can be inferred that the use of predigested formula may result in more nutrients being absorbed in the proximal small intestine and lead to decreased nutrient concentration in the distal ileum.

The replacement of gastrointestinal epithelium occurs within a short period, usually between 3 and 6 days (20). This replacement requires the delivery of adequate nutrients, and gastrointestinal epithelial cells must receive their nutritional requirements intraluminally to guarantee replacement. Villous length and crypt depth are decreased due to mucosal atrophy caused by a lack of luminal nutrients (9). These pathophysiologic processes might result in intestinal absorption and mobility insufficiency, contributing to the development of postoperative ileus following intestinal surgery (21). Compared to polymeric formula, the predigested formula may reduce the nourishing

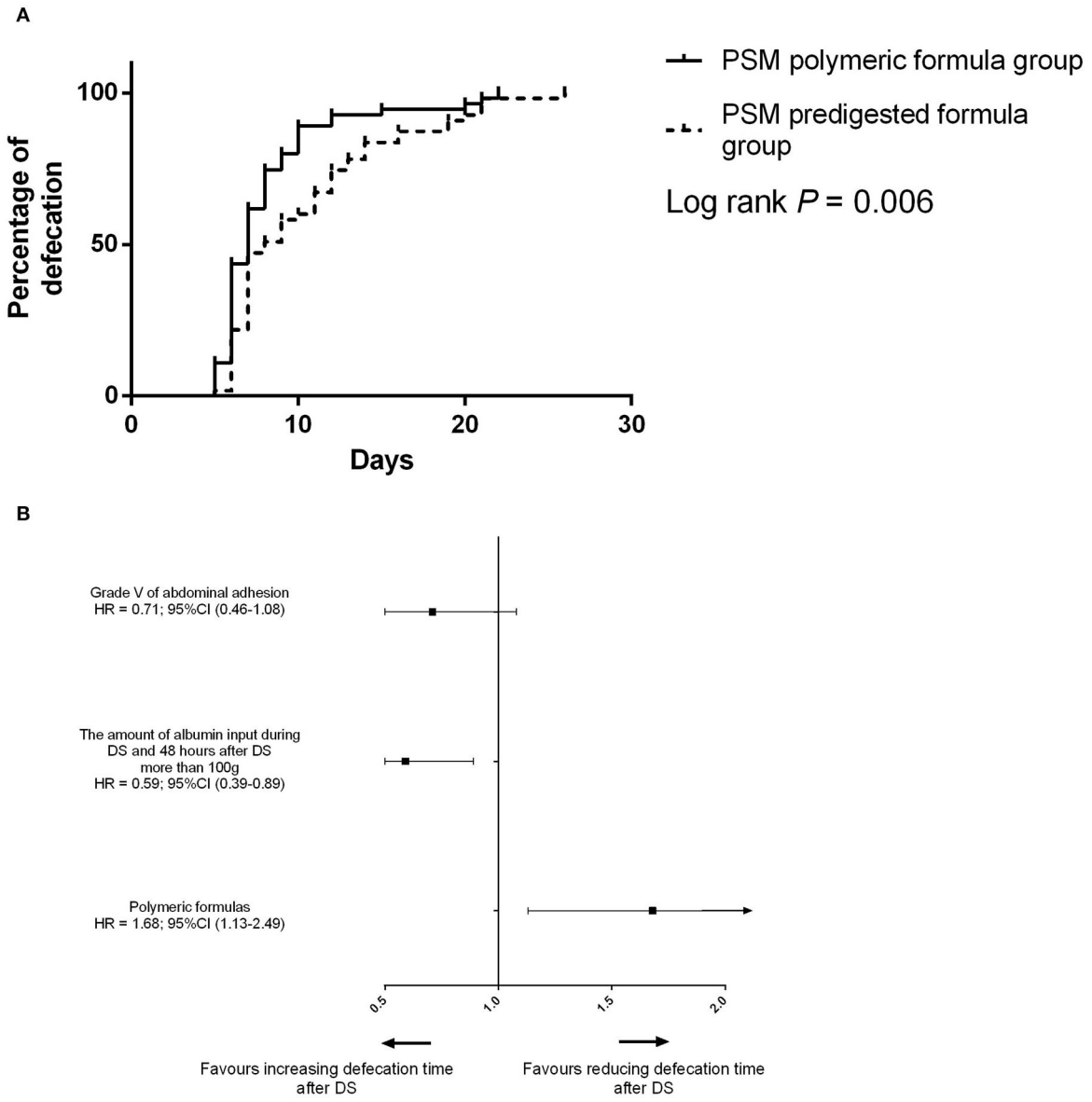

FIGURE 4
(A) Difference of the defecation time between the PSM predigested formula group and PSM polymeric formula group. **(B)** Risk factors for defecation after PSM.

effect of chyme in the digestive tract on the distal ileum, which eventually produces the potential incidence of postoperative ileus.

The problem of postoperative ileus caused by gastrointestinal disuse has been previously reported. Most of those studies focused on the complete disuse of the distal gastrointestinal tract after the temporary stoma (21–24). Unlike these studies, the present study is mainly concerned with the influence of relative disuse on gastrointestinal motility, following different types of EN formula. However, it was revealed that even with the same energy density, the difference in nutrient composition might lead to the relative disuse of distal ileum owing to the difference in absorption and digestion, which then might subsequently contribute to the varied incidence of postoperative ileus.

Our study's protocol of postoperative nutritional support was different from the current feeding strategy for gastrointestinal surgery consisting of early EN or oral administration. Surgery for EAF always has a high incidence

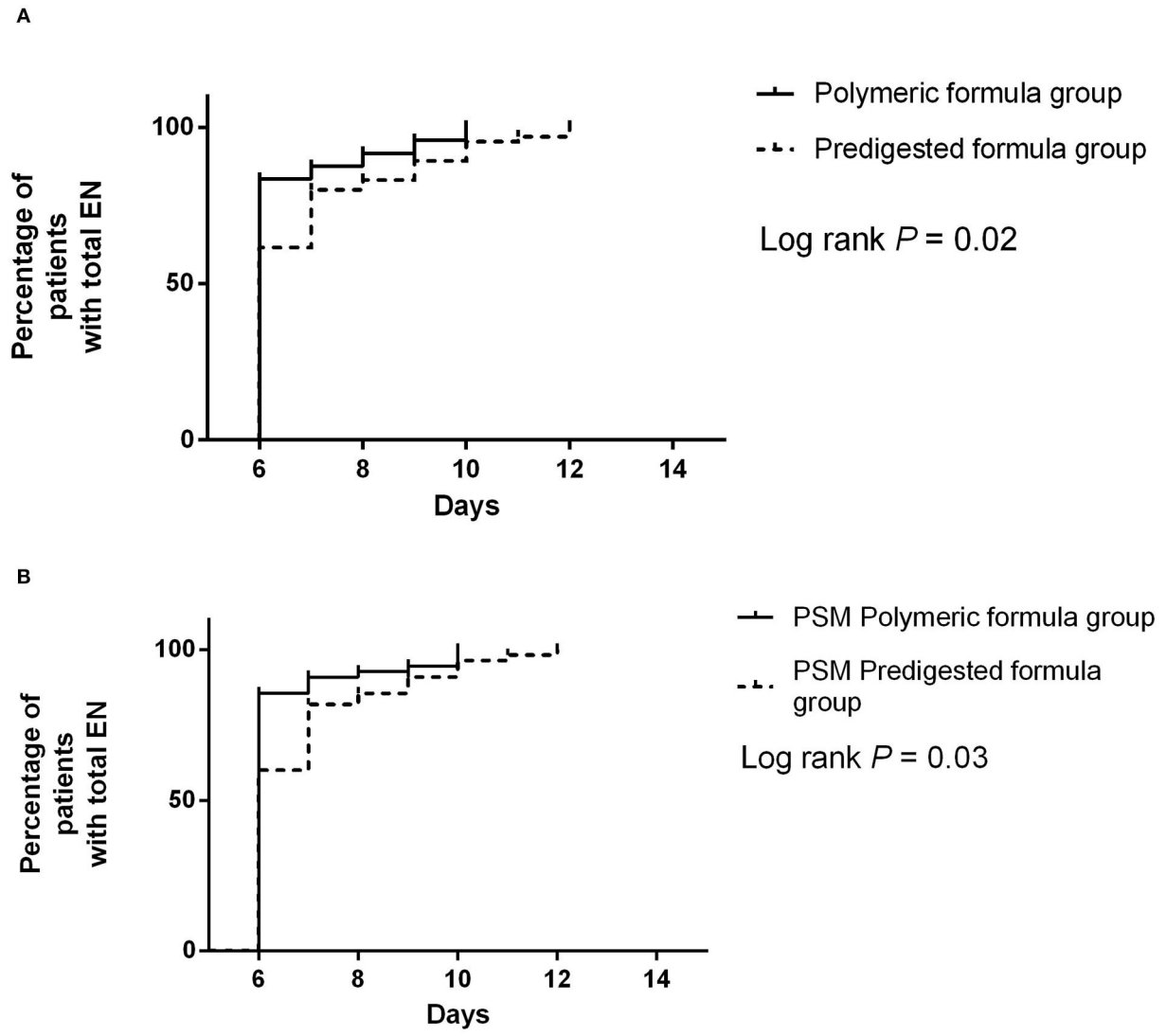

FIGURE 5
(A) Difference of the resumption from minimal EN to total EN between the predigested formula group and polymeric formula group. **(B)** Difference of the resumption from minimal EN to total EN between the PSM predigested formula group and PSM polymeric formula group.

of complications resulting from the sizeable peeling surface, the large amount of oozing blood during the surgery, and the severe postoperative intestinal edema. Oral or EN after defecation is an ancient strategy, but at least it seems safe. So, we still use this strategy when a complicated enterocutaneous fistula surgery is performed. In our study, our procedure for achieving postoperative EN feeding objectives is divided into two phases. The first component is the postoperative defecation time, whereas the second is the postoperative resumption of EN. Indeed, while there was a statistically significant difference in the length from the first postoperative restart of EN, this difference is unlikely to be clinically meaningful given that the median period for both groups was 6 days, with Figures 5A,B demonstrating a slight difference. However, when the defection time following surgery and the fraction of patients in various defecation phases were evaluated, it seemed that intervention with different components of EN solution had some impact on bowel function recovery.

Our research has certain limitations. First, the retrospective nature and the small sample size of this research might account for the deviation. A further randomized controlled study was welcome. However, to our knowledge, EAF is relatively rare, and the number of patients enrolled in our study might be the most so far. In addition, there seemed to be few studies focused on the association between the different types of EN formula and postoperative ileus. The second limitation was that in our study,

besides the different nitrogen sources, there are also disparities between the content of fat and saccharides in the two nutrient formulas. As a result, a further study employing a module diet might better distinguish which nutrients play a crucial role in gastrointestinal mucosal nutrition. The third limitation was that the morphological data of terminal ileal cells cannot be collected completely in our investigation. Consequently, comparing the morphology of terminal ileal mucosa in patients with different nutrient formulas was challenging. In fact, during the DS, when anastomosis was performed after the fistula was excised, we observed that, in the predigested formula group, the closer the fistula is to the terminal ileum, the smaller the diameter of the bowel (not shown in our study). However, this phenomenon is not apparent in the polymeric formula group. Additionally, the majority of patients in the polymeric formula group also received the predigested formula as a transition. It might result in a diminished impact of polymeric formula on reducing the postoperative ileus. Another limitation was that a possible bias existed introduced by the fact that the study cohorts were at differing time periods.

Conclusion

This study has demonstrated that compared with predigested formula, the preoperative polymeric formula appears to be associated with earlier recovery of bowel function after DS for EAF.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Jinling Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

RZ and WT provide research objects. XX and RZ collected and analyzed the data. ZY, XX, and WT wrote the main manuscript text. XX prepared figures and revised the manuscript. ZY designed the research. SL organized data. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Majercik S, Kinikini M, White T. Enteroatmospheric Fistula: from soup to nuts. *Nutr Clin Pract.* (2012) 27:507–12. doi: 10.1177/0884533612444541

2. Di Saverio S, Tarasconi A, Inaba K, Navsaria P, Coccolini F, Costa Navarro D, et al. Open abdomen with concomitant enteroatmospheric fistula: attempt to rationalize the approach to a surgical nightmare and proposal of a clinical algorithm. *J Am Coll Surg.* (2015) 220:e23–33. doi: 10.1016/j.jamcollsurg.2014.11.020

3. Marinis A, Gkiokas G, Argyra E, Fragulidis G, Polymeneas G, Voros D. "Enteroatmospheric Fistulae"–gastrointestinal openings in the open abdomen: a review and recent proposal of a surgical technique. *Scand J Surg.* (2013) 102:61–8. doi: 10.1177/1457496913482252

4. Sriussadaporn S, Sriussadaporn S, Kritayakirana K, Pak-art R. Operative management of small bowel fistulae associated with open abdomen. *Asian J Surg.* (2006) 29:1–7. doi: 10.1016/S1015-9584(09)60284-0

5. Liao Y, Tao S, Yao Z, Tian W, Xu X, Zhao R, et al. Chyme reinfusion improved outcomes after definitive surgery for small-intestinal enteroatmospheric fistula in patients with enteral nutrition. *Nutr Clin Pract.* (2022) 37:634–44. doi: 10.1002/ncp.10823

6. Tian W, Zhao R, Xu X, Zhao Y, Luo S, Tao S, et al. Chyme reinfusion reducing the postoperative complications after definitive surgery for small intestinal enteroatmospheric fistula: a cohort study. *Front Nutr.* (2022) 9:708534. doi: 10.3389/fnut.2022.708534

7. Kumpf VJ, de Aguilar-Nascimento JE, Diaz-Pizarro Graf JI, Hall AM, McKeever L, Steiger E, et al. ASPEN-FELANPE clinical guidelines. *JPEN J Parenter Enteral Nutr.* (2017) 41:104–12. doi: 10.1177/0148607116680792

8. Tang QQ, Hong ZW, Ren HJ, Wu L, Wang GF, Gu GS, et al. Nutritional management of patients with enterocutaneous fistulas: practice and progression. *Front Nutr.* (2020) 7:564379. doi: 10.3389/fnut.2020.564379

9. Koopman R, Crombach N, Gijsen AP, Walrand S, Fauquant J, Kies AK, et al. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *Am J Clin Nutr.* (2009) 90:106–15. doi: 10.3945/ajcn.2009.27474

10. Schörghuber M, Fruhwald S. Effects of enteral nutrition on gastrointestinal function in patients who are critically ill. *Lancet Gastroenterol Hepatol.* (2018) 3:281–7. doi: 10.1016/S2468-1253(18)30036-0

11. Visschers RG, Olde Damink SW, Winkens B, Soeters PB, van Gemert WG. Treatment strategies in 135 consecutive patients with enterocutaneous fistulas. *World J Surg.* (2008) 32:445–53. doi: 10.1007/s00268-007-9371-1

12. Hobson KG, DeWing M, Ho HS, Wolfe BM, Cho K, Greenhalgh DG. Expression of transforming growth factor beta1 in patients with and without previous abdominal surgery. *Arch Surg.* (2003) 138:1249–52. doi: 10.1001/archsurg.138.11.1249

13. Tian W, Yan M, Xu X, Yao Z, Zhao R. Risk factors and outcomes for postoperative ileus after small intestinal fistula excision in patients with diffuse extensive abdominal adhesions. *Front Surg.* (2021) 8:632241. doi: 10.3389/fsurg.2021.632241

14. Boelens PG, Heesakkers FF, Luyer MD, van Barneveld KW, de Hingh IH, Nieuwenhuijzen GA, et al. Reduction of postoperative ileus by early enteral nutrition in patients undergoing major rectal surgery: prospective, randomized, controlled trial. *Ann Surg.* (2014) 259:649–55. doi: 10.1097/SLA.0000000000000288

15. Wehner S, Behrendt FF, Lyutenski BN, Lysson M, Bauer AJ, Hirner A, et al. Inhibition of macrophage function prevents intestinal inflammation and postoperative ileus in rodents. *Gut.* (2007) 56:176–85. doi: 10.1136/gut.2005.089615

16. Carteron L, Samain E, Winiszewski H, Blasco G, Balon AS, Gilli C, et al. Semi-elemental versus polymeric formula for enteral nutrition in brain-injured critically ill patients: a randomized trial. *Crit Care.* (2021) 25:31. doi: 10.1186/s13054-020-03456-7

17. Ekelund KM, Ekblad E. Structural, neuronal, and functional adaptive changes in atrophic rat ileum. *Gut.* (1999) 45:236–45. doi: 10.1136/gut.45.2.236

18. Williams L, Armstrong MJ, Finan P, Sagar P, Burke D. The effect of faecal diversion on human ileum. *Gut.* (2007) 56:796–801. doi: 10.1136/gut.2006.102046

19. Miedema BW, Karlstrom L, Hanson RB, Johnson GP, Kelly KA. Absorption and motility of the bypassed human ileum. *Dis Colon Rectum.* (1990) 33:829–35. doi: 10.1007/BF02051917

20. Williamson RC. Intestinal adaptation (first of two parts). Structural, functional and cytokinetic changes. *N Engl J Med.* (1978) 298:1393–402. doi: 10.1056/NEJM197806222982505

21. Duan M, Cao L, Gao L, Gong J, Li Y, Zhu W. Chyme reinfusion is associated with lower rate of postoperative ileus in Crohn's disease patients after stoma closure. *Dig Dis Sci.* (2020) 65:243–9. doi: 10.1007/s10620-019-05753-w

22. Abrisqueta J, Abellan I, Luján J, Hernández Q, Parrilla P. Stimulation of the efferent limb before ileostomy closure: a randomized clinical trial. *Dis Colon Rectum.* (2014) 57:1391–6. doi: 10.1097/DCR.0000000000000237

23. Danielsen AK, Park J, Jansen JE, Bock D, Skullman S, Wedin A, et al. Early closure of a temporary ileostomy in patients with rectal cancer: a multicenter randomized controlled trial. *Ann Surg.* (2017) 265:284–90. doi: 10.1097/SLA.0000000000001829

24. Picot D, Layec S, Dussaulx L, Trivin F, Thibault R. Chyme reinfusion in patients with intestinal failure due to temporary double enterostomy: a 15-year prospective cohort in a referral centre. *Clin Nutr.* (2017) 36:593–600. doi: 10.1016/j.clnu.2016.04.020

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Association between glucosamine use and cancer mortality: A large prospective cohort study

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Objective: Previous studies have shown anti-cancer and anti-inflammatory benefits of glucosamine. This study was performed to prospectively evaluate the association between glucosamine supplementation and the mortality of multiple cancers based on the UK Biobank cohort study.

Materials and methods: A total of 453,645 participants aged 38–73 who had no cancer at baseline were recruited between 2006 and 2010 and followed until March 2021. We used cox and poission proportional hazards models to explore the association between habitual use of glucosamine and cancer mortality. Subgroup analyses were conducted to understand the potential effect modifications of demographics, lifestyle factors, and health outcomes. Sensitivity analyses were performed to determine the robustness of the results.

Results: Of the participants, 88,224 (19.4%) reported habitual glucosamine use at baseline. There were 9,366 cancer deaths during a median follow-up of 12.1 years, and we observed a significant association between the use of glucosamine and lower overall cancer mortality (HR = 0.95, 95% CI = 0.90–1.00, $p < 0.05$), kidney cancer (IRR = 0.68, 95% CI = 0.49–0.95, $p < 0.05$), lung cancer mortality (IRR = 0.84, 95% CI = 0.74–0.95, $p < 0.05$), and rectum cancer (IRR = 0.76, 95% CI = 0.59–0.98, $p < 0.05$). Subgroup analysis showed that habitual glucosamine supplementation was correlated with lower overall cancer mortality among participants who were aged $\geq$ 60 years, male, current smoker, without high cholesterol and not obese. Sensitivity analysis showed that the results were stable.

Conclusion: Habitual glucosamine use was significantly related to decreased overall cancer, kidney cancer, lung cancer, and rectum cancer mortality, based on data from the large-scale, nationwide, prospective UK Biobank cohort study.

KEYWORDS

glucosamine, cancer, cohort study, mortality, UK Biobank

Introduction

As a non-mineral and non-vitamin supplement, glucosamine is only available by prescription in European countries. However, in Australia and the United States, glucosamine is available over the counter (1, 2). About one in five adults in Australia and 2.6% adults in the United States regularly take glucosamine supplements (3, 4). Glucosamine is an important component in the synthesis of proteoglycans in the human articular cartilage matrix and is widely used in the treatment of osteoarthritis (5). Additionally, glucosamine can regulate various signaling pathways and play a pharmacological role in multiple diseases, including skin diseases, cancer, bacterial infections, and cardiovascular diseases (1, 6, 7). A previous study showed that glucosamine could inhibit the proliferation of a range of tumor cells by inducing cell cycle arrest and apoptosis in cancer cells (8). Some *in vitro* and *in vivo* studies have shown that glucosamine reduces the production of pro-inflammatory factors by inhibiting the mRNA transcription and/or protein expression of pro-inflammatory factors, thereby exerting anti-inflammatory and tumor suppressor effects (9). Moreover, glucosamine can regulate the activity of various important transcription factors and affect various signal transduction pathways, thereby exerting anti-tumor effects. Additionally, according to a large, prospective cohort study, regular use of glucosamine was related to decreased cancer mortality (10). Furthermore, regular glucosamine supplementation was associated with lower lung cancer mortality in the UK Biobank cohort (11) and this work was conducted to expand the evaluation to all cancers. Currently, evidence on the relationship between glucosamine and different types of cancers remained limited.

Therefore, in the present study, we aimed to understand the association of habitual glucosamine use and cancer mortality using the UK Biobank data. Additionally, the potential effect modifications of certain cancer risk factors were explored.

Materials and methods

Study population

The UK Biobank is one of the largest population studies in the world aimed at improving the prevention, diagnosis, and treatment of various diseases as well as promoting health across society. The UK Biobank data is open and has been used by researchers around the world (12–16). Over half a million participants aged 40–70 from across the UK were included in the UK Biobank between 2006 and 2010. Participants provided detailed self-reported data at baseline through touchscreen questionnaires and oral interviews with trained nurses at the assessment center. Extensive body measurements were also collected.

In this study, a total of 502,407 participants were recruited from the UK Biobank. Participants without information on the use of glucosamine were excluded (n = 4,756). Additionally, we excluded participants with one cancer diagnosis at baseline (n = 26,293) as well as those with multiple cancer diagnoses (n = 17,713). Ultimately, a total of 453,645 participants aged 38–73 were included in this study (**Figure 1**). Written consent was obtained from all participants, and the UK Biobank study was approved by the North West Multi-centre Research Ethics Committee in the United Kingdom.

Glucosamine use and measurement of outcome

Participants were asked whether they regularly took supplements including glucosamine at baseline, and those with "yes" responses were defined as habitual glucosamine users. Incidence and survival time data for tumors and deaths was obtained through links to national registries, where cancer cases were classified *via* the International Classification of Diseases, 10th Revision (ICD-10) codes. Self-reported cancer cases were also validated through interviews with trained nurses. Details on tumor mortality validation are available at https://biobank.ctsu.ox.ac.uk/showcase/label.cgi?id=2000. Participants were followed from baseline until death or 1 March 2021, whichever came first.

Other variables

Variables of interest were noted in this study, including age (in years), gender, ethnic background, average total household income, obesity, physical activity, smoking status, use of alcohol, fruit and vegetable intake, processed meat and red meat intake, supplementation, and drug use (minerals, aspirin, NSAIDS, chondroitin and vitamin). We calculated the body mass index (BMI) as the weight in kilograms (kg) divided by the square of the height in meters (m^2) and the obesity was defined as BMI $\geq$ 30 kg/m^2. According to healthy physical activity recommendations (17), we categorized the enrolled participants into two groups based on the total time spent in moderate physical activity in minutes: $<$150 or $\geq$150 min/week.

Statistical analysis

All the missing covariate values were imputed using multiple imputation with chained equations. Lilliefors tests were conducted to detect whether the data were normally distributed. Continuous variables were indicated as mean $\pm$ standard deviation (SD) for normal distributions and median and IQR for non-normal distributions. Categorical variables were indicated

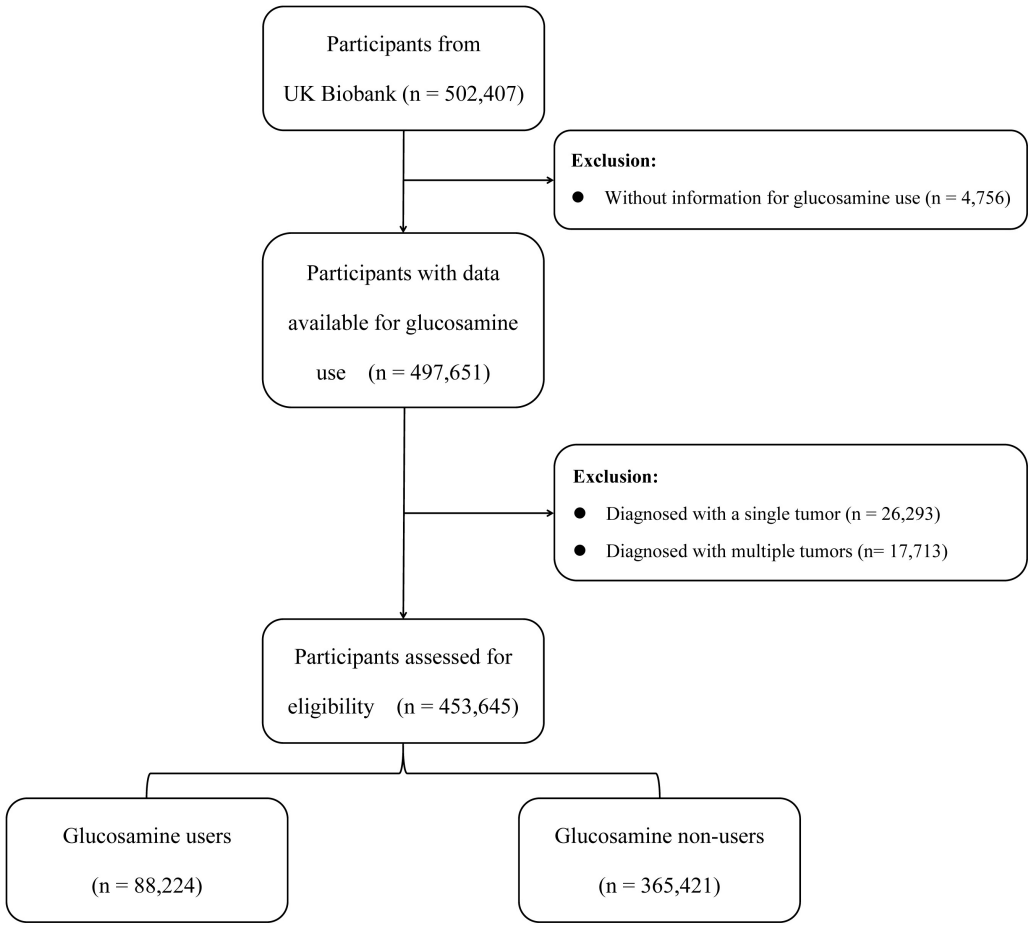

FIGURE 1
Flowchart of the participants selection.

as counts and percentages. Cox proportional hazards models were adopted to show the correlation between glucosamine use and overall cancer mortality using hazard ratios (HR) and 95%. Poission proportional hazards models were used to explore the association of glucosamine use and multiple cancers mortality using incidence rate ratio (IRR) and 95% CI. We constructed two models, the basic model and adjusted model, to evaluate the connection between regular use of glucosamine and cancer mortality. The basic model was adjusted by age (years), gender (male or female), ethnic background (white or others), and average total household income (<£18,000, £18,000–£30,999, £31,000–£51,999, £52,000–£100,000, or >£100,000) to analyze the association between glucosamine use and cancer mortality. The adjusted model was also further adjusted for obesity (BMI < 30 or BMI ≥ 30), physical activity (<150 min/week or ≥150 min/week), current smoking (yes or no), alcohol intake (<1, 1–2, 3–4, or >4 times/week), minerals supplementation (calcium, zinc, iron, and selenium) (yes or no), fruit intake (<2.0, 2.0–3.9, or ≥4.0 pieces/day), vegetable intake (<2.0, 2.0–3.9, or ≥4.0 tablespoons/day), processed meat intake (0, 0–1, or >1 times/week), red meat intake (0, 0–1, or >1 times/week), aspirin use (yes or no), NSAIDS use (yes or no), chondroitin use (yes or no), and vitamin use (yes or no). Li et al. evaluated pack years in this population previously and found that it did not alter the association between glucosamine and lung cancer (11); therefore, we did not include pack year for analysis in this study. All the results were indicated as HR/IRR, 95% CI, and *P*-values.

To reveal the potential effect modifications on the association of habitual glucosamine use and cancer mortality, we conducted several subgroup analyses by age (<60 vs. ≥60 years), ethnic background (white vs. others), gender (males vs. females), smoking (yes vs. no), diabetes (yes vs. no), high cholesterol (yes vs. no), arthritis (yes vs. no), and obesity (yes vs. no).

Additionally, we performed a series of sensitivity analyses to reveal the robustness of our results. Participants with glucosamine supplementation also tended to take other supplements more often than participants without glucosamine supplementation. Therefore, we performed sensitivity analyses by removing participants who used other supplementation. Additionally, we removed these participants with missing data

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TABLE 1 Baseline features for UK Biobank participants by glucosamine use.

Characteristics	Overall	Glucosamine non-user	Glucosamine user
No. of participants	453,645 (100%)	365,421 (80.6%)	88224 (19.4%)
Age (median [IQR])	57.00 [50.00, 63.00]	56.00 [49.00, 62.00]	60.00 [54.00, 64.00]
Female (%)	245,726 (54.2)	190,906 (52.2)	54,820 (62.1)
Ethnic background (%)			
Others	27,058 (6.0)	22,920 (6.3)	4,138 (4.7)
White	426,587 (94.0)	342,501 (93.7)	84,086 (95.3)
Average total household income (£)			
<18,000	104,696 (23.1)	85,619 (23.4)	19,077 (21.6)
18,000–30,999	116,336 (25.6)	91,256 (25.0)	25,080 (28.4)
31,000–51,999	117,769 (26.0)	94,472 (25.9)	23,297 (26.4)
52,000–100,000	90,343 (19.9)	73,781 (20.2)	16,562 (18.8)
>100,000	24,501 (5.4)	20,293 (5.6)	4,208 (4.8)
Obesity (%)	111,030 (24.5)	90,483 (24.8)	20,547 (23.3)
Physical activity (Min/Week)			
<150	184,836 (40.7)	153,841 (42.1)	30,995 (35.1)
≥150	268,809 (59.3)	211,580 (57.9)	57,229 (64.9)
Current smoking (%)	48,396 (10.7)	42,595 (11.7)	5,801 (6.6)
Alcohol intake (times/week)			
<1	139,549 (30.7)	115,251 (31.5)	24,298 (27.5)
1–2	117,466 (25.9)	95,206 (26.1)	22,260 (25.2)
3–4	105,022 (23.2)	82,919 (22.7)	22,103 (25.1)
>4	91,608 (20.2)	72,045 (19.7)	19,563 (22.2)
Minerals supplementation (%)	60,222 (13.3)	38,283 (10.5)	21,939 (24.9)
Fruit intake (pieces/day)			
<2.0	161,936 (35.7)	137,628 (37.7)	24,308 (27.6)
2.0–3.9	218,450 (48.2)	171,995 (47.1)	46,455 (52.7)
≥4.0	73,259 (16.1)	55,798 (15.3)	17,461 (19.8)
Vegetable intake (tablespoons/day)			
<2.0	28,455 (6.3)	25,084 (6.9)	3,371 (3.8)
2.0–3.9	129,515 (28.5)	107,350 (29.4)	22,165 (25.1)
≥4.0	295,675 (65.2)	232,987 (63.8)	62,688 (71.1)
Processed meat intake (times/week)			
0	42,600 (9.4)	33,712 (9.2)	8,888 (10.1)
0–1	137,779 (30.4)	107,724 (29.5)	30,055 (34.1)
>1	273,266 (60.2)	223,985 (61.3)	49,281 (55.9)
Pork intake (times/week)			
0	79,298 (17.5)	64,848 (17.7)	14,450 (16.4)
0–1	257,310 (56.7)	205,402 (56.2)	51,908 (58.8)
>1	117,037 (25.8)	95,171 (26.0)	21,866 (24.8)
Lamb mutton intake (times/week)			
0	81,655 (18.0)	66,771 (18.3)	14,884 (16.9)
0–1	256,877 (56.6)	205,673 (56.3)	51,204 (58.0)
>1	115,113 (25.4)	92,977 (25.4)	22,136 (25.1)
Beef intake (times/week)			
0	51,003 (11.2)	41,338 (11.3)	9,665 (11.0)
0–1	206,216 (45.5)	164,433 (45.0)	41,783 (47.4)
>1	196,426 (43.3)	159,650 (43.7)	36,776 (41.7)

(Continued)

TABLE 1 (Continued)

Characteristics	Overall	Glucosamine non-user	Glucosamine user
Poultry intake (times/week)			
0	23,599 (5.2)	19,241 (5.3)	4,358 (4.9)
0–1	48,508 (10.7)	39,445 (10.8)	9,063 (10.3)
>1	381,538 (84.1)	306,735 (83.9)	74,803 (84.8)
Aspirin use (%)	61,956 (13.7)	50,414 (13.8)	11,542 (13.1)
NSAIDS use (%)	52,813 (11.6)	43,898 (12.0)	8,915 (10.1)
Chondroitin use (%)	4,491 (1.0)	1,016 (0.3)	3,475 (3.9)
Vitamin use (%)	78,022 (17.2)	52,767 (14.4)	25,255 (28.6)

to observe the robustness of our results. R version 4.1.2[1] was adopted for analysis in the present study and two-sided *P*-values of <0.05 were considered statistically significant.

Results

Features of participants

A total of 453,645 participants aged 38–73 were enrolled between 2006 and 2010 and followed up until March 2021. Of the participants, 88,224 reported taking glucosamine supplements habitually, while 365,421 reported no history of regular glucosamine supplementation. The median age of all participants was 57.00 years and 54.2% were female. Additionally, 94% of the participants were white. Detailed participant features at baseline are shown in Table 1. Participants with regular glucosamine supplementation were older and more likely to be female compared with non-glucosamine users. In addition, they were more likely to consume minerals, fish oil, and vitamins (such as vitamins A, B, C, D, E, and B9) than participants without habitual glucosamine supplementation.

Relationship between glucosamine supplementation and cancer mortality

According to the results, we found that glucosamine use was significantly associated with decreased mortality in overall cancer (HR = 0.87, 95% CI = 0.83–0.92, $p < 0.05$), kidney cancer (IRR = 0.65, 95% CI = 0.47–0.90, $p < 0.05$), lung cancer (IRR = 0.68, 95% CI = 0.60–0.76, $p < 0.05$), rectum cancer (IRR = 0.75, 95% CI = 0.58–0.96, $p < 0.05$). These results were obtained from the basic model with age, gender, ethnic background, and average total household income adjusted (Figure 2A and Table 2). The model was then further adjusted by obesity, physical activity, current smoking, alcohol intake, minerals supplementation, fruit intake, vegetable intake, processed meat intake, red meat intake, aspirin use, NSAIDS use, chondroitin use, and vitamin use. With this model, we noted that supplementation of glucosamine was related to lower mortality for overall cancer (HR = 0.95, 95% CI = 0.90–1.00, $p < 0.05$), kidney cancer (IRR = 0.68, 95% CI = 0.49–0.95, $p < 0.05$), lung cancer mortality (IRR = 0.84, 95% CI = 0.74–0.95, $p < 0.05$), and rectum cancer (IRR = 0.76, 95% CI = 0.59–0.98, $p < 0.05$) (Figure 2B and Table 2).

Subgroup analysis

Several subgroup analyses were conducted to analyze the potential effect modifications among the variables of age, ethnicity, gender, smoking, diabetes, high cholesterol, arthritis, and obesity. Significant association between habitual supplementation of glucosamine and lower overall cancer mortality was observed in participants who were aged ≥ 60 years (HR = 0.92, 95% CI = 0.86–0.98, $p < 0.05$), male (HR = 0.90, 95% CI = 0.84–0.98, $p < 0.05$), current smoker (HR = 0.83, 95% CI = 0.72–0.96, $p < 0.05$), without high cholesterol (HR = 0.94, 95% CI = 0.88–1.00, $p < 0.05$), and not obese (HR = 0.90, 95% CI = 0.83–0.98, $p < 0.05$) (Figure 3A). We found that the use of glucosamine was connected to lung cancer in those ≥ 60 years (IRR = 0.80, 95% CI = 0.70–0.92, $p < 0.05$), white (IRR = 0.84, 95% CI = 0.74–0.95, $p < 0.05$), female (IRR = 0.76, 95% CI = 0.64–0.90, $p < 0.05$), current smoker (IRR = 0.79, 95% CI = 0.64–0.98, $p < 0.05$), without diabetes (IRR = 0.86, 95% CI = 0.76–0.97, $p < 0.05$), without arthritis (IRR = 0.82, 95% CI = 0.71–0.95, $p < 0.05$), and not obese (IRR = 0.79, 95% CI = 0.69–0.91, $p < 0.05$) (Figure 3B). Glucosamine supplementation was related to prostate cancer mortality in participants without diabetes (IRR = 0.76, 95% CI = 0.58–0.98, $p < 0.05$) and without high cholesterol (IRR = 0.74, 95% CI = 0.55–0.98, $p < 0.05$) (Figure 3C) and rectum cancer mortality in participants who were white (IRR = 0.76, 95% CI = 0.59–0.99, $p < 0.05$), males (IRR = 0.55, 95% CI = 0.38–0.81, $p < 0.05$), without diabetes (IRR = 0.73, 95% CI = 0.56–0.96, $p < 0.05$), without high cholesterol (IRR = 0.69, 95% CI = 0.52–0.93, $p < 0.05$), and not obese (IRR = 0.67, 95% CI = 0.49–0.92, $p < 0.05$) (Figure 3D). The association of glucosamine use with the overall cancer mortality was stronger among participants

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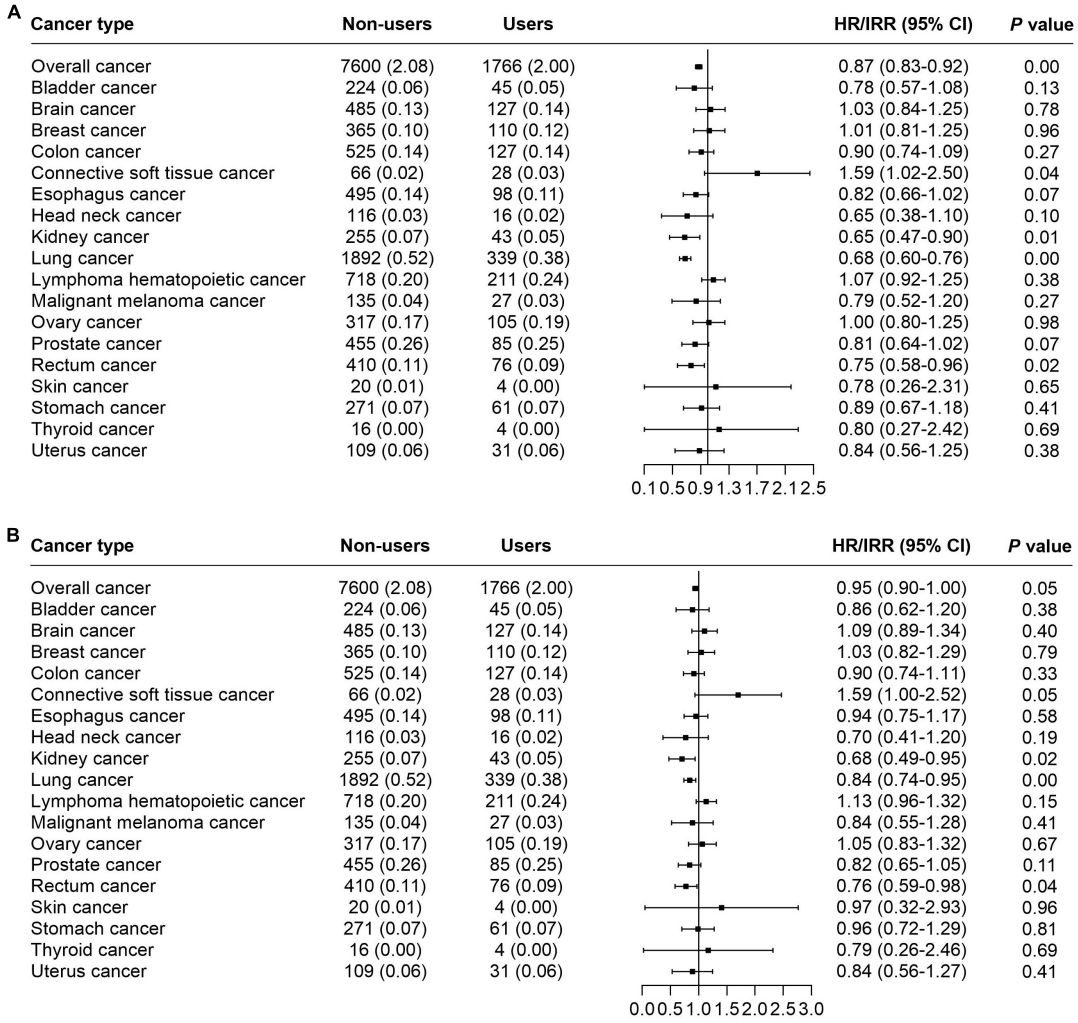

FIGURE 2
The forest plot indicating the correlation of habitual use of glucosamine and cancer mortality. **(A)** Basic model adjusted by age, gender, ethnic, and average total household. **(B)** Adjusted model performed *via* adjusting of age, gender, ethnic background, average total household income, obesity, physical activity, current smoking, alcohol intake, minerals supplementation, fruit intake, vegetable intake, processed meat intake, red meat intake, aspirin use, NSAIDS use, chondroitin use, and vitamin use.

who were more than 60 years (P for interaction = 0.01) current smoker (P for interaction = 0.02) and not obese (P for interaction = 0.02) (Figure 3). The connection between glucosamine use and the rectum cancer mortality was stronger among participants who were male (P for interaction = 0.03) (Figure 3). No more finding was observed from subgroup analysis of breast cancer (Figure 4A), colon cancer (Figure 4B), esophagus cancer (Figure 4C), lymphoma hematopoietic cancer (Figure 4D), and ovary cancer (Figure 4E).

Sensitivity analysis

The correlation between glucosamine supplementation and cancer mortality did not change substantially after we removed participants who took other supplements (Supplementary Table 1). Moreover, when we removed the participants with missing values for covariates, the conclusion were unchanged (Supplementary Table 2). The results from our sensitivity analysis indicated that the results we obtained were stable.

Discussion

Glucosamine is an amino sugar substance formed by replacing one hydroxyl group of glucose with an amino group. Endogenous glucosamine is synthesized *in vivo* through the hexosamine biosynthesis pathway (HBP), and it occurs naturally in most human tissues, with the highest levels being in healthy cartilage (18). Glucosamine is one of the most commonly

TABLE 2 Associations of glucosamine supplement use with cancer mortality.

Cancer type	Glucosamine non-users	Glucosamine users	Basic model*		Adjusted model#	
			HR/IRR (95% CI)	*P*-value	HR/IRR (95% CI)	*P*-value
Overall cancer	7,600 (2.08)	1,766 (2.00)	0.87 (0.83–0.92)	<0.01	0.95 (0.90–1.00)	0.05
Bladder cancer	224 (0.06)	45 (0.05)	0.78 (0.57–1.08)	0.13	0.86 (0.62–1.20)	0.38
Brain cancer	485 (0.13)	127 (0.14)	1.03 (0.84–1.25)	0.78	1.09 (0.89–1.34)	0.40
Breast cancer	365 (0.10)	110 (0.12)	1.01 (0.81–1.25)	0.96	1.03 (0.82–1.29)	0.79
Colon cancer	525 (0.14)	127 (0.14)	0.90 (0.74–1.09)	0.27	0.90 (0.74–1.11)	0.33
Connective softTissue cancer	66 (0.02)	28 (0.03)	1.59 (1.02–2.50)	0.04	1.59 (1.00–2.52)	0.05
Esophagus cancer	495 (0.14)	98 (0.11)	0.82 (0.66–1.02)	0.07	0.94 (0.75–1.17)	0.58
HeadNeck cancer	116 (0.03)	16 (0.02)	0.65 (0.38–1.10)	0.10	0.70 (0.41–1.20)	0.19
Kidney cancer	255 (0.07)	43 (0.05)	0.65 (0.47–0.90)	0.01	0.68 (0.49–0.95)	0.02
Lung cancer	1,892 (0.52)	339 (0.38)	0.68 (0.60–0.76)	<0.01	0.84 (0.74–0.95)	0.00
Lymphoma hematopoietic cancer	718 (0.20)	211 (0.24)	1.07 (0.92–1.25)	0.38	1.13 (0.96–1.32)	0.15
Malignant melanoma cancer	135 (0.04)	27 (0.03)	0.79 (0.52–1.20)	0.27	0.84 (0.55–1.28)	0.41
Ovary cancer	317 (0.17)	105 (0.19)	1.00 (0.80–1.25)	0.98	1.05 (0.83–1.32)	0.67
Prostate cancer	455 (0.26)	85 (0.25)	0.81 (0.64–1.02)	0.07	0.82 (0.65–1.05)	0.11
Rectum cancer	410 (0.11)	76 (0.09)	0.75 (0.58–0.96)	0.02	0.76 (0.59–0.98)	0.04
Skin cancer	20 (0.01)	4 (0.00)	0.78 (0.26–2.31)	0.65	0.97 (0.32–2.93)	0.96
Stomach cancer	271 (0.07)	61 (0.07)	0.89 (0.67–1.18)	0.41	0.96 (0.72–1.29)	0.81
Thyroid cancer	16 (0.00)	4 (0.00)	0.80 (0.27–2.42)	0.69	0.79 (0.26–2.46)	0.69
Uterus cancer	109 (0.06)	31 (0.06)	0.84 (0.56–1.25)	0.38	0.84 (0.56–1.27)	0.41

*Basic model: adjusted for age, gender, ethnicity, and average total household income.
#Adjusted model: adjusted for age, gender, ethnic background, average total household income, obesity, physical activity, current smoking, alcohol intake, minerals supplementation, fruit intake, vegetable intake, processed meat intake, red meat intake, aspirin use, NSAIDS use, chondroitin use, and vitamin use

taken dietary supplements in Australia, and it is commonly used to treat rheumatoid arthritis and osteoarthritis. The substance can regulate a variety of signaling pathways and play pharmacological roles in various diseases. However, the impact of glucosamine supplementation on cancer mortality remains unclear. Here we conducted a large prospective cohort study with more than 450,000 participants enrolled from the UK Biobank. We observed that glucosamine supplementation was correlated with a 5% lower risk of overall cancer mortality, 32% lower risk of kidney cancer mortality, 16% lower risk of lung cancer, and 24% lower risk of rectum cancer mortality. Notably, the association between glucosamine use and lung cancer mortality had previously been evaluated in this cohort with similar results (11).

According to the research progress *in vitro* and *in vivo*, glucosamine can interact with multiple molecular targets, regulate multiple cell signaling pathways, and have great therapeutic potential for various cancers (19). The anti-tumor effects of glucosamine are mainly achieved by inhibiting the proliferation of cancer cells and inducing apoptosis, inducing autophagic death of cancer cells, reversing tumor drug resistance, anti-tumor angiogenesis, and inhibiting the expression of matrix metalloproteinases (20, 21).

Several epidemiologic studies have indicated that glucosamine use is connected to cancer mortality. Bell et al. reported that use of glucosamine was related to a significant decreased risk of death from cancer in the United States (22). Li et al. found that regular use of glucosamine was associated with a lower cancer mortality in the United Kingdom (UK) (10). Additionally, Brasky et al. observed that high 10-year supplementation of glucosamine was related to decreased lung cancer risk in the United States (23). Li et al. found that regular glucosamine supplementation was associated with lower lung cancer mortality in the UK population (11). In the present study, we observed that glucosamine supplementation was associated with a 16% lower risk of lung cancer mortality, which was similar to the findings of Li et al. (11). Compared with Li et al.'s study, we constructed cox and poission proportional hazards models to explore the association between habitual use of glucosamine and multiple cancer mortalities, not merely lung cancer. We found that glucosamine supplementation was also related to a 5% lower risk of overall cancer mortality, 32% lower risk of kidney cancer mortality, 16% lower risk of lung cancer, and 24% lower risk of rectum cancer mortality. Moreover, although we did not find an association between glucosamine use and other cancers among the general population, subgroup

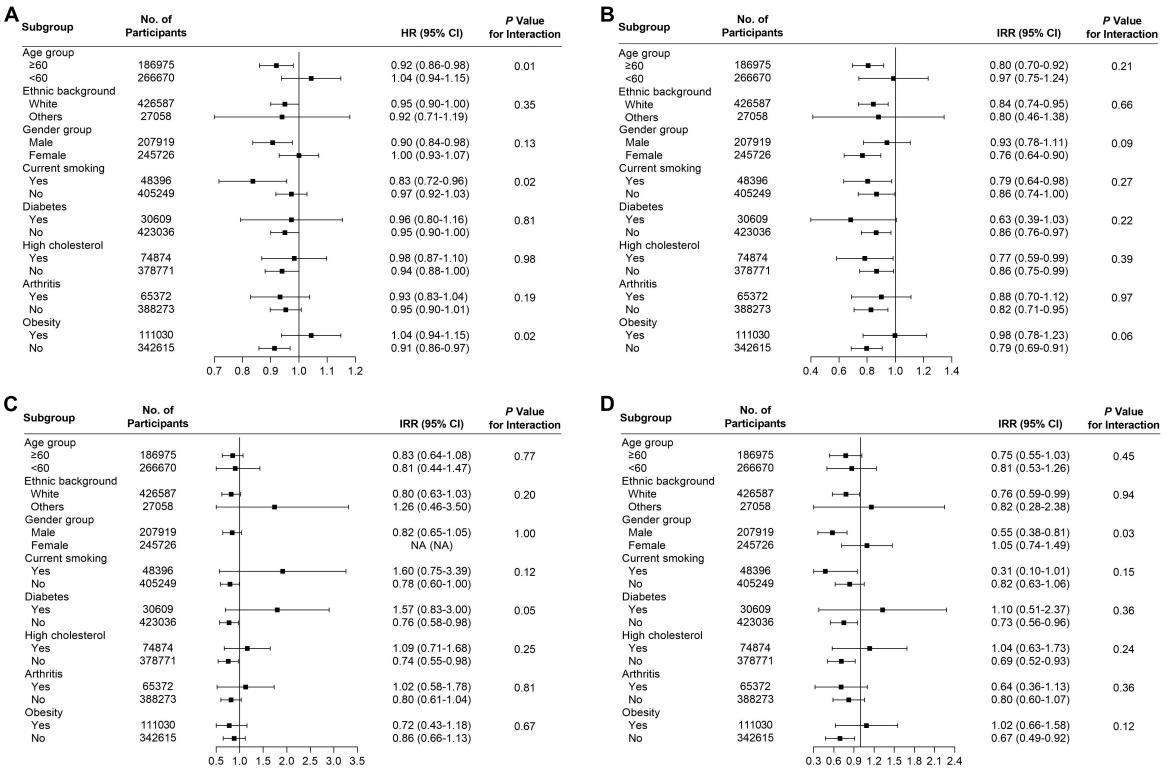

FIGURE 3
Subgroup analysis for **(A)** all cancers, **(B)** lung cancer, **(C)** prostate cancer, and **(D)** rectum cancer to analyze the potential modification effects between age, ethnic background, gender, smoking, diabetes, high cholesterol, arthritis, and obesity.

analysis indicated that its supplementation was related to a lower risk of mortality for prostate cancer in participants without diabetes/cholesterol and rectum cancer mortality in participants who were white, males, without diabetes, without high cholesterol and not obese.

Several potential mechanisms may contribute to the inverse relationship between habitual use of glucosamine and cancer mortality. Glucosamine reduces the production of pro-inflammatory factors by inhibiting the mRNA transcription and/or protein expression of these pro-inflammatory factors, thereby exerting anti-inflammatory and tumor-suppressive effects (9). Additionally, glucosamine improves the resistance of non-small cell lung cancer cells A549 to TRAIL by upregulating the expression of DR5 (24). Furthermore, glucosamine blocks the VEGF-VEGFR signaling pathway by inhibiting VEGF mRNA expression, inhibiting tumor angiogenesis, and exerting anticancer effects (25). Previous studies confirmed that glucosamine inhibited the proliferation of human non-small cell lung cancer A549 cells and inhibited the expression of downstream transcription factors FoxO1 and FoxO3 (26). Glucosamine also promotes NK cell differentiation through the expression of CD3-CD56 + subsets, promotes T cell differentiation through the expression of CD4 + subsets, induces the secretion of IL-2 and IFN-γ, and activates NK cells and T cells at the same time. Thus, exerting its immune regulation and anti-tumor activity (25).

In addition, subgroup analysis indicated glucosamine use was associated with lower overall cancer mortality among participants who were aged ≥ 60 years, male, current smoker, without high cholesterol and not obese. Further clarification on the mechanisms of this association may be necessary. Furthermore, a significant relationship between glucosamine uses and lower risk of lung cancer mortality was observed in those ≥ 60 years, white, female, current smoker, without diabetes, without arthritis and not obese. The potential explanation for stronger effect against cancer observed among current smokers might be that those smoker are at a state of higher inflammatory stress. Therefore, the anti-inflammatory effect from glucosamine may provide stronger benefit.

Glucosamine and other supplements were often taken together, which we hypothesized may affect the relationship. Therefore, sensitivity analyses were conducted to detect the correlation between glucosamine use alone (excluding participants who took other supplementation) with cancer mortality. We observed that the estimates did not change significantly. In addition, when we excluded the participants with missing values for covariates, the conclusion did not substantially change, making it likely that

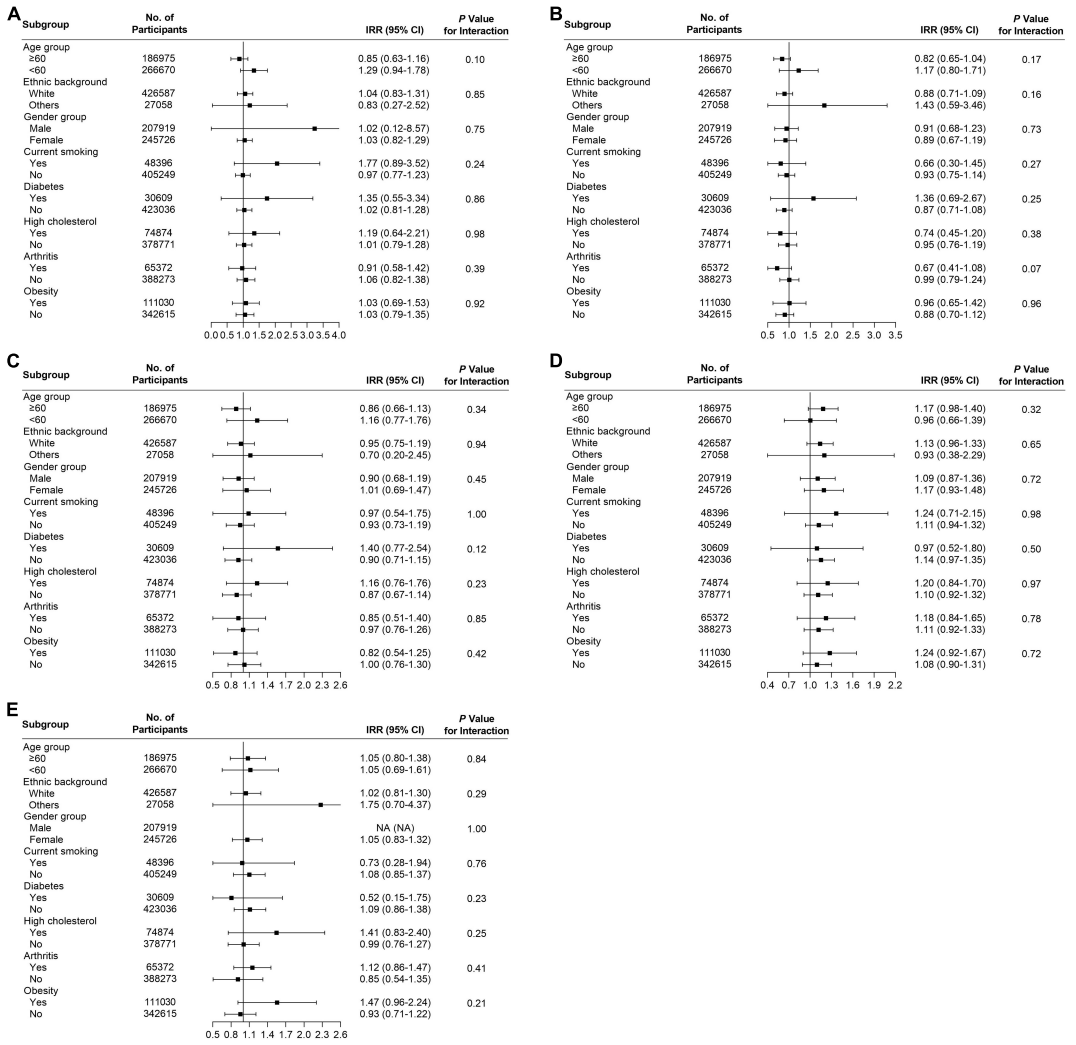

FIGURE 4
Subgroup analysis for **(A)** breast cancer, **(B)** colon cancer, **(C)** esophagus cancer, **(D)** lymphoma hematopoietic cancer, and **(E)** ovary cancer to analyze the potential modification effects between age, ethnic background, gender, smoking, diabetes, high cholesterol, arthritis, and obesity.

glucosamine supplementation may decrease cancer mortality regardless of the use of other supplementation or missing values for covariates.

Strengths and limitations

There were several major strengths of this study, including a minimal loss of follow-up, a large sample size, and a population-based prospective cohort study design. Additionally, the rich information on socioeconomic factors, disease history, and lifestyle allowed us to conduct a comprehensive subgroup analysis. However, there were some limitations in our study. First, there was no detailed information presented on the dose, form, or duration of glucosamine use. Second, although we carefully adjusted for potential confounders related to lifestyle in our analysis, we could not remove the possibility that the results we obtained were confounded by unmeasured factors related to lifestyle. Third, this study evaluated glucosamine use and cancer mortality in the UK. The UK uses a prescription grade formula and the results we obtained may not be generalizable to other populations that use over the counter formulations.

Conclusion

In conclusion, the present study indicated that regular use of glucosamine supplements was significantly related to decreased overall cancer, kidney cancer, lung cancer, and rectum cancer mortality. Further pharmacological studies are needed to increase our understanding of the potential benefits of glucosamine.

Data availability statement

This research has been conducted using the UK Biobank resource (https://www.ukbiobank.ac.uk) under application number: 80610.

Ethics statement

The UK Biobank received ethical approval from the research Ethics Committee (REC reference for UK Biobank 11/NW/0382) and participants provided written informed consent. The patients/participants provided their written informed consent to participate in this study.

Author contributions

TL designed the study and performed the analysis. JZ and RW drafted the manuscript. ZW, ZL, WW, and TL contributed to the revision of the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.947818/full#supplementary-material

References

1. Ma H, Li X, Sun D, Zhou T, Ley SH, Gustat J, et al. Association of habitual glucosamine use with risk of cardiovascular disease: prospective study in UK Biobank. *BMJ.* (2019) 365:l1628.

2. Ma H, Li X, Zhou T, Sun D, Liang Z, Li Y, et al. Glucosamine use, inflammation, and genetic susceptibility, and incidence of type 2 diabetes: a prospective study in UK Biobank. *Diabetes Care.* (2020) 43:719–25. doi: 10.2337/dc19-1836

3. Sibbritt D, Adams J, Lui CW, Broom A, Wardle J. Who uses glucosamine and why? A study of 266,848 Australians aged 45 years and older. *PLoS One.* (2012) 7:e41540. doi: 10.1371/journal.pone.0041540

4. Clarke TC, Black LI, Stussman BJ, Barnes PM, Nahin RL. Trends in the use of complementary health approaches among adults: United States, 2002-2012. *Natl Health Stat Rep.* (2015) 79:1–16.

5. Jordan KM, Arden NK, Doherty M, Bannwarth B, Bijlsma JW, Dieppe P, et al. Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). *Ann Rheum Dis.* (2003) 62:1145–55. doi: 10.1136/ard.2003.011742

6. Valinezhad SF, Palizban A, Mosaffa F, Jamialahmadi K. Glucosamine attenuates drug resistance in Mitoxantrone-resistance breast cancer cells. *J Pharm Pharmacol.* (2021) 73:922–7. doi: 10.1093/jpp/rgaa032

7. Bissett DL. Glucosamine: an ingredient with skin and other benefits. *J Cosmet Dermatol.* (2006) 5:309–15. doi: 10.1111/j.1473-2165.2006.00277.x

8. Wang LS, Chen SJ, Zhang JF, Liu MN, Zheng JH, Yao XD. Anti-proliferative potential of Glucosamine in renal cancer cells via inducing cell cycle arrest at G0/G1 phase. *BMC Urol.* (2017) 17:38. doi: 10.1186/s12894-017-0221-7

9. Dalirfardouei R, Karimi G, Jamialahmadi K. Molecular mechanisms and biomedical applications of glucosamine as a potential multifunctional therapeutic agent. *Life Sci.* (2016) 152:21–9. doi: 10.1016/j.lfs.2016.03.028

10. Li ZH, Gao X, Chung VC, Zhong WF, Fu Q, Lv YB, et al. Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study. *Ann Rheum Dis.* (2020) 79:829–36.

11. Li G, Zhang X, Liu Y, Zhang J, Li L, Huang X, et al. Relationship between glucosamine use and the risk of lung cancer: data from a nationwide prospective cohort study. *Eur Respir J.* (2022) 59:2101399. doi: 10.1183/13993003.01399-2021

12. Li X, Zhou T, Ma H, Huang T, Gao X, Qi L, et al. Healthy sleep patterns and risk of incident arrhythmias. *J Am Coll Cardiol.* (2021) 78:1197–207.

13. Li X, Wang M, Song Y, Ma H, Zhou T, Liang Z, et al. Obesity and the relation between joint exposure to ambient air pollutants and incident type 2 diabetes: a cohort study in UK Biobank. *PLoS Med.* (2021) 18:e1003767. doi: 10.1371/journal.pmed.1003767

14. Li X, Zhou T, Ma H, Liang Z, Fonseca VA, Qi L. Replacement of sedentary behavior by various daily-life physical activities and structured exercises: genetic risk and incident type 2 diabetes. *Diabetes Care.* (2021) [Online ahead of print]. doi: 10.2337/dc21-0455

15. Li X, Xue Q, Wang M, Zhou T, Ma H, Heianza Y, et al. Adherence to a healthy sleep pattern and incident heart failure: a prospective study of 408 802 UK biobank

participants. *Circulation.* (2021) 143:97–9. doi: 10.1161/CIRCULATIONAHA.120.050792

16. Liu D, Li ZH, Shen D, Zhang PD, Song WQ, Zhang WT, et al. Association of sugar-sweetened, artificially sweetened, and unsweetened coffee consumption with all-cause and cause-specific mortality : a large prospective cohort study. *Ann Intern Med.* (2022) 175:909–17. doi: 10.7326/M21-2977

17. World Health Organization. *Global Recommendations On Physical Activity For Health*. Geneva: World Health Organization (2010).

18. Vasiliadis HS, Tsikopoulos K. Glucosamine and chondroitin for the treatment of osteoarthritis. *World J Orthop.* (2017) 8:1–11.

19. Masuda S, Azuma K, Kurozumi S, Kiyose M, Osaki T, Tsuka T, et al. Anti-tumor properties of orally administered glucosamine and N-acetyl-D-glucosamine oligomers in a mouse model. *Carbohydr Polym.* (2014) 111:783–7. doi: 10.1016/j.carbpol.2014.04.102

20. Jung CW, Jo JR, Lee SH, Park YK, Jung NK, Song DK, et al. Anti-cancer properties of glucosamine-hydrochloride in YD-8 human oral cancer cells: induction of the caspase-dependent apoptosis and down-regulation of HIF-1alpha. *Toxicol In Vitro.* (2012) 26:42–50. doi: 10.1016/j.tiv.2011.10.005

21. Pohlig F, Ulrich J, Lenze U, Mühlhofer HM, Harrasser N, Suren C, et al. Glucosamine sulfate suppresses the expression of matrix metalloproteinase-3 in osteosarcoma cells in vitro. *BMC Complement Altern Med.* (2016) 16:313. doi: 10.1186/s12906-016-1315-6

22. Bell GA, Kantor ED, Lampe JW, Shen DD, White E. Use of glucosamine and chondroitin in relation to mortality. *Eur J Epidemiol.* (2012) 27:593–603.

23. Brasky TM, Lampe JW, Slatore CG, White E. Use of glucosamine and chondroitin and lung cancer risk in the VITamins And Lifestyle (VITAL) cohort. *Cancer Causes Control.* (2011) 22:1333–42. doi: 10.1007/s10552-011-9806-8

24. Liang Y, Xu W, Liu S, Chi J, Zhang J, Sui A, et al. Acetyl-glucosamine sensitizes non-small cell lung cancer cells to trail-induced apoptosis by activating death receptor 5. *Cell Physiol Biochem.* (2018) 45:2054–70. doi: 10.1159/000488042

25. Xu W, Jiang C, Kong X, Liang Y, Rong M, Liu W. Chitooligosaccharides and N-acetyl-D-glucosamine stimulate peripheral blood mononuclear cell-mediated antitumor immune responses. *Mol Med Rep.* (2012) 6:385–90. doi: 10.3892/mmr.2012.918

26. Yu Z, Ju Y, Liu H. Antilung cancer effect of glucosamine by suppressing the phosphorylation of FOXO. *Mol Med Rep.* (2017) 16:3395–400. doi: 10.3892/mmr.2017.6976

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Association between chili pepper consumption and risk of gastrointestinal-tract cancers: A meta-analysis

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Background: Stimulating food is emerging as an important modifiable factor in the development of gastrointestinal (GI) tract cancers, but the association between chili pepper consumption and the risk of GI cancers is unclear. We aimed to evaluate the direction and magnitude of the association between chili pepper consumption and the risk of GI cancers.

Methods: A literature search was performed in PubMed, Embase, and Web of Science databases from inception to 22 December 2021. Observational studies reporting the association between chili pepper consumption and the risk of gastric cancer (GC), esophageal cancer (EC), and/or colorectal cancer (CRC) in adults were eligible for inclusion. Data extraction and quality assessment were conducted independently by two reviewers for the included literature. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a random-effects model. Subgroup analyses were also performed based on the cancer type, study design, region of the study, study quality, and adjustments.

Results: A total of 11,421 studies were screened, and 14 case-control studies were included involving 5009 GI cancers among 11,310 participants. The summary OR showed that high consumption of chili pepper was positively related to the risk of GI cancers (OR = 1.64; 95% CI: 1.00–2.70). A stronger positive relationship was observed between chili pepper consumption and EC risk (OR = 2.71; 95% CI: 1.54–4.75), but there was no statistically significant association between GC and CRC risk. In analyses stratified by geographical location, a positive association was found between chili pepper consumption and the risk of GI cancers in Asian studies (OR = 2.50; 95% CI: 1.23–5.08), African studies (OR = 1.62; 95% CI: 1.04–2.52), and North American studies (OR = 2.61; 95% CI: 1.34–5.08), but an inverse association was seen in South American studies (OR = 0.50; 95% CI: 0.29–0.87) and European studies (OR = 0.30; 95% CI: 0.15–0.61).

Conclusion: This meta-analysis suggests that chili pepper is a risk factor for certain GI cancers (e.g., EC). Geographical regions influence the risk of GI cancers, especially in Asian, African, and North American populations, which require more attention during dietary guidance.

Systematic review registration: [https://www.crd.york.ac.uk/PROSPERO/], identifier [CRD42022320670].

KEYWORDS

chili pepper, gastrointestinal tract cancer, systematic review, meta-analysis, risk

Introduction

Globally, gastrointestinal (GI) tract cancers are a significant cause of morbidity and mortality, of which the most prevalent are colorectal cancer (CRC), gastric cancer (GC), and esophageal cancer (EC), ranking third, fifth, and eighth in incidence, respectively, but second, fourth, and sixth in mortality in both sexes combined according to GLOBOCAN estimates for 2020 (1). Despite the availability of multiple therapeutic options such as radiation, chemotherapy, curative resection, and immunotherapy, the early signs of GI cancers are generally undetectable and identified at an advanced stage, leaving patients with limited treatment options, and a poor prognosis (2). Therefore, early identification of risk factors for GI cancers is of great significance to public health.

Diet plays a major role in the development of these diseases. Chili pepper is one of the major vegetables and spices consumed around the world (3). Chili peppers are rich in the bioactive component capsaicin (CAP), which has been reported to have diverse biological properties such as anti-obesity, anti-oxidant, and anti-inflammatory effects *in vitro* and *vivo* experiments (4–6). Increasing evidence suggests that CAP facilitates the growth and migration of esophageal squamous cell carcinoma (ESCC) and human colon cancer cells (7, 8). Because of the CAP content, the association between chili pepper consumption and the risk of GI cancers is unclear, and it is important to clarify this question from a public health perspective. Previous individual studies, however, have reported the association between chili pepper exposure and the risk of GI cancers, with controversial results. This may be explained by heterogeneity among the studies, which is attributed to differences in the methods of exposure assessment, study area, sample sizes, and adjustments. For instance, Galvan-Portillo et al. (9) conducted a study of 726 subjects in Mexico, adjusted for energy, age, sex, and education, and showed a positive association between chili pepper and GC risk, whereas Munoz et al. (10) included 191 participants in Italy and observed an inverse association between chili pepper and GC risk after adjustment for sex, age, area of residence, and education. This difference can lead to confusion among dietitians and the general public, as well as challenges in translating into dietary advice.

Previous meta-analyses focusing on the association between chili pepper consumption and GC risk have yielded conflicting findings. For example, most studies showed a positive effect on GC risk (11–13), and a meta-analysis performed by Chen et al. (14) reported a null association. However, to date, no systematic review has been published that specifically explored the relationship between GI cancer risk and chili pepper consumption. Additionally, the validity of the above meta-analyses has been questioned due to the inclusion of studies that mixed chili pepper with other foods (12, 13), used kimchi or CAP instead of chili pepper as the interesting exposure (11–13), and extracted effect estimates incorrectly (12, 14), thus an extensive systematic review and meta-analysis is needed to obtain a more accurate estimate.

Hence, we performed a systematic review and meta-analysis to evaluate the association between chili pepper consumption and the risk of GI cancers by combining all available data from eligible studies. When possible, we used meta-analysis to quantify the effects and explore the possible sources of heterogeneity among the studies.

Methods

The study was complemented following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (15). This review was registered at PROSPERO as CRD42022320670.

Search strategy

We searched PubMed, Embase, and Web of Science for studies in humans on the association between chili pepper consumption and the risk of GI cancers from inception until 22 December 2021, using ("spicy" OR "chili" OR "chilli" OR "pepper" OR "capsaicin" OR "paprika") AND ("malignancy" OR "cancer" OR "carcinoma" OR "tumor" or "neoplasm") as search terms. Additionally, references to relevant articles and recent reviews were manually searched to identify other eligible articles.

Selection criteria

Studies that satisfied the following criteria were included in this meta-analysis: (1) participants were adults; (2) studies were observational (cohort, case-control, or cross-sectional studies); (3) information was available on the relationship between chili pepper consumption as the exposure of interest and the risk of EC, GC, and/or CRC as the outcome of interest; and (4) studies reported available risk estimates in the form of relative risk (RR), odds ratio (OR), or hazard ratio (HR) with 95% confidence intervals (CIs). When overlapping populations were included in multiple articles, only the most recent or largest population was used to avoid duplications.

Non-English articles were excluded. Studies were excluded if they were reviews, letters, posters, meetings, or conference abstracts. We also excluded studies that included patients with precancerous lesions as an outcome of interest, mixed chili pepper with other foods (e.g., hot pepper-soybean stew) as the exposure of interest, had a sample size of fewer than 20 cases, and were conducted on children or adolescents. Additionally, studies with insufficient data were excluded. All searches were performed independently by two authors (CC and MZ), and inconsistencies were resolved through discussion.

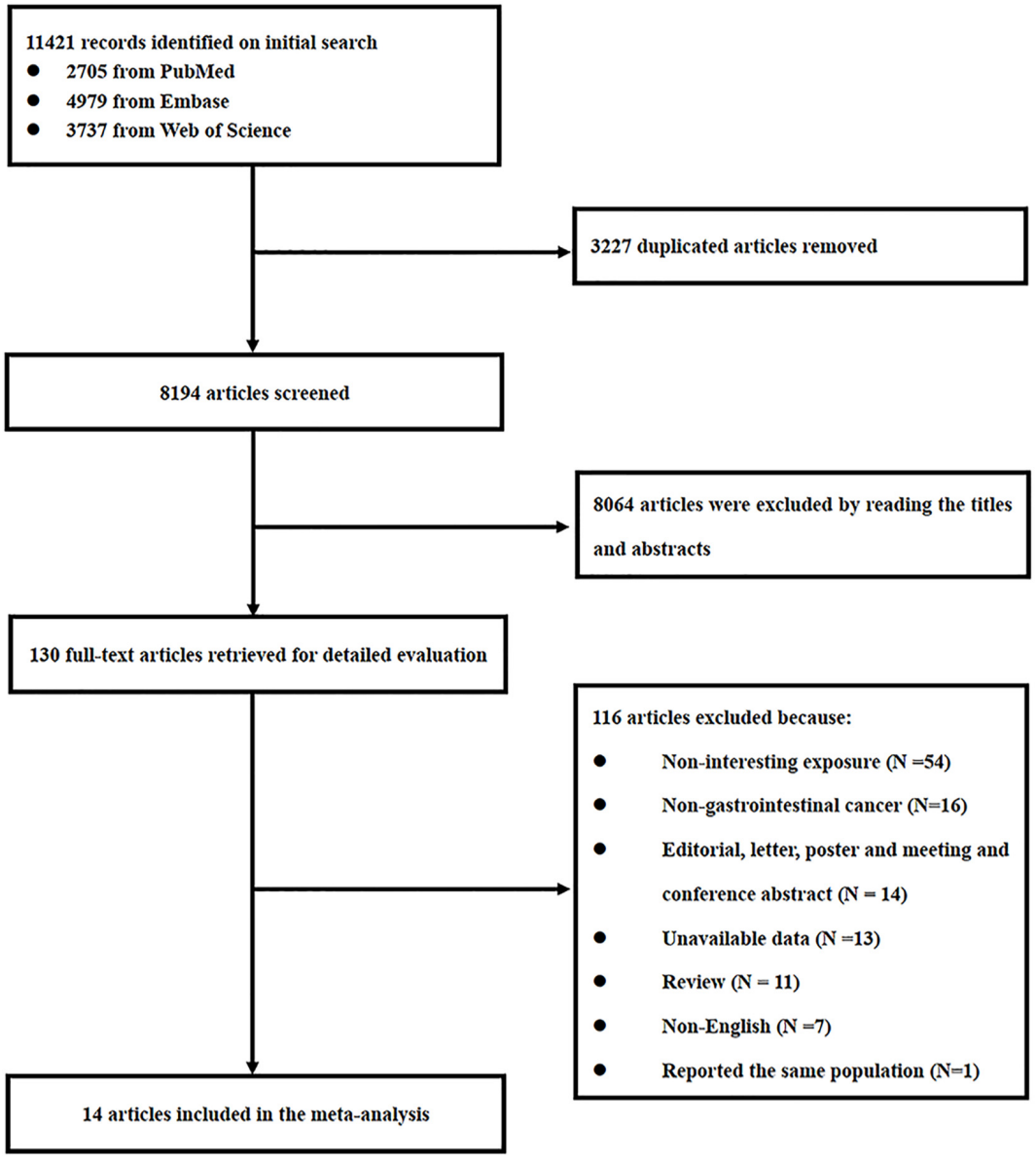

FIGURE 1
Flowchart of the study selection process.

TABLE 1 Characteristics of studies included in the meta-analysis.

References	Country	Study design	Sample sizes/cases	Age, male ratio	Assessment of exposure	Cancer type	Risk estimate (95% CI)	Adjustments
Liu et al. (32)	China	HCCS	1666/833	60 (53−67) vs. 60 (53−66), 58%	Q	CRC	Sweet pepper category: OR <0.75 kg/year: 1 0.75−2.60 kg/year: 0.54 (0.37−0.78) 2.60−5.20 kg/year: 0.52 (0.35−0.75) ≥5.20 kg/year: 0.48 (0.33−0.70)	BMI, colon cancer in first-degree relative, smoking status, alcohol drinking, eating breakfast, fried food, grilled food, hot and spicy food intake, total energy, total fruits, milk product, and red meat intake
Mmbaga et al. (24)	Tanzania	HCCS	942/471	59 (47−69) vs. 55 (45−65), 69%	FFQ	EC	Spicy chilies category: OR <daily: 1 Daily: 1.62 (1.04−2.52)	NA
Yang et al. (33)	China	PCCS	800/400	55.7 ± 11.08 vs. 55.74 ± 11.19, 58.2%	Q	CRC	Chili peppers category: OR ≤2 times/week: 1 3−7 times/week: 1.20 (0.75−2.00) > 7 times/week: 1.40 (0.84−2.20)	Intake of red meat, cured meat, pickles, tea, bean, fruit, vegetables, high-fat food, sweetmeats, daily sitting time, smoking regularly, drinking regularly, exercise regularly, and family history of CRC
Galván-Portillo et al, 2009 (9)	Mexico	PCCS	726/248	58 (mean), 54%	FFQ	GC	Chili category: OR No: 1 Regular: 1.19 (0.77−1.84) Much: 1.96 (1.26−3.05)	Energy, age, sex, and education
Goh et al. (27)	Malaysia	HCCS	261/87	61.4 ± 13.0 vs. 58.9 ± 10.8, 49%	Q	GC	Chili category: OR Low/none: 1 Heavy: 1.81 (0.74−4.43)	Race, *H. pylori* status, education, smoking, fresh fruits/vegetables, and salted fish/vegetables
Wang et al. (26)	China	PCCS	763/355	61.51 ± 7.94 vs. 60.75 ± 8.30, 62.3%	Q	EC	Chili category (men): OR Seldom: 1 Often: 3.38 (2.12−5.39) Chili category (women): OR Seldom: 1 Often: 1.61 (0.66−3.89)	Age, marital status, and education years
Phukan et al. (31)	India	HCCS	1506/502	55.0 ± 8.1 vs. 54.5 ± 7.8, NA	FFQ	EC	Chili category: OR Moderate user: 1 Non-user: 0.10 (0.05−5.80) Very chili: 3.60 (1.80−8.60)	Education, income, chewing betel nut and tobacco, smoking, and alcohol use
Muñoz et al. (22)	Venezuela	PCCS	777/292	> 35, NA	FFQ	GC	Chili category: OR Not often: 1 Often: 0.50 (0.30−0.90)	Age, sex, and socio-economic status
Mathew et al. (25)	India	HCCS	499/194	> 20, 76.0%	Q	GC	Chili category: OR Blank: 1 Medium: 1.80 (1.00−3.10) Very hot: 7.40 (4.00−13.50)	Age, sex, religion, education, smoking, and alcohol habits
López-Carrillo et al. (23)	Mexico	PCCS	972/220	57.2 vs. 59.2 (mean), 43.2%	FFQ	GC	Chili pepper (none of alcohol per day) category: OR No: 1 Yes: 4.50 (1.92−10.71) Chili pepper (<5 g of alcohol per day) category: OR No: 1 Yes: 2.90 (0.84−9.96)	Age, sex
Muñoz et al. (10)	Italy	HCCS	191/88	≤75, NA	Q	GC	Peppers category: OR 0 time/week: 1 1 time/week: 0.42 (0.21−0.86) ≥2 times/week: 0.31 (0.12−0.83)	Sex, age, area of residence, and education
Fernandez et al. (29)	Italy	HCCS	220/112	≤75, 57.7%	Q	CRC	Peppers category: RR Low: 1 Intermediate: 0.40 (0.20−0.70) High: 0.30 (0.10−0.70)	Sex, age, and area of residence

(Continued)

TABLE 1 (Continued)

References	Country	Study design	Sample sizes/cases	Age, male ratio	Assessment of exposure	Cancer type	Risk estimate (95% CI)	Adjustments
Gajalakshmi et al. (28)	India	HCCS	776/388	NA, 73.9%	Q	GC	Chilies category: OR Medium: 1 Hot: 2.80 (1.73−4.54)	Smoking, drinking alcohol, chewing habit, factors significant in the multivariate model of dietary item analysis, income group, educational level, and area of residence
Notani et al. (30)	India	PCCS	1211/819	NA, 100%	Q	EC	Red chili powder category: RR <75 g/cu/month: 1 75−99 g/cu/month: 1.94 (0.80−4.90) 100−149 g/cu/month: 1.99 (1.00−4.00) ≥150 g/cu/month: 2.85 (1.50−5.50)	Age, tobacco habits

GC, gastric cancer; EC, esophagus cancer; CRC, colorectal cancer; g/cu/month, grams per consumption unit per month; Q, questionnaire; FFQ, food frequency questionnaire; OR, odds ratio; RR, relative risk; CI, confidence interval; NA, not available; BMI, body mass index; HCCS, hospital case-control study; PCCS, population case-control study.

Data extraction

Two investigators (CC and MZ) independently reviewed and performed the data extraction from all the included studies. The extracted characteristics and data were composed of the first author's last name, publication year, country, study design (hospital case-control, population case-control, or cohort study), number of study populations and cases, mean/median age of participants, male ratio, assessment method of exposure, GI cancer type, risk estimates and corresponding 95% CI, and covariates adjusted in multivariate analysis. For studies reporting several multivariate-adjusted risk estimates, the risk estimates that were maximally adjusted for underlying confounders were the top priority for use. Any discrepancies during the data extraction process were determined through discussion with a third investigator (XZ).

Assessment of study quality

The quality of the included studies was evaluated using a modified version of the Newcastle–Ottawa Quality Assessment Scale (NOS) (16) with a nine-star scoring system. The following items were taken into consideration: selection of the study groups (up to four stars), comparability of the study groups (up to two stars), and confirmation of chili pepper exposure (up to three stars). We considered NOS scores above or equal to the median as high-quality studies (low risk of bias) and those with NOS scores below the median were regarded as low-quality (high risk of bias) (17). The results of study quality were not used as exclusion criteria.

Statistical analysis

The OR and 95% CI were identified as effect sizes to evaluate the association between chili pepper consumption and the risk of GI cancers. We used the maximally adjusted OR reported in the original research when the OR was directly available. Heterogeneity among the included studies was evaluated by I-square (I^2) statistic (18). When I^2 was greater than 50%, there was significant heterogeneity between studies, so a random-effects model was selected. Otherwise, a fixed-effects model was performed (19). Publication bias was evaluated through a combination of qualitative and quantitative approaches, involving funnel plots and the Egger regression test (20).

A sensitivity analysis was conducted using the leave-one-out method to determine the influence of a single study. Subgroup analyses were also performed to explore whether pooled risk estimates were affected by cancer subgroups (EC, GC, or CRC), study design (population-based or hospital-based case-control study), region of the study (Asian, African, North American, South American, or European studies), study quality (high-quality or low-quality studies), adjustment for alcohol intake (Yes or No), and adjustment for smoking (Yes or No). Statistical analyses were done using Stata 16.0 software (StataCorp LLC, College Station, TX, USA). All P-values were two-sided, with $P < 0.05$ considered statistically significant.

Result

Literature search

Our search strategy retrieved 11,421 studies from 3 databases, and 3,227 duplicates were excluded. A further 8,194

TABLE 2 Quality of studies according to the modified Newcastle-Ottawa Scale (NOS).

	Case-control studies													
	Liu et al. (32)	**Mmbaga et al. (24)**	**Yang et al. (33)**	**Galván-Portillo et al. (9)**	**Goh et al. (27)**	**Wang et al. (26)**	**Phukan et al. (31)**	**Muñoz et al. (22)**	**Mathew et al. (25)**	**López-Carrillo et al. (23)**	**Muñoz et al. (10)**	**Fernandez et al. (29)**	**Gajalakshmi et al. (28)**	**Notani et al. (30)**
Selection														
1. Is the case definition adequate?	•	★	★	★	★	•	★	★	★	★	★	★	★	•
2. Representativeness of the cases	★	★	★	★	★	★	★	★	★	★	★	★	★	★
3. Selection of controls	•	•	★	★	•	★	•	★	•	★	•	★	•	★
4. Definition of controls	★	★	★	★	•	★	•	★	★	•	★	★	★	★
Comparability														
5. Study controls for the most important factor	★	★	★	★	★	★	★	★	★	★	★	★	★	★
6. Study controls for the second important factor	★	★	★	★	★	★	★	★	★	★	★	★	★	★
Exposure														
7. Was the measurement method of chili pepper described?	★	★	★	★	★	★	★	★	★	★	★	★	★	★
8. Were the methods of measurements same for cases and controls?	★	★	★	★	★	★	★	★	★	★	★	★	★	★
9. Non-response rate	•	★	★	•	•	★	•	•	•	•	•	•	•	•
Summary score	6/9	8/9	9/9	8/9	6/9	8/9	6/9	8/9	7/9	7/9	7/9	7/9	7/9	7/9
(Risk of bias)	(high)	(low)	(low)	(low)	(high)	(low)	(high)	(low)	(low)	(low)	(low)	(low)	(low)	(low)

★ was awarded when the respective information was available.
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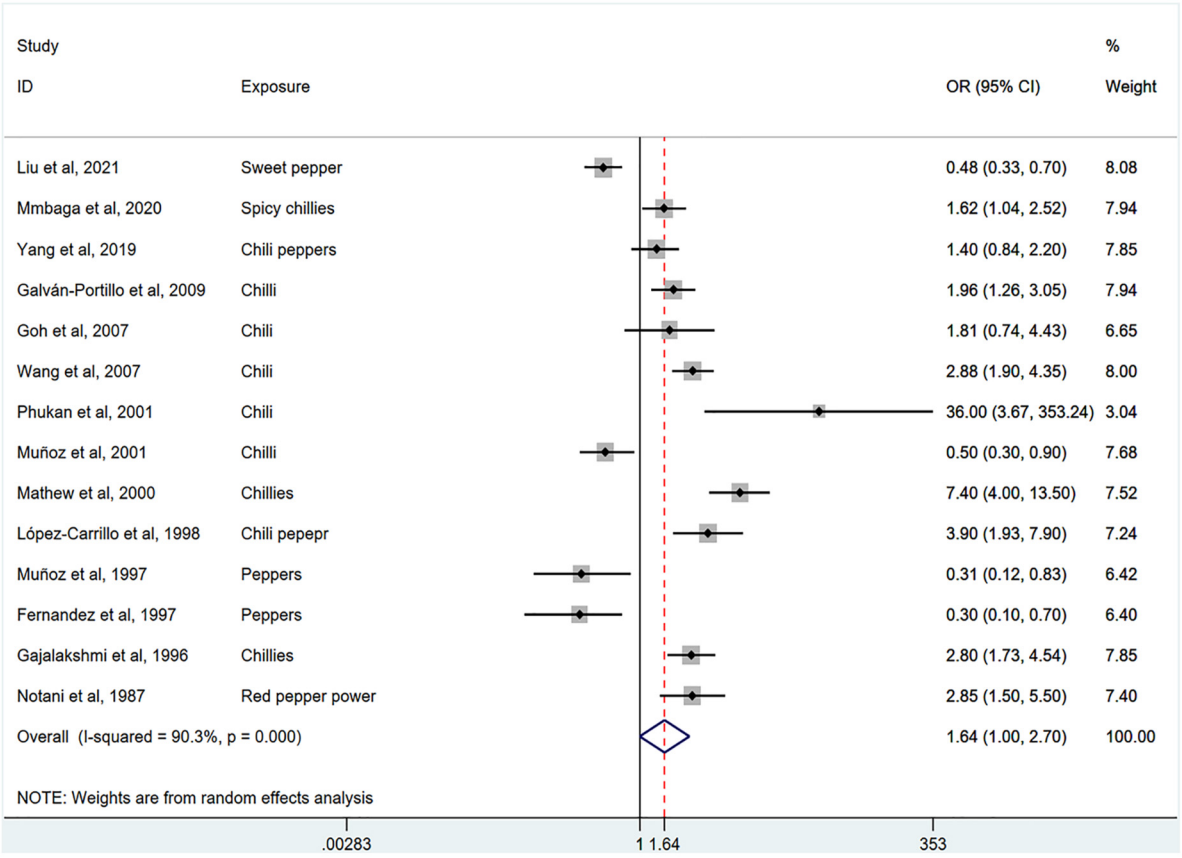

FIGURE 2
Pooled analysis showing associations between chili pepper consumption and the risk of GI cancers.

studies were screened based on titles and abstracts, of which 8,064 articles were excluded because they did not meet the eligibility criteria. The remaining 130 studies were identified for full-text review, and 116 studies were excluded due to 54 being non-chili exposure, 16 being non-GI cancers, 14 studies being editorial, letter, poster, meeting, and conference abstract, the data of 13 studies being unavailable, 11 studies being review, 7 studies being published in a non-English language, and 1 study reporting the same population (21) with one of the included studies in this meta-analysis. In total, 14 studies (9, 10, 22–33) were included in our final analysis, and the flow diagram of the literature search is shown in **Figure 1**.

Study characteristics

Table 1 summarizes the main characteristics of the included studies. The included studies, which were published between 1987 and 2021, included 14 case-control studies with 5009 GI cancers among 11,310 participants. The number of GI cases enrolled in these articles ranged from 87 to 833, and the number of participants ranged from 191 to 1666. Of the 14 case-control studies, eight studies were conducted in Asia (25–28, 30–33), two in Europe (10, 29), two in North America (9, 23), one in Africa (24), and one in South America (22). As to study design, most of these studies were population-based controls (9, 22, 23, 26, 30, 33), and the remaining six studies used a hospital-based case-control design (10, 24, 25, 27–29, 31, 32). Moreover, seven studies examined the association between chili pepper intake and the risk of GC (9, 10, 22, 23, 25, 27, 28), three on CRC (29, 32, 33), and four on EC (24, 26, 30, 31). In terms of the assessment methods of exposure, five studies (9, 22–24, 31) used the FFQ, while nine studies used frequency-reported questionnaires (10, 25–30, 32, 33). Almost all studies reported OR, except for two studies that reported RR (29, 30). All studies were adjusted or matched for age and sex, with only one study not adjusted for sex because all participants were male (30). Smoking (24, 25, 28, 31–33) and alcohol consumption (24, 25, 27, 28, 30–33) have been controlled in several studies.

The detailed quality assessment of the included studies by the modified NOS for case-control studies is shown in **Table 2**. The median NOS score is 7. Eleven studies (9, 10, 21, 22, 24–26, 28–30, 33) with a NOS score of 7 or higher were evaluated as high methodological quality (low risk of bias), and three studies

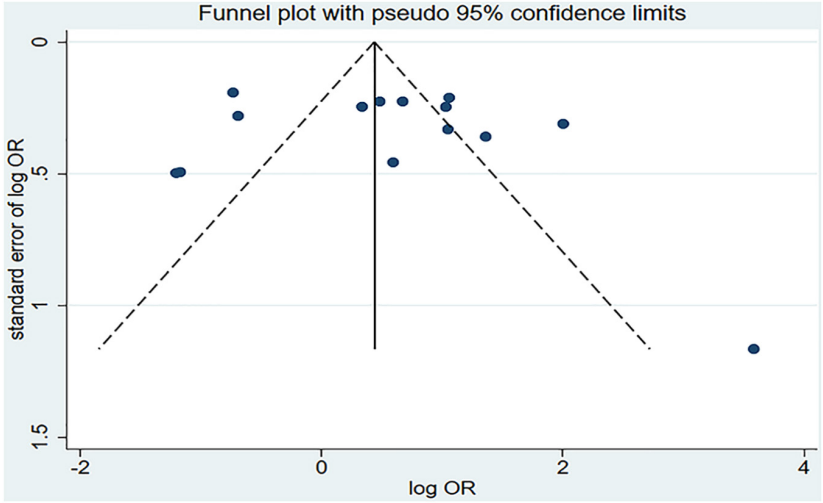

FIGURE 3
Funnel plot for evaluation publication bias. OR, odds ratio.

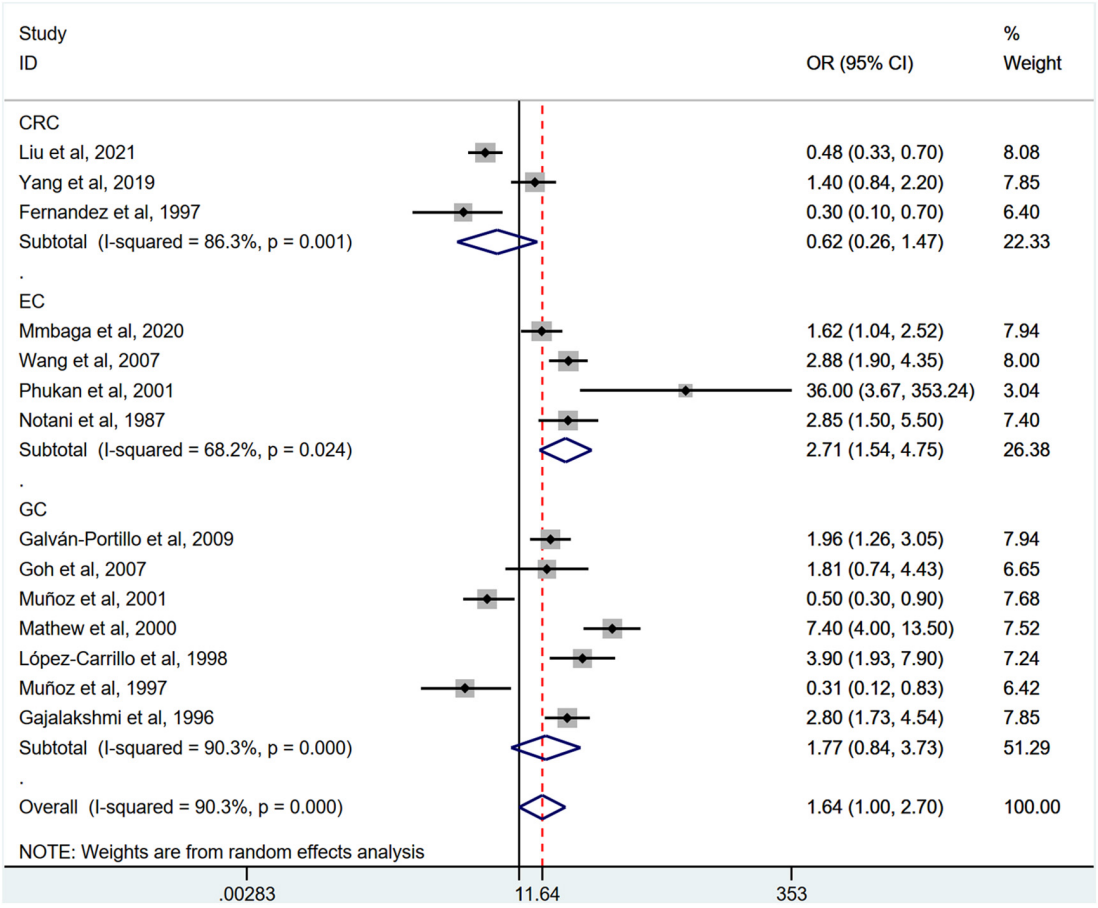

FIGURE 4
Subgroup analysis showing associations between chili pepper consumption and the risk of GI cancers based on the cancer type. GC, gastric cancer; EC, esophageal cancer; CRC, colorectal cancer; OR, odds ratio; CI, confidence interval.

(27, 31, 32) with a score lower than 7 were assessed as low methodological quality (high risk of bias).

Chili pepper consumption and the risk of gastrointestinal cancers

Figure 2 shows the results of the pooled analysis of the eligible studies. The association between chili pepper consumption and the risk of GI cancers was evaluated in 14 studies, consisting of 11,310 participants and 5009 cases. Pooled results showed that the highest category of chili pepper consumption was associated with an increased risk of GI cancers (OR = 1.64; 95% CI: 1.00–2.70), compared with the lowest category (or no intake). Significant heterogeneity existed across the studies (I^2 = 90.3%; $P < 0.001$). No evidence of publication bias was found based on Egger's test ($P = 0.651$) and symmetrical funnel plot (Figure 3).

Subgroup analysis

Stratification by cancer type showed that higher chili pepper consumption was associated with an elevated risk of EC (OR = 2.71; 95% CI: 1.54–4.75), but not with GC (OR = 1.77; 95% CI: 0.84–3.73) and CRC risk (OR = 0.62; 95% CI: 0.26–1.47) (Figure 4). When stratified by study design (Figure 5), population-based case-control studies showed a positive association between chili pepper consumption and the risk of GI cancers (OR = 1.86; 95% CI: 1.07–3.22), whereas hospital-based case-control studies showed a null association (OR = 1.52; 95% CI: 0.65–3.52). In the subgroup analysis of the region of the study (Figure 6), chili pepper consumption obviously increased the risk of GI cancers in Asian studies (OR = 2.50; 95% CI: 1.23–5.08), North American studies (OR = 2.61; 95% CI: 1.34–5.08), and African studies (OR = 1.62; 95% CI: 1.04–2.52). However, a significantly lower risk of GI cancers was observed in South American studies (OR = 0.50; 95% CI: 0.29–0.87) and European studies (OR = 0.30; 95% CI: 0.15–0.61). We further performed subgroup analysis by study quality (Figure 7) and adjustment factors (Figures 8, 9), finding a significant positive association between the highest chili pepper consumption compared with the lowest and the risk of GI cancers was seen in high-quality studies (OR = 1.65; 95% CI: 1.02–2.69), as well as in studies that adjusted for alcohol intake (OR = 2.29; 95% CI: 1.15–4.57). However, a null association was seen between chili pepper consumption and GI cancer risk in low-quality studies (OR = 2.15; 95% CI: 0.39–11.77), and studies not adjusted for alcohol intake (OR = 1.06; 95% CI: 0.47–2.39). A non-significant

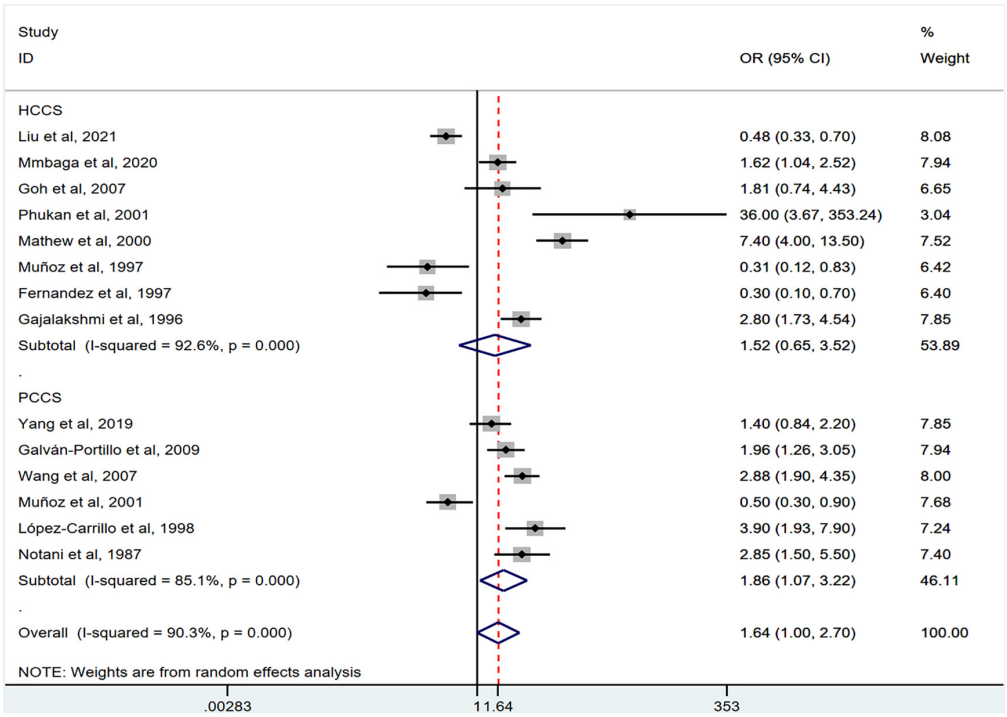

FIGURE 5
Subgroup analysis showing associations between chili pepper consumption and the risk of GI cancers based on the study design. HCCS, hospital case-control study; PCCS, population case-control study; OR, odds ratio; CI, confidence interval.

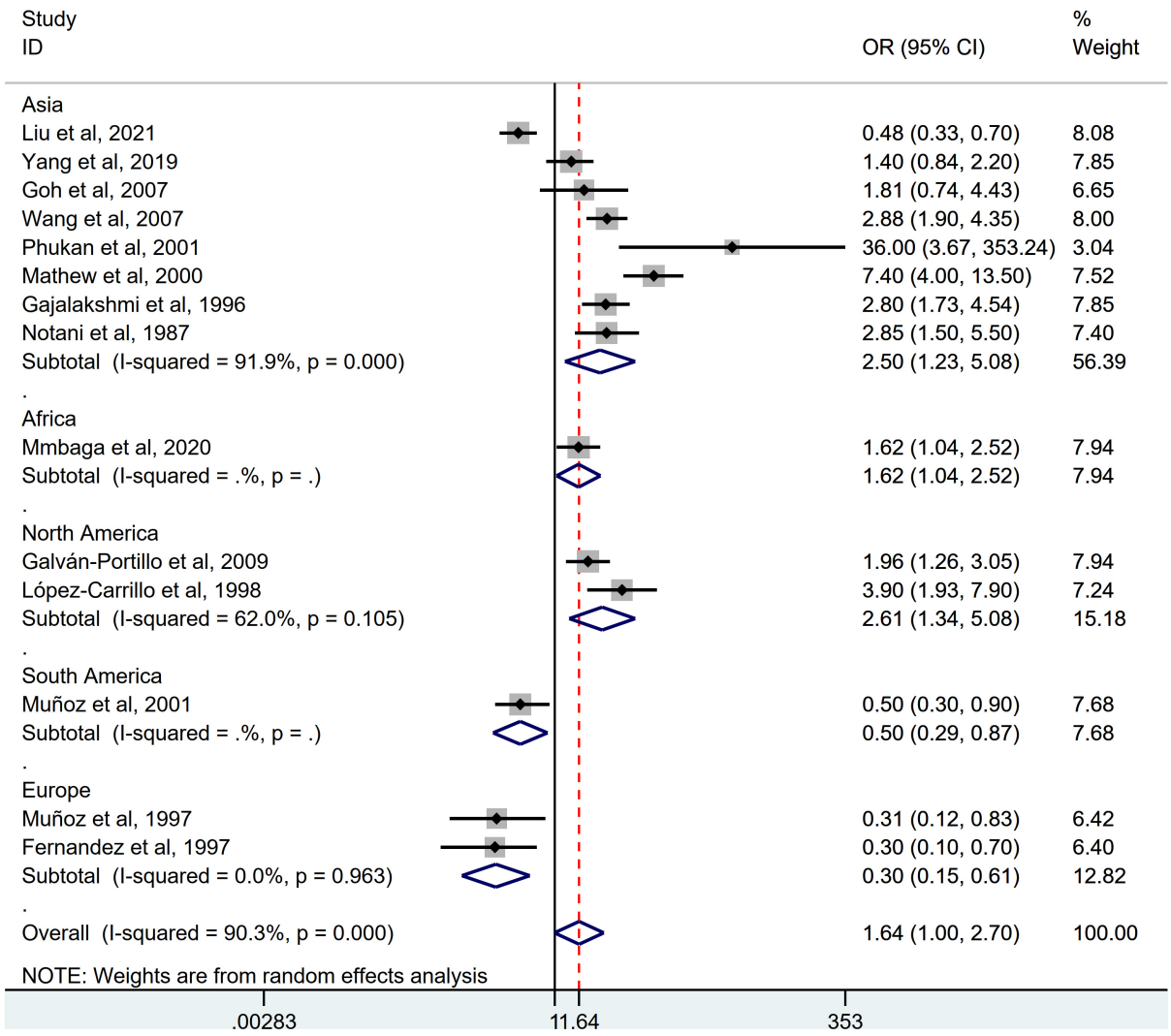

FIGURE 6
Subgroup analysis showing associations between chili pepper consumption and the risk of GI cancers based on the region of the study. OR, odds ratio; CI, confidence interval.

association was also seen in studies either adjusted for smoking or not.

Discussion

This systematic review and meta-analysis was designed to evaluate the association between chili pepper consumption and the risk of GI cancers. The evaluation of 14 case-control studies involving 11,310 participants found a positive association between chili pepper consumption and the risk of GI cancers. In the subgroup analysis, this correlation between high chili pepper consumption and rising GI cancer risk was applied to EC, but not to GC and CRC. Especially in Asia, Africa, and North America, chili pepper intake showed a significant positive correlation with GI cancer risk.

In this study, the intake of chili pepper was positively associated with EC risk. The same finding was also observed for the consumption of chili pepper and GI cancer risk. Given that EC is a part of all GI cancers, the observed increased association with GI cancers appears to be related to EC. Several mechanisms could explain why higher chili pepper consumption was significantly associated with an increased risk of EC, but not with GC and CRC. The different effects could be due to differences in cancer sites. Chili peppers are rich in CAP, which has an intensely pungent flavor, further leading to a sensation of tingling and burning pain by stimulating transient receptor potential vanilloid 1 (TRPV1) (34–36). The stomach and intestine share a common endodermal origin

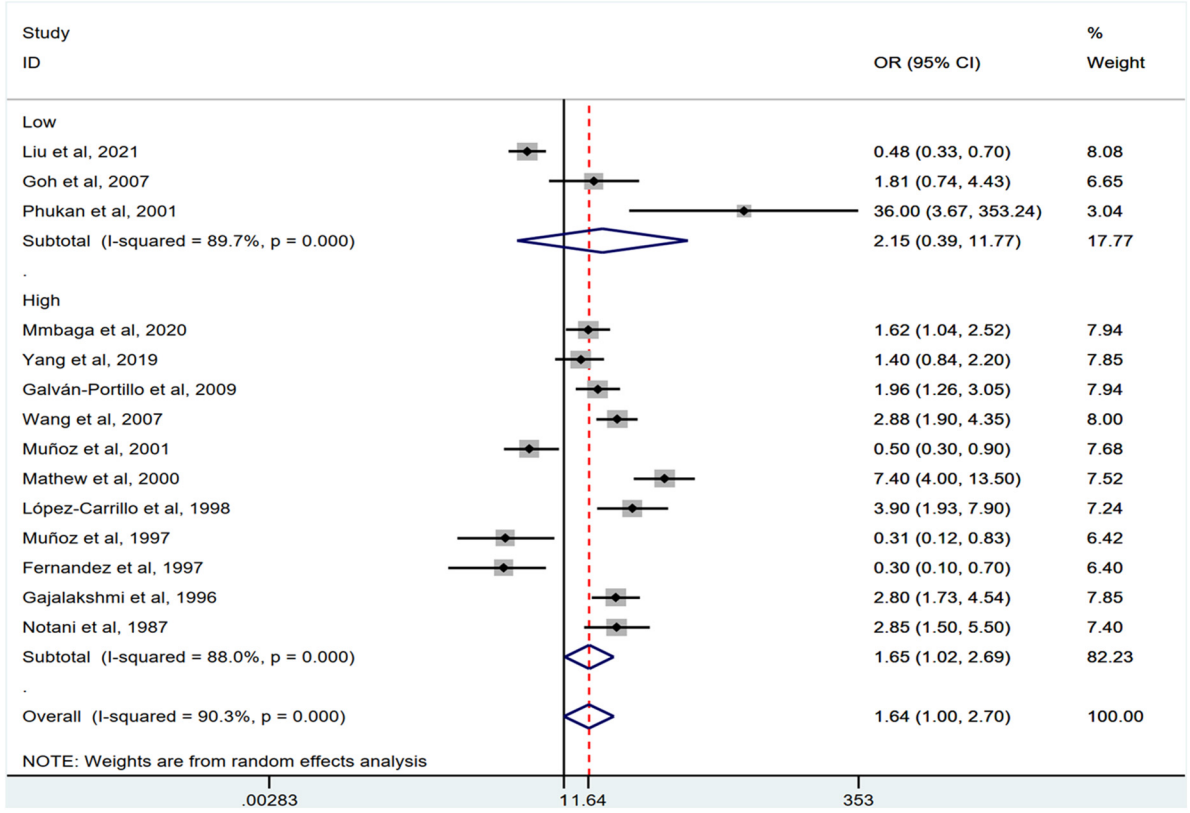

FIGURE 7
Subgroup analysis showing associations between chili pepper consumption and the risk of GI cancers based on the study quality. OR, odds ratio; CI, confidence interval.

and their epithelium is renewed more rapidly than that of the esophagus (37). Therefore, the stomach and colorectum are less affected than the esophagus. In addition, the differences are associated with different signaling pathways. Studies have shown that oral intake of CAP increases NF-κB expression (38). The methyldiazonium ion is the ultimate dimethylhydrazine (DMH) oncogenic metabolite, which is responsible for the methylation of DNA bases, leading to increased proliferation of colonic epithelial cells and triggering NF-κB activation (39, 40). NF-κB can exert numerous pro-tumorigenic functions, such as stimulating cell growth and inducing cell proliferation (41). Conversely, CAP also induces the expression of NF-κB inhibitors, of which the downregulation of Smad4 plays a role in the suppression of cell growth and invasion (42). This may explain why chili pepper is not associated with GC and CRC. For EC, several studies have demonstrated the carcinogenic effects of CAP on EC. For example, Huang et al. showed that thermo-TRPVs are functionally expressed in Eca109 and TE-1 ESCC cell lines. Hyperactivation of TRPV1 and TRPV4 facilitates the growth and/or migration of ESCC (8).

Several meta-analyses have investigated the relationship between the frequency of chili pepper consumption and GI cancer risk, with controversial results. When comparing the highest with lowest categories, most meta-analyses revealed a positive association between chili pepper intake and GC risk (11–13), while Chen et al. (14) showed a null association with the risk of GC. This discrepancy between different meta-analyses may be relevant to the inaccurate inclusion of the original literature. We investigated the eligibility of the studies included in previous meta-analyses, the results of which are summarized in **Table 3**. The aforementioned meta-analyses included studies analyzing CAP or kimchi instead of chili pepper as exposure (11–13), chili pepper mixed with other foods as exposure (12, 13), incorrect extraction of risk estimates (12, 14), and precancerous lesions rather than GI cancers as interesting outcomes (12), which may be considered as a limitation. Moreover, in a previous meta-analysis (13), the researchers inappropriately substituted continuous variables for categorical variables (highest vs. lowest) to calculate the effect estimates. To address these limitations, we performed a systematic review and meta-analysis by solely including studies that specifically reported chili pepper consumption as the exposure and GI cancers as the outcome.

When stratified by the region of the studies, those studies conducted in Asia, North America, and Africa indicated that participants consuming the highest category of chili

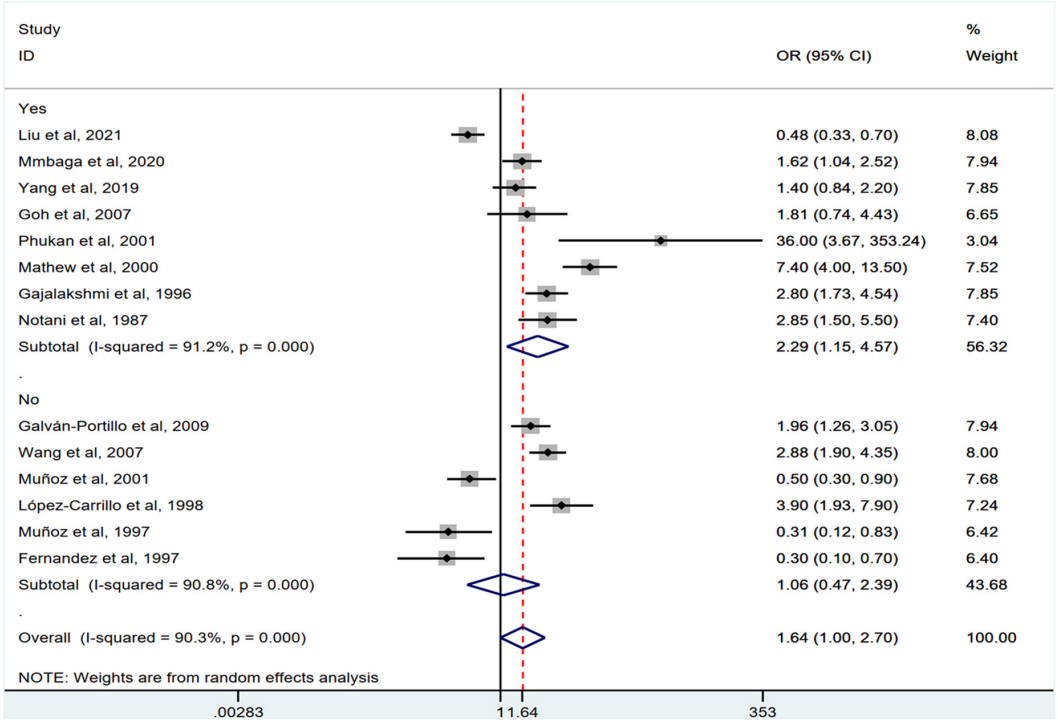

FIGURE 8
Subgroup analysis showing associations between chili pepper consumption and the risk of GI cancers based on the adjustment for alcohol intake.

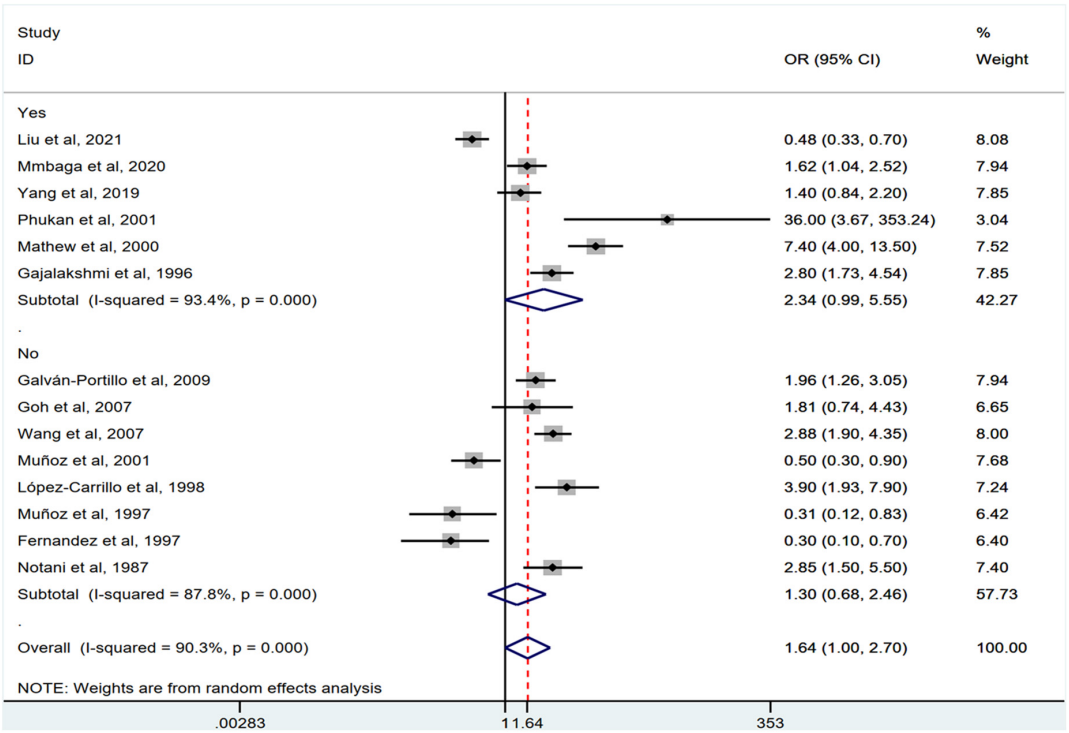

FIGURE 9
'Subgroup analysis showing associations between chili pepper consumption and the risk of GI cancers based on the adjustment for smoking.

TABLE 3 Eligibility survey of the original included literature on the relationship between chili pepper consumption and GI cancers.

Meta 1 (13)	Meta 2 (12)	Meta 3 (14)	Meta 4 (11)	Rationality of literature inclusion	Reasons
	Trujillo Rivera et al. (59)			Unreasonable	Examined the relationship between capsaicin consumption and gastric cancer
Al-qadasi et al. (60)		Al-qadasi et al. (60)		Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
	Wu et al. (61)			Unreasonable	Assessed the association between spicy food intake with precancerous lesion of gastric cancer
Xue et al. (62)				Reasonable	Chinese literature
Peng et al. (63)				Reasonable	Chinese literature
	López-Carrillo et al. (60)			Unreasonable	Examined the relationship between capsaicin consumption and gastric cancer
	Zhang et al. (65)			Unreasonable	Explored the relationship between kimchi and gastric cancer
Gómez Zuleta et al. (66)			Gómez Zuleta et al. (66)	Unclear	Non-english literature
Galván-Portillo et al. (9)			Galván-Portillo et al. (9)	Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
		Wang et al. (26)		Reasonable	A case-control study assessing the association between chili pepper consumption and esophageal squamous cell carcinoma cancer
Goh et al. (27)	Goh et al. (27)	Goh et al. (27)		Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
Bermúdez et al. (67)			Bermúdez et al. (67)	Unclear	Non-english literature
	Nan et al. (68)			Unreasonable	The study determining the risk relationship between kimchi and gastric cancer
López-Carrillo et al. (69)	López-Carrillo et al. (69)		López-Carrillo et al. (69)	Unreasonable	Examined the relationship between capsaicin consumption and gastric cancer
	Lee et al. (70)			Unreasonable	The study determining the risk relationship between kimchi and gastric cancer
Stefani et al. (71)				Unreasonable	Red pepper as a continuous variable rather than high versus low category
Muñoz et al. (22)			Muñoz et al. (22)	Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
		Phukan et al. (31)		Reasonable	A case-control study assessing the association between chili pepper consumption and esophageal cancer
Mathew et al. (25)	Mathew et al. (25)	Mathew et al. (25)		Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
Botterweck et al. (72)				Unreasonable	Red pepper as a continuous variable rather than high versus low category
López-Carrillo et al. (23)				Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
Gajalakshmi et al. (28)	Gajalakshmi et al. (28)	Gajalakshmi et al. (69)		Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
Lee et al. (73)	Lee et al. (73)			Unreasonable	The study measuring the association between hot pepper-soybean paste stew and gastric cancer

(Continued)

TABLE 3 (Continued)

Meta 1 (13)	Meta 2 (12)	Meta 3 (14)	Meta 4 (11)	Rationality of literature inclusion	Reasons
López-Carrillo et al. (21)	López-Carrillo et al. (21)	López-Carrillo et al. (21)	López-Carrillo et al. (21)	Reasonable	A case-control study investigating the association between chili pepper consumption and gastric cancer
		Notani et al. (30)		Reasonable	A case-control study investigating the association between chili pepper powder consumption and esophageal cancer
	Tajima et al. (74)	Tajima et al. (74)		Unreasonable	Incorrect risk estimates extracted

pepper had a greater risk of GI cancers, whereas three studies conducted in South America and Europe reported a significantly lower risk of GI cancers (10, 22, 29). One possible reason is that the number of included studies was relatively small, although an extensive search was done. Most original studies were conducted in Asian countries (25–28, 30–33), with only two in Europe (10, 29), two in North America (9, 23), one in Africa (24), and one in South America (22). The results should be cautiously interpreted. Moreover, most original studies reporting a lower risk of GI cancers were conducted in Europe. The evidence has shown that the estimated daily mean CAP intake in Europe was approximately 1.5 mg, which was less than the consumption level in Asia (e.g., Thailand) and North America (e.g., Mexico) (25–200 mg/person/day CAP) (43, 44). Therefore, the results of the highest than lowest or no chili pepper intake in studies conducted in Europe were more likely to obtain a protective effect, whereas the opposite effect was found in studies from Asia, North America, etc. Carcinogenicity or anticancer differences in chili pepper may depend on the dose. Further confirmation is needed to determine whether there is a U-shaped curve relationship, suggesting that a low dose of chili pepper intake might reduce GI cancer risk while a high dose intake might not.

In addition, subgroup analysis revealed that studies adjusted for alcohol consumption in the final model examining chili pepper intake and the risk of GI cancers had a positive association. Meanwhile, a seemingly stronger association between chili pepper consumption and the risk of GI cancers was observed in studies with adjustment for smoking than in those without such adjustment. The small number of original studies focusing on adjustment for smoking (n = 8) or alcohol consumption (n = 6) may be a possible reason. Another explanation is that numerous studies have found alcohol consumption or smoking to be related to a higher risk of GI cancers (45–48). However, there is currently no consensus on whether GI cancer risk is strongly associated with alcohol and smoking, because the evidence for heterogeneity by sex, age, cancer site, age at initiation, clinical stage of cancer, cancer grade, alcohol or smoking intensity, and duration is mixed (49–52). The mechanisms underlying the effects of alcohol consumption and smoking on GI cancers have not been comprehensively elucidated. These factors may affect the accuracy of the analysis. Regarding the study design, a significant association was found in the population-based case-control studies between chili pepper consumption and the risk of GI cancers but not in hospital-based case-control studies. The lack of representativeness may account for this difference.

Although a series of prespecified subgroup analyses were conducted, some heterogeneity generally persisted and could not be reduced. There were some other reasons for the heterogeneity among the included studies. First, the types of chili peppers consumed in different regions may have contributed to the heterogeneity among the results. However, few of the included studies reported specific types of chili peppers, except for two studies, in which the types of chili peppers were reported as sweet pepper (32) and red pepper powder (30), respectively. Second, stratified analysis of Helicobacter pylori (*H. pylori*) infection status was limited as data on *H. pylori* infection were only provided in one original study (27). *H. pylori* infection is a major risk factor for GI cancers. Experimental studies have suggested that combined *H. pylori* infection and CAP contribute to gastric inflammation and lead to GC with 50% incidence by regulating the expression of interleukin-6 (IL-6) and IFN-γ (53). On the other hand, chili pepper consumption may affect the *H. pylori* infection rate (54). Thus, *H. pylori* infection may act as a mediator and confound the association between chili pepper consumption and cancer risk.

Certain limitations of this study should be acknowledged. First, given the observational nature of the included studies, it is possible that the associations we found reflected residual confounding. Although a large number of potential confounders, such as cancer type, study design, and region of the study, were adjusted for in most studies, we cannot exclude that some other dietary biologically active components may partly or wholly affect the association. Second, recall bias associated

with the assessment methods of chili pepper exposure should be considered because FFQ or frequency-reported questionnaires are subject to measurement errors, which can attenuate or overestimate the observed association (55). Additional limitations related to different cooking and processing methods for chili pepper. Several studies have examined various cooking methods (roasting, boiling, steaming, and stir-frying), cooking time, and temperature of chili pepper affect their phytonutrient content (56–58). However, the studies we included did not investigate the effect of chili pepper preparation methods, which prevented us from further exploring the sources of heterogeneity. Third, the dose–response analysis could not be conducted due to the insufficient number of available studies. Finally, only studies published in English were included, which may lead to the exclusion of related studies in other languages.

Conclusion

Our results suggest that chili pepper consumption is associated with an increased risk of certain GI cancers. An increased EC risk was observed when high levels of chili pepper were ingested. However, no significant association was found between chili pepper consumption and the risk of GC and CRC. More prospective cohort studies are necessary to clarify the dose-response effect of chili pepper on the risk of GI cancers.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

CC and MZ designed the study, performed the literature search, extracted data, conducted the statistical analysis, and drafted the manuscript. CC, XZ, and HL performed data screening, interpreted the data, and performed the writing review of the manuscript. All authors contributed to the articles and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2021) 71:209–49. doi: 10.3322/caac.21660

2. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, et al. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37513025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet.* (2018) 391:1023–75. doi: 10.1016/S0140-6736(17)33326-3

3. Jarret RL, Barboza GE, Da Costa Batista FR, Berke T, Chou Y, Hulse-Kemp A, et al. Capsicum—an abbreviated compendium. *J Am Soc Hortic Sci.* (2019) 144:3–22.

4. Srinivasan K. Biological activities of red pepper (*Capsicum annuum*) and its pungent principle capsaicin: a review. *Crit Rev Food Sci Nutr.* (2016) 56:1488–500. doi: 10.1080/10408398.2013.772090

5. Li R, Lan Y, Chen C, Cao Y, Huang Q, Ho CT, et al. Anti-obesity effects of capsaicin and the underlying mechanisms: a review. *Food Funct.* (2020) 11:7356–70. doi: 10.1039/d0fo01467b

6. Ghiasi Z, Esmaeli F, Aghajani M, Ghazi-Khansari M, Faramarzi MA, Amani A. Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: an in vivo study. *Int J Pharm.* (2019) 559:341–7. doi: 10.1016/j.ijpharm.2019.01.043

7. Liu NC, Hsieh PF, Hsieh MK, Zeng ZM, Cheng HL, Liao JW, et al. Capsaicin-mediated tNOX (ENOX2) up-regulation enhances cell proliferation and migration in vitro and in vivo. *J Agric Food Chem.* (2012) 60:2758–65. doi: 10.1021/jf204869w

8. Huang R, Wang F, Yang Y, Ma W, Lin Z, Cheng N, et al. Recurrent activations of transient receptor potential vanilloid-1 and vanilloid-4 promote cellular proliferation and migration in esophageal squamous cell carcinoma cells. *Febs Open Bio.* (2019) 9:206–25. doi: 10.1002/2211-5463.12570

9. Galván-Portillo MV, Cantoral A, Oñate-Ocaña LF, Chen J, Herrera-Goepfert R, Torres-Sanchez L, et al. Gastric cancer in relation to the intake of nutrients involved in one-carbon metabolism among MTHFR 677 TT carriers. *Eur J Nutr.* (2009) 48:269–76. doi: 10.1007/s00394-009-0010-5

10. Muñoz SE, Ferraroni M, La Vecchia C, Decarli A. Gastric cancer risk factors in subjects with family history. *Cancer Epidemiol Biomarkers Prev.* (1997) 6:137–40.

11. Bonequi P, Meneses-González F, Correa P, Rabkin CS, Camargo MC. Risk factors for gastric cancer in Latin America: a meta-analysis. *Cancer Causes Control.* (2013) 24:217–31. doi: 10.1007/s10552-012-0110-z

12. Du Y, Lv Y, Zha W, Hong X, Luo Q. Chili consumption and risk of gastric cancer: a meta-analysis. *Nutr Cancer.* (2021) 73:45–54. doi: 10.1080/01635581.2020.1733625

13. Luo L, Yan J, Wang X, Sun Z. The correlation between chili pepper consumption and gastric cancer risk: a meta-analysis. *Asia Pac J Clin Nutr.* (2021) 30:130–9. doi: 10.6133/apjcn.202103_30(1).0016

14. Chen YH, Zou XN, Zheng TZ, Zhou Q, Qiu H, Chen YL, et al. High spicy food intake and risk of cancer: a meta-analysis of case-control studies. *Chin Med J.* (2017) 130:2241–50. doi: 10.4103/0366-6999.213968

15. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. *BMJ.* (2009) 339:b2535. doi: 10.1136/bmj.b2535

16. Islam MA, Alam F, Wong KK. Comorbid association of antiphospholipid antibodies and migraine: a systematic review and meta-analysis. *Autoimmun Rev.* (2017) 16:512–22. doi: 10.1016/j.autrev.2017.03.005

17. Wu GC, Liu HR, Leng RX, Li XP, Li XM, Pan HF, et al. Subclinical atherosclerosis in patients with systemic lupus erythematosus: a systemic review and meta-analysis. *Autoimmun Rev.* (2016) 15:22–37. doi: 10.1016/j.autrev.2015.10.002

18. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

19. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2

20. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629

21. López-Carrillo L, Hernández AM, Dubrow R. Chili pepper consumption and gastric cancer in Mexico: a case-control study. *Am J Epidemiol.* (1994) 139:263–71. doi: 10.1093/oxfordjournals.aje.a116993

22. Muñoz N, Plummer M, Vivas J, Moreno V, De Sanjosé S, Lopez G, et al. A case-control study of gastric cancer in Venezuela. *Int J Cancer.* (2001) 93:417–23. doi: 10.1002/ijc.1333

23. López-Carrillo L, López-Cervantes M, Ramírez-Espitia A, Rueda C, Fernández-Ortega C, Orozco-Rivadeneyra S. Alcohol consumption and gastric cancer in Mexico. *Cad Saude Publica.* (1998) 14(Suppl 3.):25–32. doi: 10.1590/s0102-311x1998000700004

24. Mmbaga EJ, Mushi BP, Deardorff K, Mgisha W, Akoko LO, Paciorek A, et al. A Case-Control study to evaluate environmental and lifestyle risk factors for esophageal cancer in tanzania. *Cancer Epidemiol Biomark Prev.* (2021) 30:305–16. doi: 10.1158/1055-9965.EPI-20-0660

25. Mathew A, Gangadharan P, Varghese C, Nair MK. Diet and stomach cancer: a case-control study in South India. *Eur J Cancer Prev.* (2000) 9:89–97. doi: 10.1097/00008469-200004000-00004

26. Wang JM, Xu B, Rao JY, Shen HB, Xue HC, Jiang QW. Diet habits, alcohol drinking, tobacco smoking, green tea drinking, and the risk of esophageal squamous cell carcinoma in the Chinese population. *Eur J Gastroenterol Hepatol.* (2007) 19:171–6. doi: 10.1097/MEG.0b013e32800ff77a

27. Goh KL, Cheah PL, Md N, Quek KF, Parasakthi N. Ethnicity and H. Pylori as risk factors for gastric cancer in Malaysia: a prospective case control study. *Am J Gastroenterol.* (2007) 102:40–5. doi: 10.1111/j.1572-0241.2006.00885.x

28. Gajalakshmi CK, Shanta V. Lifestyle and risk of stomach cancer: a hospital-based case-control study. *Int J Epidemiol.* (1996) 25:1146–53. doi: 10.1093/ije/25.6.1146

29. Fernandez E, La Vecchia C, D'Avanzo B, Negri E, Franceschi S. Risk factors for colorectal cancer in subjects with family history of the disease. *Br J Cancer.* (1997) 75:1381–4. doi: 10.1038/bjc.1997.234

30. Notani PN, Jayant K. Role of diet in upper aerodigestive tract cancers. *Nutr Cancer.* (1987) 10:103–13. doi: 10.1080/01635588709513945

31. Phukan RK, Chetia CK, Ali MS, Mahanta J. Role of dietary habits in the development of esophageal cancer in Assam, the north-eastern region of India. *Nutr Cancer.* (2001) 39:204–9. doi: 10.1207/S15327914nc392_7

32. Liu Y, Li S, Jiang L, Zhang Y, Li Z, Shi J. Solanaceous vegetables and colorectal cancer risk: a hospital-based matched case-control study in Northeast China. *Front Nutr.* (2021) 8:688897. doi: 10.3389/fnut.2021.688897

33. Yang Y, Zhang J, Weiss NS, Guo L, Zhang L, Jiang Y, et al. The consumption of chili peppers and the risk of colorectal cancer: a matched case-control study. *World J Surg Oncol.* (2019) 17:71–7. doi: 10.1186/s12957-019-1615-7

34. Lu M, Cao Y, Ho CT, Huang Q. Development of organogel-derived capsaicin nanoemulsion with improved bioaccessibility and reduced gastric mucosa irritation. *J Agric Food Chem.* (2016) 64:4735–41. doi: 10.1021/acs.jafc.6b01095

35. Lu M, Cao Y, Ho CT, Huang Q. The enhanced anti-obesity effect and reduced gastric mucosa irritation of capsaicin-loaded nanoemulsions. *Food Funct.* (2017) 8:1803–9. doi: 10.1039/c7fo00173h

36. Jordt SE, Julius D. Molecular basis for species-specific sensitivity to "hot" chili peppers. *Cell.* (2002) 108:421–30. doi: 10.1016/s0092-8674(02)00637-2

37. Wang L, Moore DC, Huang J, Wang Y, Zhao H, D-H YJ, et al. SHP2 regulates the development of intestinal epithelium by modifying OSTERIX(+) crypt stem cell self-renewal and proliferation. *Faseb J.* (2021) 35:e21106. doi: 10.1096/fj.202001091R

38. Yang MH, Jung SH, Sethi G, Ahn KS. Pleiotropic pharmacological actions of capsazepine, a synthetic analogue of capsaicin, against various cancers and inflammatory diseases. *Molecules.* (2019) 24:995. doi: 10.3390/molecules24050995

39. Perše M, Cerar A. Morphological and molecular alterations in 1,2 dimethylhydrazine and azoxymethane induced colon carcinogenesis in rats. *J Biomed Biotechnol.* (2011) 2011:473964. doi: 10.1155/2011/473964

40. Tanwar L, Vaish V, Sanyal SN. Chemoprevention of 1,2-dimethylhydrazine-induced colon carcinogenesis by a non-steroidal anti-inflammatory drug, etoricoxib, in rats: inhibition of nuclear factor kappaB. *Asian Pac J Cancer Prev.* (2009) 10:1141–6.

41. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. *Mol Cancer.* (2013) 12:86. doi: 10.1186/1476-4598-12-86

42. Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. *Nat Rev Cancer.* (2017) 17:79–92. doi: 10.1038/nrc.2016.126

43. Kwon Y. Estimation of dietary capsaicinoid exposure in Korea and assessment of its health effects. *Nutrients.* (2021) 13:2461–76. doi: 10.3390/nu13072461

44. Scientific Committee on food. *Opinion of the scientific committee on food on capsaicin.* (2002). Available online at: https://www.semanticscholar.org/paper/Opinion-of-the-Scientific-Committee-on-Food-on/ae75389416978d3a657779a9a9279932bfb2b4d1 (accessed February 26, 2002).

45. Deng W, Jin L, Zhuo H, Vasiliou V, Zhang Y. Alcohol consumption and risk of stomach cancer: a meta-analysis. *Chem Biol Interact.* (2021) 336:109365–73. doi: 10.1016/j.cbi.2021.109365

46. Zhou X, Wang L, Xiao J, Sun J, Yu L, Zhang H, et al. Alcohol consumption, DNA methylation and colorectal cancer risk: results from pooled cohort studies and mendelian randomization analysis. *Int J Cancer.* (2022) 151:33945–56. doi: 10.1002/ijc.33945

47. Rex DK, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American college of gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *Am J Gastroenterol.* (2009) 104:739–50. doi: 10.1038/ajg.2009.104

48. Oze I, Matsuo K, Ito H, Wakai K, Nagata C, Mizoue T, et al. Cigarette smoking and esophageal cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol.* (2012) 42:63–73. doi: 10.1093/jjco/hyr170

49. Huang YM, Wei PL, Ho CH, Yeh CC. Cigarette smoking associated with colorectal cancer survival: a nationwide, population-based cohort study. *J Clin Med.* (2022) 11:913–25. doi: 10.3390/jcm11040913

50. Yaegashi Y, Onoda T, Morioka S, Hashimoto T, Takeshita T, Sakata K, et al. Joint effects of smoking and alcohol drinking on esophageal cancer mortality in Japanese men: findings from the Japan collaborative cohort study. *Asian Pac J Cancer Prev.* (2014) 15:1023–9. doi: 10.7314/apjcp.2014.15.2.1023

51. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. *Ann Oncol.* (2011) 22:1958–72. doi: 10.1093/annonc/mdq653

52. Cho E, Smith-Warner SA, Ritz J, van den Brandt PA, Colditz GA, Folsom AR, et al. Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Ann Intern Med.* (2004) 140:603–13. doi: 10.7326/0003-4819-140-8-200404200-00007

53. Aziz F, Xin M, Gao Y, Chakroborty A, Khan I, Monts J, et al. Induction and prevention of gastric cancer with combined helicobacter pylori and capsaicin administration and DFMO treatment, respectively. *Cancers.* (2020) 12:816–34. doi: 10.3390/cancers12040816

54. Monno R, De Laurentiis V, Trerotoli P, Roselli AM, Ierardi E, Portincasa P. Helicobacter pylori infection: association with dietary habits and socioeconomic conditions. *Clin Res Hepatol Gastroenterol.* (2019) 43:603–7. doi: 10.1016/j.clinre.2018.10.002

55. Archer E, Marlow ML, Lavie CJ. Controversy and debate: memory-based methods paper 1: the fatal flaws of food frequency questionnaires and other

memory-based dietary assessment methods. *J Clin Epidemiol.* (2018) 104:113–24. doi: 10.1016/j.jclinepi.2018.08.003

56. Hamed M, Kalita D, Bartolo ME, Jayanty SS. Capsaicinoids, polyphenols and antioxidant activities of capsicum annuum: comparative study of the effect of ripening stage and cooking methods. *Antioxidants.* (2019) 8:364–82. doi: 10.3390/antiox8090364

57. Hwang IG, Shin YJ, Lee S, Lee J, Yoo SM. Effects of different cooking methods on the antioxidant properties of red pepper (*Capsicum annuum* L.). *Prev Nutr Food Sci.* (2012) 17:286–92. doi: 10.3746/pnf.2012.17.4.286

58. Chuah AM, Lee Y, Yamaguchi T, Takamura H, Yin L, Matoba T. Effect of cooking on the antioxidant properties of coloured peppers. *Food Chem.* (2008) 111:20–8. doi: 10.1016/j.foodchem.2008.03.022

59. Trujillo Rivera A, Sampieri CL, Morales RJ, Montero H, Acosta MH, Cruz RN, et al. Risk factors associated with gastric cancer in Mexico: education, breakfast and chili. *Rev Esp Enferm Dig.* (2018) 110:372–9. doi: 10.17235/reed.2018.5042/2017

60. Al-Qadasi FA, Shah SA, Ghazi HF. Tobacco chewing and risk of gastric cancer: a case-control study in Yemen. *East Mediterr Health J.* (2017) 22:719–26. doi: 10.26719/2016.22.10.719

61. Wu Y, Fan Y, Jiang Y, Wang Y, Liu H, Wei M. Analysis of risk factors associated with precancerous lesion of gastric cancer in patients from Eastern China: a comparative study. *J Cancer Res Ther.* (2013) 9:205–9. doi: 10.4103/0973-1482.113351

62. Xue, GP, Pan XF, Li SG, Zhao Y, Chang H, Zhao ZM, et al. Association between lifestyle factors and behaviors and risk of gastric cancer, Sichuan province. *Modern Prevent Med.* (2015) 42:1257–60.

63. Peng H, Huang F, Zhang YY, Yuan H, Zhuang Q. A casecontrol study on risk factors of stomach cancer in Jiading district. *Chin J Prev Contr Chron Dis.* (2012) 06:668–71. doi: 10.16386/j.cjpccd.issn.1004-6194.2012.06.045

64. López-Carrillo L, Camargo MC, Schneider BG, Sicinschi LA, Hernández-Ramírez RU, Correa P, et al. Capsaicin consumption, helicobacter pylori CagA status and IL1B-31C>T genotypes: a host and environment interaction in gastric cancer. *Food Chem Toxicol.* (2012) 50:2118–22. doi: 10.1016/j.fct.2012.02.043

65. Zhang YW, Eom SY, Kim YD, Song YJ, Yun HY, Park JS, et al. Effects of dietary factors and the NAT2 acetylator status on gastric cancer in Koreans. *Int J Cancer.* (2009) 125:139–45. doi: 10.1002/ijc.24328

66. Gómez Zuleta M, Otero-Regino W, Ruiz-Lobo X. Risk factors for gastric cancer in Colombian patients. *Revista Colombiana de Gastroenterologia.* (2009) 24:134–43.

67. Bermúdez C, Jesús I, Gamarra G. Blood group a and gastric cancer risk in the hospital universitario de santander (Bucaramanga, Colombia). *Acta Médica Colombiana.* (2006) 31:400–10.

68. Nan HM, Park JW, Song YJ, Yun HY, Park JS, Hyun T, et al. Kimchi and soybean pastes are risk factors of gastric cancer. *World J Gastroenterol.* (2005) 11:3175–81. doi: 10.3748/wjg.v11.i21.3175

69. López-Carrillo L, López-Cervantes M, Robles-Díaz G, Ramírez-Espitia A, Mohar-Betancourt A, Meneses-García A, et al. Capsaicin consumption, helicobacter pylori positivity and gastric cancer in Mexico. *Int J Cancer.* (2003) 106:277–82. doi: 10.1002/ijc.11195

70. Lee SA, Kang D, Shim KN, Choe JW, Hong WS, Choi H. Effect of diet and helicobacter pylori infection to the risk of early gastric cancer. *J Epidemiol.* (2003) 13:162–8. doi: 10.2188/jea.13.162

71. De Stefani E, Correa P, Boffetta P, Ronco A, Brennan P, Deneo-Pellegrini H, et al. Plant foods and risk of gastric cancer: a case-control study in Uruguay. *Eur J Cancer Prev.* (2001) 10:357–64. doi: 10.1097/00008469-200108000-00009

72. Botterweck AA, van den Brandt PA, Goldbohm RA. A prospective cohort study on vegetable and fruit consumption and stomach cancer risk in the Netherlands. *Am J Epidemiol.* (1998) 148:842–53. doi: 10.1093/oxfordjournals.aje.a009709

73. Lee JK, Park BJ, Yoo KY, Ahn YO. Dietary factors and stomach cancer: a case-control study in Korea. *Int J Epidemiol.* (1995) 24:33–41. doi: 10.1093/ije/24.1.33

74. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res.* (1985) 76:705–16.

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The prognostic impact of preoperative body mass index changes for patients with esophageal squamous cell carcinoma who underwent esophagectomy: A large-scale long-term follow-up cohort study

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Background: This study aims to investigate the relationship between preoperative body mass index changes (ΔBMI) and prognosis in patients with esophageal squamous cell carcinoma who underwent esophagectomy.

Methods: We identified 1,883 patients with esophageal squamous cell carcinoma who underwent curative resection in our department between January 2005 and December 2013. Patients were grouped into a stable body mass index (ΔBMI = 0) group and a decreased body mass index (ΔBMI < 0) group. Risk factors for ΔBMI were assessed using logistic regression analysis. The impact of ΔBMI on survival was investigated using Kaplan–Meier curves and Cox regression. A nomogram for survival prediction was constructed and validated.

Results: The results showed that T stage (OR: 1.30, 95% CI: 1.16–1.45, $P < 0.001$) and N stage (OR: 1.24, 95% CI: 1.11–1.38, $P < 0.001$) were independent risk factors for ΔBMI. The ΔBMI < 0 group had worse overall survival than the stable body mass index group (HR: 1.25, 95% CI: 1.08–1.44, $P = 0.002$). When stratified by stage, ΔBMI had the greatest prognostic impact in stage I tumors (HR: 1.82, 95%: 1.05–3.15, $P = 0.033$). In addition, multiple comparisons showed that decreasing ΔBMI correlated with worse prognosis. The ΔBMI-based nomogram presented good predictive ability with a C-index of 0.705.

Conclusion: This study demonstrates that ΔBMI < 0 had an adverse impact on the long-term survival of patients with esophageal squamous cell carcinoma undergoing esophagectomy. These results may support further investigation of preoperative nutrition support.

KEYWORDS

esophageal squamous cell carcinoma, body mass index changes, prognosis, nomogram, nutrition

Introduction

Esophageal cancer is the seventh most common malignant tumor and the sixth leading cause of cancer-related deaths worldwide (1, 2). The recently published 10-year outcome of the CROSS study reported that 49% of patients had overall disease progression in the neoadjuvant chemoradiotherapy plus surgery group (3). The prognosis for patients with esophageal cancer remains unsatisfactory. Unlike many other malignancies, esophageal cancer is more likely to cause malnutrition in patients because an obstructing tumor leads to different degrees of dysphagia. The nutritional state has been shown to be related to poor prognosis in multiple cancers (4–6). Thus, a better understanding of how the nutritional state influences cancer survival may open novel therapeutic strategies to improve cancer outcomes for patients with esophageal cancer.

Body mass index is a crucial diagnostic criterion of cancer-associated weight loss and is based on body weight and height (7). Although several studies have demonstrated the effect of preoperative body mass index (BMI) on the outcomes of patients who underwent esophagectomy for esophageal cancer (8, 9), BMI alone is not a reliable indicator of survival (10, 11). A better measure than weight alone, BMI accounts for how height might influence the effects of weight on a health response. Therefore, body mass index changes (ΔBMI) may better help to estimate the correlation between nutritional state and prognosis in various populations.

The aim of this study was to investigate the relationship between preoperative ΔBMI and prognosis in patients with esophageal squamous cell carcinoma who underwent curative esophagectomy. These findings may be helpful for the development of new potential therapeutic strategies.

Materials and methods

Study population

We conducted a retrospective review of our prospectively collected database to identify consecutive patients who underwent curative esophagectomy at West China Hospital of Sichuan University between January 2005 and December 2013. Eligible patients were previously diagnosed with esophageal cancer. Inclusion criteria included (1) esophageal squamous cell carcinoma; (2) receiving esophagectomy with or without neoadjuvant or adjuvant therapy; (3) R0 resection; and (4) at least 15 lymph nodes should be removed and assessed to achieve adequate nodal staging. Exclusion criteria included the coexistence of other malignancies. Ethics approval for this study was granted by the Ethics Committee of West China Hospital, Sichuan University (No. 2019641), and informed consent was waived.

Body mass index changes measurement

BMI was defined as weight (kg) divided by height squared (m^2). Diagnostic BMI was based on weight and height at the first visit to the outpatient clinic. BMI at 3 months before diagnosis was also recorded as the baseline BMI at the same visit, which was based on patient-reported weight and height. ΔBMI was calculated as diagnostic BMI minus baseline BMI.

Surgery

Tumor staging was based on esophagoscopy, contrast-enhanced computed tomography of the neck, chest, and abdomen, endoscopic ultrasonography, bone scan, and magnetic resonance imaging of the brain. Standard surgery included minimally invasive esophagectomy or open thoracotomy. The surgical approach, whether minimally invasive or open, was not associated with completeness of resection. The extent of lymphadenectomy included two-field lymph node dissections and was conducted in most patients. Three-field lymph node dissections were performed in only a few patients who had suspicious cervical node disease. Postoperative patients with lymph node involvement were recommended to receive adjuvant therapy.

Data collection and follow-up

We classified tumor stage according to the 7th edition of the TNM staging system of esophageal cancer (11). We recorded tumor recurrence, mortality, and survival status. Overall survival was measured as the time from operation to death. Patients alive or lost to follow-up were censored at the date of the last follow-up. The patients were followed up every 3 months for the first 2 years and every 6 months thereafter. Follow-up information was available over 5 years postoperatively or at the date of death.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (version 24, IBM, Armonk, NY) and the R programming language (version 3.6.3, Vienna, Austria). Normally distributed continuous variables are presented as the mean ± standard deviation (SD), non-normally distributed continuous variables as the median with interquartile range (IQR), and categorical variables as frequencies and percentages. Categorical variables were compared using the χ^2-test, while continuous variables were analyzed by Student's *t*-test. Risk factors were identified using Cox regression modeling. Survival analyzes were analyzed using the survival package, and Kaplan–Meier survival curves were plotted using the survminer package in R. We used the rsm package in R to develop the prediction model. For multiple comparisons, the *P*-value was adjusted using the Benjamini and Hochberg method by the fdrtool package in R. A bootstrap with 1,000 resamples was used to perform internal validation. The C-index was used to measure the prediction performance. Calibration was assessed by using the Hosmer–Lemeshow goodness-of-fit test (12). Statistical significance was set at a two-sided *P*-value less than 0.05.

Results

Basic characteristics and risk factors for ΔBMI

Of 1,883 eligible patients, 1,162 patients (61.7%) were grouped into the ΔBMI = 0 group, and 721 patients (38.3%) were grouped into the ΔBMI < 0 group. No patient had an increased body mass index in the ΔBMI < 0 group. The characteristics of the ΔBMI = 0 and ΔBMI < 0 groups are summarized in Table 1. There was no significant difference in baseline BMI between the two groups ($P = 0.083$).

On univariate analysis, T stage (OR: 1.385, 95% CI: 1.249–1.535, $P < 0.001$), N stage (OR: 1.348, 95% CI: 1.216–1.495, $P < 0.001$), and differentiation (OR: 1.195, 95% CI: 1.027–1.390, $P = 0.021$) were identified as potential risk factors and included in multivariate analysis (Table 2). On multivariate analysis, our results indicated that T stage (OR: 1.296, 95% CI: 1.163–1.445, $P < 0.001$) and N stage (OR: 1.241, 95% CI: 1.113–1.383, $P < 0.001$) were independent risk factors for body mass index changes (Table 2).

TABLE 1 Patient characteristics ($n = 1,883$).

Characteristics	Δ BMI = 0 ($n = 1,162$)	Δ BMI < 0 ($n = 721$)	*P*
Age (years)			0.360
≤55/>55	258/904	166/555	
Gender			0.355
Male/Female	953/209	597/124	
Baseline BMI	21.9 ± 2.9	22.1 ± 3.2	0.083
Tumor location			0.639
Upper/Middle/Lower	119/688/355	65/427/229	
pT stage			<0.001
T1/T2/T3	227/216/719	78/108/535	
pN stage			<0.001
N0/N1/N2/N3	676/293/155/38	338/201/127/55	
Differentiation			0.010
High/Moderate/Low	193/692/277	83/454/184	
LVI			0.221
No/Yes	1,111/51	683/38	
Surgical approach			0.914
Open/Minimal/Hybrid	1,105/15/42	684/11/26	
Adjuvant therapy			0.773
No/Yes	700/462	431/290	

ΔBMI, body mass index changes; BMI, body mass index; LVI, lymphovascular invasion.

TABLE 2 Risk factors for body mass index changes identified by logistic regression.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
T stage	1.39	1.25–1.53	<0.001	1.30	1.16–1.45	<0.001
N stage	1.35	1.22–1.50	<0.001	1.24	1.11–1.38	<0.001
Differentiation	1.20	1.03–1.40	0.021	1.06	0.91–1.25	0.443

OR, odds ratio; CI, confidence interval.

ΔBMI and prognosis in different stages

The median follow-up for the entire cohort was 34.6 months (95% CI: 32.8–36.4) using the reverse Kaplan–Meier method. The median overall survival was 36 months (95% CI: 32.4–44.4) among 1,162 patients without body mass index changes and 28.8 months (95% CI: 25.2–33.6) among 721 patients with body mass index changes. There was a significant difference in overall survival between these two groups (HR: 1.25, 95% CI: 1.08–1.44, $P = 0.002$) (Figure 1A).

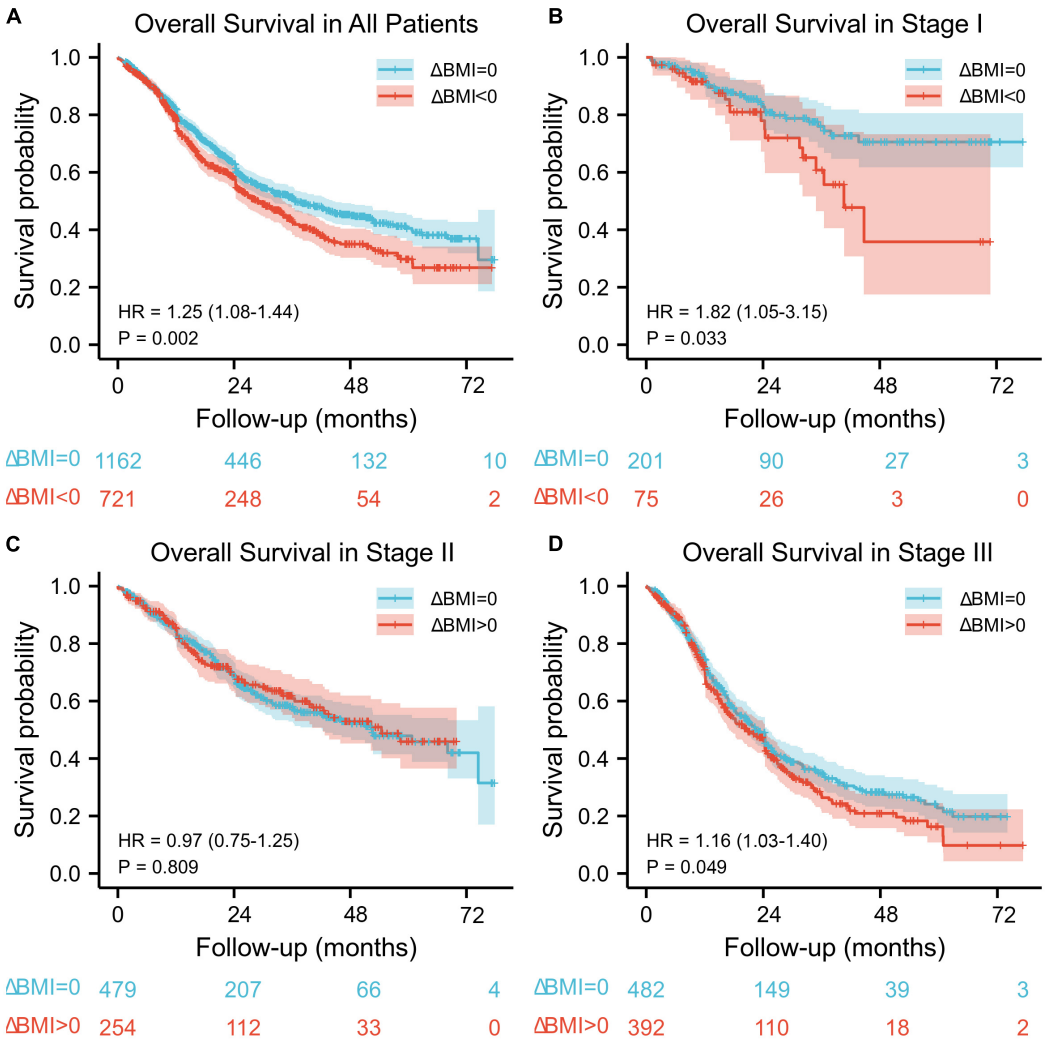

FIGURE 1
Kaplan–Meier overall survival curve according to preoperative body mass index changes for all patients **(A)** and for stage I **(B)**, stage II **(C)**, and stage III **(D)** patients.

When stratified by stage, there was a significant difference in overall survival between the ΔBMI = 0 group and ΔBMI < 0 group with stage I (HR: 1.82, 95%: 1.05–3.15, P = 0.033) and stage III (HR: 1.16, 95%: 1.03–1.40, P = 0.049) disease (Figures 1B,D), but no significant difference was found in stage II (HR: 0.97, 95%: 0.75–1.25, P = 0.809) (Figure 1C).

Determination of the optimal ΔBMI cutoff

Based on the results from the X-tile program, the optimal cutoff points for overall survival were determined to be −1.5 and −2.3 kg/m^2 (Figure 2). Accordingly, patients with 0 > ΔBMI ≥ −1.5 kg/m^2 were defined as the minor ΔBMI group, −1.5 kg/m^2 > ΔBMI ≥ −2.3 kg/m^2 moderate ΔBMI group, and ΔBMI < −2.3 kg/m^2 severe ΔBMI group. Then, we validated the cutoff points for survival stratification. The results of the Kaplan–Meier overall survival curve showed that decreasing BMI changes were associated with worse prognosis (severe ΔBMI vs. minor ΔBMI, adjusted P < 0.001; severe ΔBMI vs. moderate ΔBMI, adjusted P = 0.019; moderate ΔBMI vs. minor ΔBMI, adjusted P = 0.049) (Figure 3).

Establishment of a model

Variables with statistical significance in univariate analysis were entered into multivariate analysis. Multivariate Cox regression models determined that T stage (HR: 1.325, 95% CI: 1.210–1.451; P < 0.001), N stage (HR: 1.206, 95% CI: 1.114–1.306; P < 0.001), ΔBMI (HR: 1.174, 95% CI: 1.014–1.358;

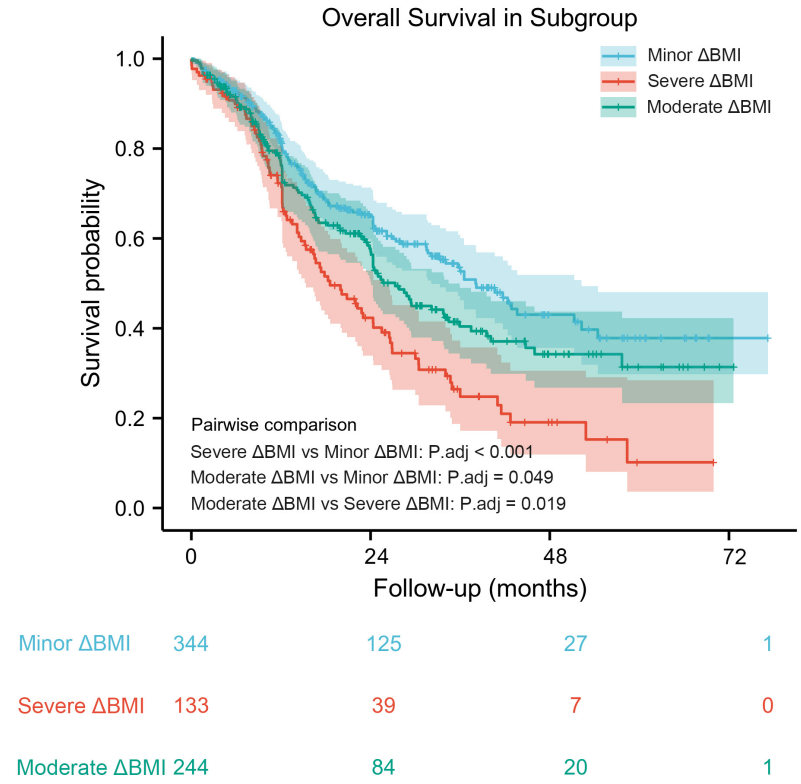

FIGURE 2
A preoperative body mass index changes-based nomogram for predicting prognosis after curative resection of esophageal squamous cell carcinoma.

P = 0.032), differentiation (HR: 1.134, 95% CI: 1.001–1.284; P = 0.047), presence of lymphovascular invasion (HR: 1.415, 1.034–1.936; P = 0.03), and adjuvant therapy (HR: 0.819, 95% CI: 0.768–0.874; $P < 0.001$) were significant predictors of overall survival (**Table 3**).

From this model, we developed a ΔBMI-based nomogram to predict the prognosis for patients with esophageal squamous cell carcinoma who underwent surgery (**Figure 4**). The Harrell C-index for the predictive nomogram was 0.706 (95% CI, 0.693–0.721) in the training cohort, which was 0.710 by bootstrapping validation. The nomogram calibration plot indicates that the nomogram was well calibrated, with mean predicted probabilities for each subgroup close to observed probabilities (**Figure 5**). The Hosmer–Lemeshow goodness-of-fit test P-value for the logistic regression model was not significant, indicating a good model fit.

Discussion

In this large-scale, long-term follow-up, retrospective, cohort study, we found that patients with ΔBMI = 0 had a median overall survival of approximately 7 months longer than the ΔBMI < 0 group. Several studies have revealed that preoperative weight loss was associated with worse long-term prognosis (13–15). Rather than measuring weight changes alone, BMI also takes into account how height might affect an individual's health. However, few studies have focused on the impact of body mass index changes on the survival of esophageal squamous cell carcinoma patients. Loehrer et al. (16) found that BMI gain compared with average adult BMI was associated with poor esophageal adenocarcinoma survival. However, the definition of BMI changes was quite different from the current study, and the sample size was too small (285 patients) to make robust conclusions.

In the subgroup analysis, we demonstrated that ΔBMI was a strong predictor of poor prognosis for stage I disease. In contrast, ΔBMI has less prognostic impact for stage III disease. Unexpectedly, no statistical significance in mortality was found between the two groups for patients with stage II disease. These findings may be due to the inherent poor prognosis of locally advanced disease diluting the strength of ΔBMI. These results also parallel the previous detection by Zhang et al. (14), in which preoperative weight loss correlated with worse survival in patients with early-stage esophageal cancer.

In the current study, the optimal ΔBMI cutoff points were also determined. Kaplan–Meier survival analysis showed that a larger body mass index decrease was associated with worse

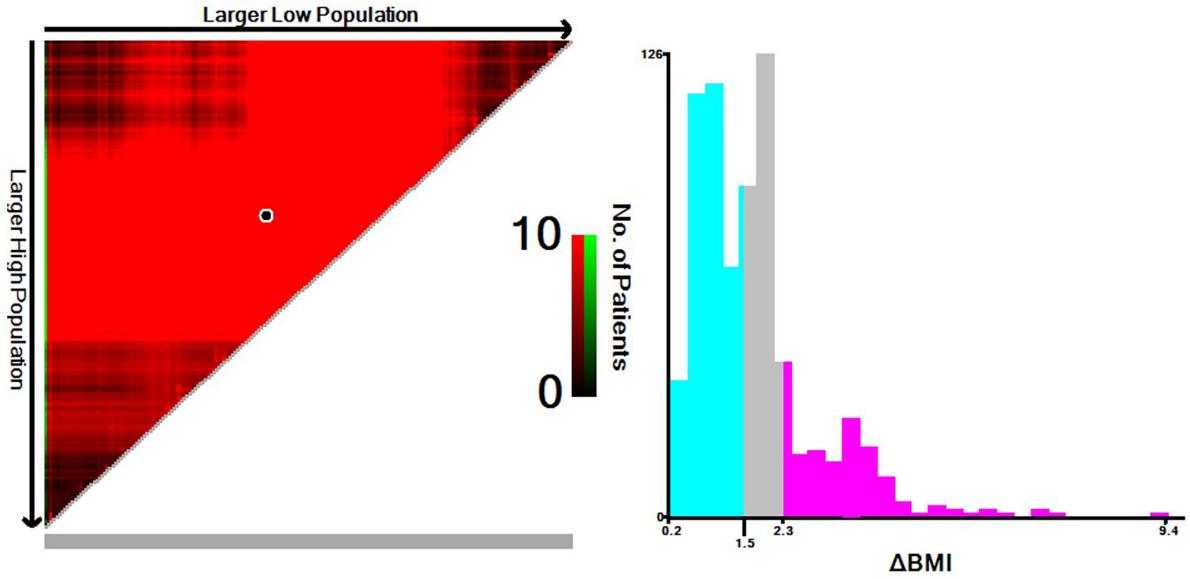

FIGURE 3
Kaplan–Meier overall survival curve stratified by optimal cutoff points in patients with decreased body mass index.

TABLE 3 Cox regression analyses of survival for 1,883 patients with esophageal cancer.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender	0.78	0.64–0.96	0.016	0.91	0.73–1.12	0.353
Age	1.23	1.03–1.48	0.02	1.11	0.93–1.33	0.256
ΔBMI	1.38	1.19–1.59	<0.001	1.17	1.01–1.36	0.032
Tumor location	0.98	0.87–1.11	0.795	–	–	–
T stage	1.53	1.41–1.67	<0.001	1.33	1.21–1.45	<0.001
N stage	1.51	1.40–1.62	<0.001	1.21	1.11–1.31	<0.001
Differentiation	1.25	1.11–1.40	<0.001	1.13	1.00–1.28	0.047
LVI	1.38	1.01–1.89	0.043	1.42	1.03–1.94	0.030
Surgical approach	1.03	0.86–1.24	0.713	–	–	–
Adjuvant therapy	0.93	0.85–0.99	0.028	0.82	0.77–0.87	<0.001

ΔBMI, body mass index changes; LVI, lymphovascular invasion; HR, hazard ratio; CI, confidence interval.

survival. These results might warrant further investigation of preoperative nutrition support for patients with severe body mass index changes, especially in early-stage (I–II) esophageal squamous cell carcinoma.

The potential weakness might be that the impact of neoadjuvant therapy on ΔBMI was not explored. Neoadjuvant chemoradiotherapy formed the standard treatment based on the CROSS trial (17) published in 2012 and the NEOCRTEC5010 trial (18) published in 2018. However, this long-term follow-up study screened patients from 2005 to 2013, and neoadjuvant therapy was not well established in that period. Most patients enrolled in this study received adjuvant therapy. However, we would like to emphasize that it would not affect the applicability of our main conclusion. To our knowledge, few studies have reported the relationship between ΔBMI and survival outcomes during neoadjuvant therapy in esophageal cancer, except for breast, rectal, and pancreatic cancer (19–21). The predictive value of ΔBMI during neoadjuvant therapy requires further investigation in future studies.

The strengths of this study include its large scale with long-term follow-up compared with previous literature (16, 22, 23). Moreover, we evaluated whether the relationship between body mass index changes and prognosis varies with tumor stage. In addition, we found that the optimal cutoff points of body mass index changes can be used to stratify patient survival. Nomograms have long been proposed as a tool for estimating an individual risk or prognosis based on clinical variables (24). Accordingly, a body mass index changes-based nomogram was established to facilitate individualized prediction of 3- and 5-year survival probability.

There were also some limitations in our study. First, it was retrospective and observational, with inherent flaws. Second, the study population was predominantly Chinese people. Globally, esophageal squamous cell carcinoma remains the most common histological type in Asia, while adenocarcinoma is the major histology in North America and Europe (25). There are differences in body mass index between the Western and Eastern populations. Thus, the generalizability of these data to the Western population requires

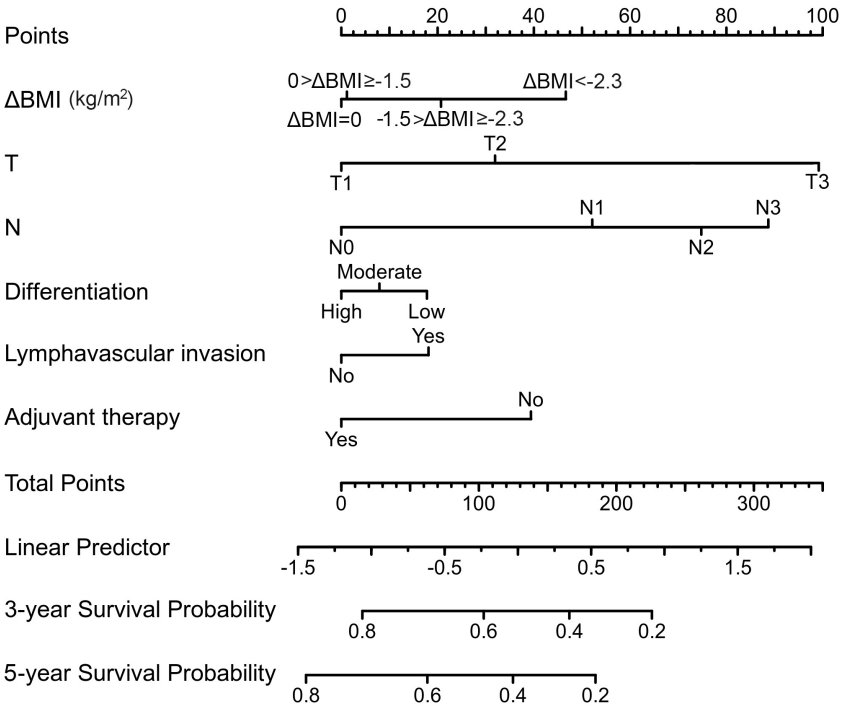

FIGURE 4
A preoperative body mass index changes-based nomogram for predicting prognosis after curative resection of esophageal squamous cell carcinoma.

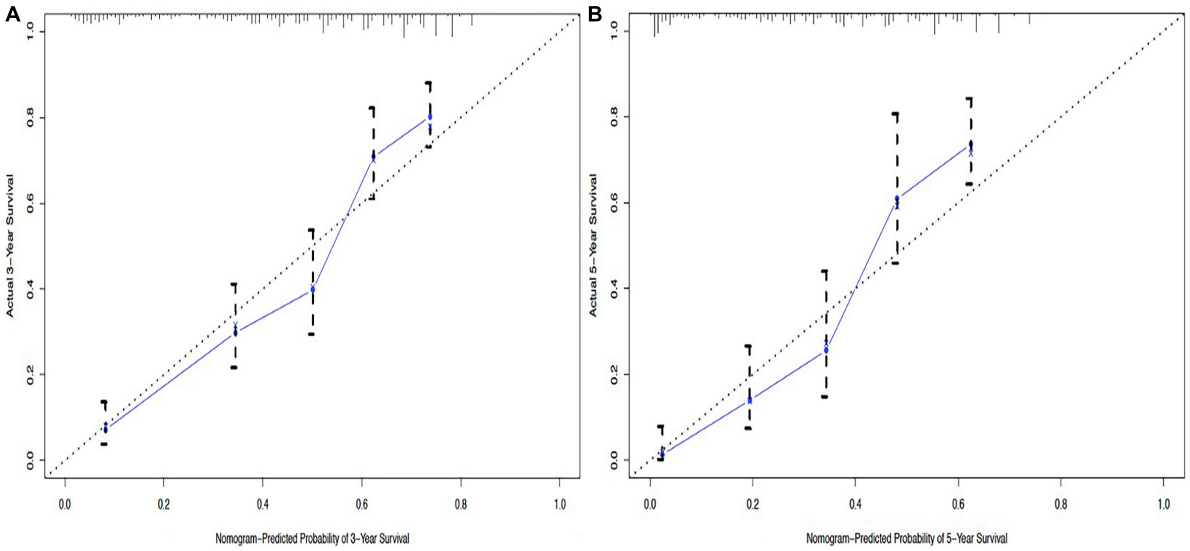

FIGURE 5
Calibration curves of the nomogram to predict 3-year overall survival **(A)** and 5-year overall survival **(B)**.

further validation. Finally, although the prediction accuracy of the model was validated internally, external validation was not performed.

In conclusion, ΔBMI < 0 had an adverse impact on long-term survival in patients with esophageal squamous cell carcinoma who underwent esophagectomy. The earlier the tumor stage was, the greater the impact. In addition, decreasing ΔBMI was associated with worse prognosis. These results might warrant further investigation of preoperative nutrition support, especially in early cancer with severe Δ BMI.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Hospital, Sichuan University. The Ethics Committee waived the requirement of written informed consent for participation.

Author contributions

L-QC and Y-MG conceptualized the study, revised the manuscript, and supervised the study. Y-MG and Q-XS collected the data, drafted the manuscript, and made the figures. H-LZ, Y-SY, YY, YH, and G-WC revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2018) 68:394–424. doi: 10.3322/caac.21492

2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. *CA Cancer J Clin.* (2016) 66:115–32. doi: 10.3322/caac.21338

3. Eyck BM, van Lanschot JJB, Hulshof M, van der Wilk BJ, Shapiro J, van Hagen P, et al. Ten-year outcome of neoadjuvant chemoradiotherapy plus surgery for esophageal cancer: the randomized controlled CROSS trial. *J Clin Oncol.* (2021) 39:1995–2004. doi: 10.1200/jco.20.03614

4. Caan BJ, Cespedes Feliciano EM, Prado CM, Alexeeff S, Kroenke CH, Bradshaw P, et al. Association of muscle and adiposity measured by computed tomography with survival in patients with nonmetastatic breast cancer. *JAMA Oncol.* (2018) 4:798–804. doi: 10.1001/jamaoncol.2018.0137

5. Thomson CA, E Crane T, Wertheim BC, Neuhouser ML, Li W, Snetselaar LG, et al. Diet quality and survival after ovarian cancer: results from the women's health initiative. *J Natl Cancer Inst.* (2014) 106:dju314. doi: 10.1093/jnci/dju314

6. Heckler M, Klaiber U, Hüttner FJ, Haller S, Hank T, Nienhüser H, et al. Prospective trial to evaluate the prognostic value of different nutritional assessment scores for survival in pancreatic ductal adenocarcinoma (NURIMAS pancreas SURVIVAL). *J Cachexia Sarcopenia Muscle.* (2021) 12:1940–7. doi: 10.1002/jcsm.12796

7. Martin L, Senesse P, Gioulbasanis I, Antoun S, Bozzetti F, Deans C, et al. Diagnostic criteria for the classification of cancer-associated weight loss. *J Clin Oncol.* (2015) 33:90–9. doi: 10.1200/jco.2014.56.1894

8. Pan W, Sun Z, Xiang Y, Fang W. The correlation between high body mass index and survival in patients with esophageal cancer after curative esophagectomy: evidence from retrospective studies. *Asia Pac J Clin Nutr.* (2015) 24:480–8. doi: 10.6133/apjcn.2015.24.3.05

9. Zhang SS, Yang H, Luo KJ, Huang QY, Chen JY, Yang F, et al. The impact of body mass index on complication and survival in resected oesophageal cancer: a clinical-based cohort and meta-analysis. *Br J Cancer.* (2013) 109:2894–903. doi: 10.1038/bjc.2013.666

10. Deng HY, Alai G, Li G, Luo J, Zhuo ZG, Lin YD. High BMI has no impact on the survival of Chinese patients with lower thoracic esophageal adenocarcinoma treated with curative esophagectomy: a propensity score-matched study. *Dis Esophagus.* (2019) 32:doy059. doi: 10.1093/dote/doy059

11. Hasegawa T, Kubo N, Ohira M, Sakurai K, Toyokawa T, Yamashita Y, et al. Impact of body mass index on surgical outcomes after esophagectomy for patients with esophageal squamous cell carcinoma. *J Gastrointest Surg.* (2015) 19:226–33. doi: 10.1007/s11605-014-2686-y

12. Kramer AA, Zimmerman JE. Assessing the calibration of mortality benchmarks in critical care: the Hosmer-Lemeshow test revisited. *Crit Care Med.* (2007) 35:2052–6. doi: 10.1097/01.Ccm.0000275267.64078.B0

13. van der Schaaf MK, Tilanus HW, van Lanschot JJ, Johar AM, Lagergren P, Lagergren J, et al. The influence of preoperative weight loss on the postoperative course after esophageal cancer resection. *J Thorac Cardiovasc Surg.* (2014) 147:490–5. doi: 10.1016/j.jtcvs.2013.07.072

14. Zhang S, Tan Y, Cai X, Luo K, Wu Z, Lu J. Preoperative weight loss is associated with poorer prognosis in operable esophageal cancer patients: a single-center retrospective analysis of a large cohort of Chinese patients. *J Cancer.* (2020) 11:1994–9. doi: 10.7150/jca.40344

15. Liu B, Cheng B, Wang C, Chen P, Cheng Y. The prognostic significance of metabolic syndrome and weight loss in esophageal squamous cell carcinoma. *Sci Rep.* (2018) 8:10101. doi: 10.1038/s41598-018-28268-2

16. Loehrer EA, Giovannucci EL, Betensky RA, Shafer A, Christiani DC. Prediagnostic adult body mass index change and esophageal adenocarcinoma survival. *Cancer Med.* (2020) 9:3613–22. doi: 10.1002/cam4.3015

17. van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med.* (2012) 366:2074–84. doi: 10.1056/NEJMoa1112088

18. Yang H, Liu H, Chen Y, Zhu C, Fang W, Yu Z, et al. Neoadjuvant chemoradiotherapy followed by surgery versus surgery alone for locally advanced squamous cell carcinoma of the esophagus (NEOCRTEC5010): a phase III multicenter, randomized, open-label clinical trial. *J Clin Oncol.* (2018) 36:2796–803. doi: 10.1200/jco.2018.79.1483

19. Kogawa T, Fujii T, Fouad TM, Liu DD, Harano K, Masuda H, et al. Impact of change in body mass index during neoadjuvant chemotherapy and survival among breast cancer subtypes. *Breast Cancer Res Treat.* (2018) 171:501–11. doi: 10.1007/s10549-018-4853-4

20. Liu H, Wei R, Li C, Zhao Z, Guan X, Yang M, et al. BMI may be a prognostic factor for local advanced rectal cancer patients treated with long-term neoadjuvant chemoradiotherapy. *Cancer Manag Res.* (2020) 12:10321–32. doi: 10.2147/cmar.S268928

21. Naumann P, Habermehl D, Welzel T, Debus J, Combs SE. Outcome after neoadjuvant chemoradiation and correlation with nutritional status in patients with locally advanced pancreatic cancer. *Strahlenther Onkol.* (2013) 189:745–52. doi: 10.1007/s00066-013-0393-3

22. Pan YP, Kuo HC, Hsu TY, Lin JY, Chou WC, Lai CH, et al. Body mass index-adjusted body weight loss grading predicts overall survival in esophageal squamous cell carcinoma patients. *Nutr Cancer.* (2021) 73:1130–7. doi: 10.1080/01635581.2020.1792950

23. Hynes O, Anandavadivelan P, Gossage J, Johar AM, Lagergren J, Lagergren P. The impact of pre- and post-operative weight loss and body mass index on prognosis in patients with oesophageal cancer. *Eur J Surg Oncol.* (2017) 43:1559–65. doi: 10.1016/j.ejso.2017.05.023

24. Kattan MW, Leung DH, Brennan MF. Postoperative nomogram for 12-year sarcoma-specific death. *J Clin Oncol.* (2002) 20:791–6. doi: 10.1200/jco.2002.20.3.791

25. Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. *Lancet.* (2013) 381:400–12. doi: 10.1016/s0140-673660643-6

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Meta-analysis of omega-3 polyunsaturated fatty acids on immune functions and nutritional status of patients with colorectal cancer

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This meta-analysis assessed the clinical significance of omega-3 polyunsaturated fatty acids (PUFAs) in the management of patients with colorectal cancer (CRC) after radical resection. We comprehensively searched electronic databases, such as EMBASE, PubMed, MEDLINE and Cochrane Library, China National Knowledge Infrastructure (CNKI), China Biomedical Database (CBM), Wanfang Electronic Database, and VIP Medical Information System (VIP) from inception to 10 April 2022. Randomized controlled trials (RCTs) of omega-3 PUFAs and conventional nutrition or blank treatments were selected. The following were evaluated in the pooled analysis: immune function-related indices (IgA, IgG, IgM, $CD3^+$, $CD4^+$, $CD8^+$, and ratio of $CD4^+/CD8^+$), nutritional status-related indices [total protein (TP), albumin (ALB), and prealbumin (PA)], and their corresponding 95% confidence intervals (CIs). Next, we conducted heterogeneity detection, sensitivity analysis, contour-enhanced funnel plot to detect possible publication bias, and meta-regression analysis. In all, 20 studies, including 1,613 patients (809 in the omega-3 PUFAs group and 804 in the control group), were selected in the final analysis. The results of the pooled analysis showed that omega-3 PUFAs significantly increased the humoral immune function indices, including IgA [standardized mean difference (SMD) = 0.54, 95% CI 0.10–0.99], IgM (SMD = 0.52, 95% CI 0.05–0.99), IgG (SMD = 0.65, 95% CI 0.47–0.84); T cell immune function indices, including $CD3^+$ (SMD = 0.73, 95% CI 0.54–0.92), $CD4^+$ (SMD = 0.76, 95% CI 0.53–0.98), and ratio of $CD4^+/CD8^+$ (SMD = 0.66, 95% CI 0.39–0.92). However, $CD8^+$ was markedly reduced after intervention of omega-3 PUFAs (SMD = −0.28, 95% CI −0.66–0.09). In addition, pooled analysis indicated that omega-3 PUFAs markedly improved

the nutritional status indicators, including TP (SMD = 0.53, 95% CI 0.17–0.88), ALB (SMD = 0.43, 95% CI 0.15–0.70), and PA (SMD = 0.46, 95% CI 0.01–0.90). The meta-regression analysis revealed that the covariates of the small sample affected the robustness and credibility of the $CD4^+$ results. Conclusively, this study suggested that omega-3 PUFAs have the potential to be used as a valid immunonutritional therapy/support for treating patients with CRC postoperatively. This meta-analysis protocol was registered in PROSPERO (no. CRD42021288487).

Systematic review registration: [https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021288487], identifier [CRD42021288487].

KEYWORDS

colorectal cancer, omega-3 polyunsaturated fatty acids, humoral immunity, T cell immunity, nutritional status, meta-analysis

Introduction

Colorectal cancer (CRC) is the most malignant tumor of the digestive system and threatens human health worldwide. The global statistical data reveal that there are approximately 1,148,515 and 732,210 new cases of colon cancer and rectal cancer, respectively, and the mortality rate of patients with CRC is approximately 9.4% (1). Furthermore, according to the American Cancer Society, CRC ranks third in the incidence of cancer among the US population. In 2021, there were 104,270 new cases of colon cancer and 45,230 new cases of rectal cancer in the United States. From 2012 to 2016, patients with CRC < 50 years increased by 2% per year while those between 50 and 64 increased by 1% per year in the United States (2). By 2035, the mortality rate from colon cancer is expected to increase by 60% and the death rate from rectal cancer is expected to increase by 71.5% (3).

The National Comprehensive Cancer Network guidelines (4, 5) and the European Society for Medical Oncology guidelines (6, 7) recommend radical surgery as a first-line treatment regimen for patients with CRC. However, the long time spent by the CRC tumor before radical resection, stress responses caused by surgical trauma, and insufficient nutritional intake may make patients susceptible to malnutrition, reduced immune function, postoperative complications, and intestinal dysfunction. Previous research revealed that the incidence of malnutrition in patients with cancer was 15–45% at the time of diagnosis, and in advanced cases, it was 80–90% (8). Additionally, the prevalence of malnutrition in patients with CRC was between 45 and 60% (9) and markedly increased with radical surgery (10). Furthermore, surgery-induced immunosuppression and immune dysfunction significantly trigger postoperative complications. Numerous studies have attributed malnutrition and immune dysfunction to postoperative complications, such as surgical site infection, anastomotic leak, intra-abdominal abscess, ureteral injury, bleeding, enteric fistula, and postoperative bowel obstruction (11–14). Thus, these complications significantly increase the hospital stay and associated medical costs as well as significantly decline the patient's quality of life and increase cancer recurrence (15, 16).

Accumulating clinical research indicates that immunonutritional therapy/support is highly effective for enhancing the nutritional status, improving immune functions, and reducing syndromes or recrudesce in patients with postoperative CRC (17–20). Omega-3 polyunsaturated fatty acids (PUFAs) are key immunonutrients that are an essential source of energy for the intestines and thereby improve intestinal functions. Studies have shown that omega-3 PUFAs have inhibitory and lethal effects on a wide variety of tumors, such as colorectal, prostate, and breast cancers (21); In addition, long-term intake of high levels of omega-3 PUFAs can effectively reduce the incidence of CRC, breast cancer, and other malignant tumor diseases (22). Furthermore, omega-3 PUFAs improve the nutritional levels after radical resection of CRC in addition to inhibiting the inflammatory response. Omega-3 PUFAs are known for the immunostatic regulation of nutrients and improve the nutritional status of patients by accelerating the synthesis of serum albumin. Moreover, they reduce the common adverse reactions associated with enteral nutrition therapy, such as nausea, vomiting, and abdominal pain, to maintain normal gastrointestinal functions and good nutritional status, thereby improving the prognosis and accelerating the recovery of patients (23). In contrast, other studies showed that omega-3 PUFAs do not markedly improve the quality of life and postoperative complications in patients with CRC (24–26). However, there are no specific guidelines for the application of omega-3 PUFAs, in terms

of time and dosage of supplementation, for patients with CRC, even the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines. The current clinical studies are heterogenous in study populations, study designs, sample quantities, and systematic approaches; hence, it is difficult to cross-examine them. Therefore, this study conducted a meta-analysis of randomized controlled trials (RCTs) in patients with CRC who received omega-3 PUFAs after radical surgery to resolve these ambiguities and assess the clinical significance of omega-3 PUFAs in these patients. Moreover, it provided substantial evidence of the effects of omega-3 PUFAs on immune functions and nutritional status in patients with postoperative CRC.

Materials and methods

Protocol registration

We previously registered the protocol in PROSPERO in January 2022 (number: CRD42021288487, https://www.crd.york.ac.uk/PROSPERO).

Inclusion criteria

The type of study was RCTs clinically involving omega-3 PUFAs in patients with postoperative CRC. The inclusion criteria should be in line with the principles of "PICOS" and be qualified as follows: (1) P: the subjects of study were definitively diagnosed with CRC (including colon and rectal cancer) and underwent radical surgery. (2) I: the experimental group added omega-3 PUFAs to the control group treatment, if the two groups of subjects received general adjuvant therapy at the same time, the adjuvant therapy should be completely consistent. (3) C: the control group was treated with conventional nutrition or blank treatment (fluid supportive therapy). (4) O: the primary outcome measures included immune function-related indicators (IgA, IgG, IgM, $CD3^+$, $CD4^+$, $CD8^+$, and the ratio of $CD4^+/CD8^+$). Secondary outcome measures included those associated with nutritional status [including total protein (TP), albumin (ALB), and prealbumin (PA)]. (5) S: patients can come from outpatient clinics or wards, and the hospital level is not limited.

Exclusion criteria

The exclusion criteria were as follows: (1) there are repetitive publications in the literature. (2) Clinical case reports, animal experiments, review papers, letters, laboratory studies, meta-analysis, reviews, or conference papers. (3) Studies with unclear diagnostic criteria and efficacy criteria. (4) Literature with incomplete or erroneous data that cannot be combined.

Search methodology

The PubMed, EMBASE, MEDLINE and Cochrane Library, Chinese Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI), Wanfang electronic databases, and VIP medicine information system (VIP) were comprehensively searched until 10 April 2022. According to the search strategies of different databases, the search was carried out by combining titles, keywords, abstracts, subject words, and the following free words: (Colon/Rectal/colorectal/neoplasm/carcinoma/tumor) AND (Omega-3 PUFAs/Ω3-PUFAs/n-3 PUFAs/Omega 3 fatty acids/Omega-3 polyunsaturated fatty acid/Fish oil) AND (immune/immunity/IgA/IgG/ IgM/ $CD3^+/CD4^+/CD8^+$/the ratio of $CD4^+/CD8^+$) AND (nutrition/nourishment/sustenance/diet/TP/Total protein/ALB/albumin/PA/prealbumin) AND (random/randomized/clinical trial/RCTs). Furthermore, citations that may be relevant were also obtained manually, and this search strategy was not limited by the language of the publications.

Risk of bias in literature screening, data extraction, and inclusion studies

Two researchers (Tinghui Yue and Kai Xiong) independently screened the literature according to the exclusion criteria for inclusion and used the data extraction table for data extraction. The contents of the extraction table include (1) the basic information of the included studies, including the author's name and publication time. (2) The basic characteristics of the study subjects (male and female), such as the number of participants, age, and number of cases in each group. (3) The specific measures and timing of the intervention. (4) The outcome indicators concerned. (5) The key elements of bias risk assessment. Bias risk assessment for inclusion of RCTs: Cochrane 5.1.0 bias risk tool was used to assess the methodological quality of the implementation literature (27), and the RevMan 5.3 software was used to generate bias risk plots. The content includes random sequence generation, allocation concealment, blinding implementation, data integrity, selective publication, and other biases (small sample size, non-equilibrium baseline), which can be divided into three levels: "low risk," "unclear," and "high risk." Cross-check the above results and discuss solutions if there are differences.

Statistical analysis

Stata version 15.0 (Stata) was used for data analysis. The immune function and nutritional status-related indicators observed in this institute are continuous variables using standardized mean difference (SMD) and its 95% confidence

interval (CI) as the statistical effect amount. Before determining pooled effects, heterogeneity between the included literature was assessed using the Q-test and the I^2 test. If $I^2 \leq 50\%$ and $P \geq 0.1$, the heterogeneity between the studies was better, and a fixed-effects model was selected for pooled analysis, and if $I^2 > 50\%$, $P < 0.1$, there is statistical heterogeneity in the results, a random-effects model was selected for pooled analysis (28, 29). The significance of pooled effects was determined using a Z–test, and $P < 0.05$ indicates a statistically significant difference. Sensitivity analysis was performed to verify the robustness of the combined results; make contour-enhanced funnel plot to analyze whether there was potential publication bias in the included studies.

Results

Results of articles screening

In all, we acquired 508 related studies from different databases. All documents were imported into the EndnoteX9 document management software and 154 duplicate studies were excluded. After reviewing the title and abstract, 209 unrelated studies were excluded for being review articles, conference abstracts, animal experiments, or case reports. Of the remaining 145 articles, 125 articles were excluded after a full-text review based on the inclusion and exclusion criteria. Finally, 20 articles were included in the meta-analysis. Figure 1 depicts the literature screening flowchart.

Study features

In all, 1,761 patients participated in the 20 studies (22, 24, 30–47), which were published between 2002 and 2021 years Among them, 809 were assigned to the omega-3 PUFAs group and 804 were assigned to the conventional nutrition or blank group. Four trials (30–33) administrated omega-3 PUFAs through enteral nutrition (EN) while 16 trials (22, 24, 34–47) administrated *via* parenteral nutrition (PN). Regarding the results of humoral immunity-related indicators, seven experiments (22, 24, 30, 32, 33, 43, 46) reported IgA indicator, six trials (22, 24, 30, 33, 43, 46) reported IgM indicator, and seven trials reported IgG indicator (22, 24, 30, 32, 33, 43, 46). Furthermore, concerning the results of T-cell immunity-related contents, seven trials reported $CD3^+$ content (22, 24, 31, 33, 39, 42, 46), 10 trials reported $CD4^+$ content (22, 24, 31, 33, 39, 42, 43, 45–47), 9 trials reported $CD8^+$ content (22, 24, 33, 39, 42, 43, 45–47), and 9 trials reported the ratio of $CD4^+/CD8^+$ (22,

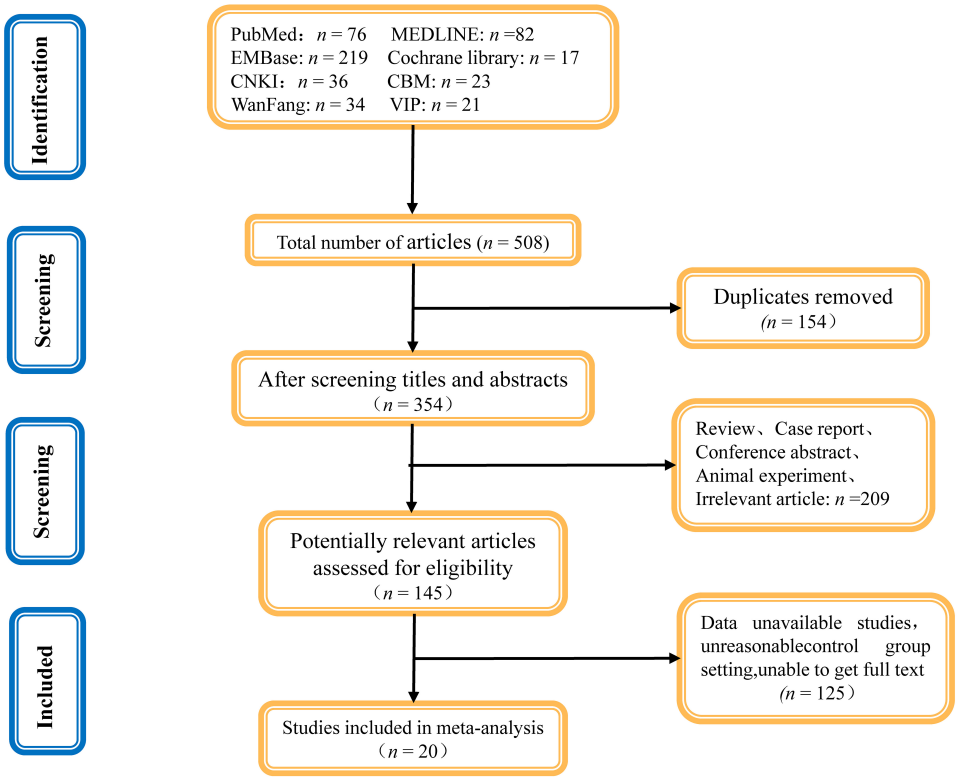

FIGURE 1
Literature screening flowchart.

24, 31, 33, 39, 42, 43, 45, 47). As for the outcomes of nutritional status, 9 trials (22, 30, 31, 36–38, 40, 44, 46) reported TP, 15 trials (22, 30–34, 36–38, 40, 41, 43–46) reported the ALB indicator, and 11 trials (22, 30, 32–35, 37, 38, 40, 41, 43) reported PA indicator. The main features of the included studies material are shown in **Table 1**.

Study quality assessment

The formation of the randomized sequence was recognized in all enrolled studies (**Figure 2A**). The allocation concealment had moderate risk in the majority of RCTs. In addition, there was no single-blinding or double-blinding in all RCTs, and none of them contained incomplete results or biased reporting. Consequently, the risk of performance bias was high, the risk of detection bias was moderate, and the implementation of concealment and blinding of some studies was unclear. Few studies did not report sample shedding, and no study selectively reported outcomes found in all trials. **Figures 2A,B** show the schematic diagram of the literature methodology quality assessment and the ratio of literature methodology quality assessment.

GRADE system evaluation results

Using the Cochrane Collaboration Network GRADE, we assessed the quality of evidence for systematic analysis. Accordingly, we used the GRADE system to assess whether omega-3 PUFAs would enhance the immune functions and improve the nutritional status of patients with CRC after radical surgery (**Figure 3**). On evaluating 10 indicators, we observed that the evidence levels of IgA, IgM, IgG, $CD3^+$, $CD4^+/CD8^+$, and TP were low, of CD8 + was very low, while that of $CD4^+$, ALB, and PA were moderate. The decrease in evidence levels is probably because of the following reasons: (1) the included studies had large deviations in randomization, allocation concealment, and blinding; (2) significant heterogeneity (48); (3) small sample size; and (4) wide confidence interval. Downgrading to a certain degree indicates selection bias of included studies. The specific degree of heterogeneity between studies and sample size results in a downgrading of the level of evidence (**Figure 3**).

Results of meta-analysis

Effect of omega-3 polyunsaturated fatty acids on humoral immune function in patients with postoperative colorectal cancer

Figure 4 shows the aggregated analysis and SMD presentation of the humoral immunity-related indicators, namely, IgA, IgM, and IgG. Heterogeneity was examined before performing a pooled analysis of these indicators. The results showed a remarkable heterogeneity for IgA (I^2 test = 81.5% and Q-test $P = 0.000$) and IgM (I^2 test = 81.9% and Q-test $P = 0.000$, **Figure 4B**); thus, we performed an aggregated analysis using the random-effects model. However, there was no remarkable heterogeneity for IgG (I^2 test = 0.0% and Q-test $P = 0.000$, **Figure 4C**); therefore, we performed an aggregated analysis using the fixed-effects model. We observed that the omega-3 PUFAs group had significantly higher IgA content ($Z = 5.042$, $P = 0.000$; SMD = 0.54, 95% CI 0.10–0.99; **Figure 4A**), IgM content ($Z = 3.887$, $P = 0.000$; SMD = 0.52, 95% CI 0.05–0.99; **Figure 4B**), and IgG content ($Z = 6.930$, $P = 0.495$; SMD = 0.65, 95% CI 0.47–0.84; **Figure 4C**) compared with the matching group. These results suggested that omega-3 PUFAs improve the humoral immune functions in patients with postoperative CRC.

Effect of omega-3 polyunsaturated fatty acids on T cell immune function in patients with postoperative colorectal cancer

Figure 5 shows the aggregated analysis and SMD presentation of the T-cell immunity-related indicators, namely, $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$. Routine heterogeneity tests were performed for each index before aggregated analysis. The results showed no remarkable heterogeneity for $CD3^+$ (I^2 test = 0.0% and Q-test $P = 0.000$, **Figure 5A**); thus, we performed an aggregated analysis using the fixed-effects model. However, there was mild heterogeneity for $CD4^+$ (I^2 test = 53.2% and Q-test $P = 0.000$, **Figure 5B**) and ratio of $CD4^+/CD8^+$ (I^2 test = 59.7% and Q-test $P = 0.000$, **Figure 5D**), while remarkable heterogeneity for $CD8^+$ (I^2 test = 82.2% and Q-test $P = 0.001$, **Figure 5C**). Hence, we performed an aggregated analysis using the random-effects model. We observed that the omega-3 PUFAs group had significantly higher $CD3^+$ index ($Z = 7.465$, $P = 0.833$; SMD = 0.73, 95% CI 0.54–0.92; **Figure 5A**), $CD4^+$ index ($Z = 10.014$, $P = 0.023$; SMD = 0.76, 95% CI 0.53–0.98; **Figure 5B**), and ratio of $CD4^+/CD8^+$ ($Z = 8.033$, $P = 0.011$; SMD = 0.66, 95% CI 0.39–0.92; **Figure 5D**) compared with the matching group. In contrast, the $CD8^+$ index was significantly decreased ($Z = -3.253$, $P = 0.000$; SMD = –0.28, 95% CI: –0.66 to 0.09; **Figure 5C**) in the omega-3 PUFAs cohort compared with the matching group.

Effect of omega-3 polyunsaturated fatty acids on nutritional status in patients with postoperative colorectal cancer

Figure 6 shows the aggregated analysis and SMD presentation of the nutritional status-related indicators, namely, TP, ALB, and PA. Routine heterogeneity tests were performed for each indicator before aggregated analysis. The results showed remarkable heterogeneity for TP (I^2 test = 81.6% and Q-test $P = 0.000$, **Figure 6A**), ALB (I^2 test = 82.1% and

TABLE 1 The main features of the included studies material.

Study ID	Sample size (n)		Ages (year)		Dose of omega-3 PUFAs	Route of administration	Tumor types	Nationality	Type of omega 3 PUFAs administered	Outcomes
	Treatment	Control	Treatment	Control						
Braga et al. (34)	50 (M/F: 28/22)	50 (M/F: 29/21)	60.5 ± 11.5	62.2 ± 10.4	POD_{1-5}: 3.3 g/L	PN	CRC (CC = NR, RC = NR)	UK	NR	⑨⑩
Gianotti et al. (37)	101 (M/F: 60/41)	102 (M/F: 56/46)	65.6 ± 11.5	63.4 ± 11.9	POD: 1 L/d	PN	CRC (CC = NR, RC = NR)	UK	NR	⑧⑨⑩
Liang et al. (39)	20 (M/F: 10/10)	21 (M/F: 15/6)	59.19 ± 10.61	55.8 ± 10.1	AOD_{1-7}: 1.2 g/(kg·d)	PN	CRC (CC = NR, RC = NR)	China	ALA, EPA, DHA	①②③④
Liu et al. (40)	20 (M/F:15/5)	20 (M/F: 14/6)	54 ± 11.3	57 ± 9.5	AOD_{1-7}: 10 g/d	PN	CRC (CC = NR, RC = NR)	China	ALA, EPA, DHA	⑧⑨⑩
Zhu et al. (47)	29 (M/F: 16/13)	28 (M/F: 17/11)	69.8 ± 10.5	70.8 ± 6.4	AOD_{1-7}: 0.2 g/(kg·d)	PN	CRC (CC = 40, RC = 17)	China	ALA, EPA, DHA	②③④
Zhuang et al. (33)	20 (M/F: NR)	20 (M/F: NR)	43–78		AOD_{1-7}: NR	EN	CRC (CC = 17, RC = 23)	China	ALA, EPA, DHA	①②③④⑤⑥⑦⑧⑨⑩
Cai et al. (32)	20 (M/F: NR)	20 (M/F: NR)	37–76		AOD_{1-7}: 3 g/d	EN	CRC (CC = 15, RC = 25)	China	NR	⑤⑥⑨⑩
Teng et al. (42)	40 (M/F: NR)	40 (M/F:NR)	42–80		AOD_{1-7}: 100 mL/d	PN	CRC (CC = NR, RC = NR)	China	EPA, DHA	①②③④
Cheng et al. (36)	30 (M/F: 18/12)	30 (M/F: 19/11)	52.63 ± 6.23	53.24 ± 8.12	$AOD_{24weeks}$: 100 mL/d	PN	CC	China	ALA, EPA, DHA	⑧⑨
Chen et al. (35)	49 (M/F: NR)	48 (M/F: NR)	18–70		POD_{1-7}-AOD_{1-3}: 2 mL/kg·d	PN	CC	China	NR	⑩
Sun et al. (41)	48 (M/F: 30/18)	48 (M/F: 35/13)	60.1 ± 5.7	61.7 ± 6.5	AOD_{1-7}: 2 ml/(Kg.d)	PN	CRC (CC = 40, RC = 56)	China	EPA, DHA	⑨⑩
Yespoli et al. (45)	70 (M/F: NR)	70 (M/F: NR)	59.3 ± 8.2	55.3 ± 9.1	AOD_{1-7}: 1.59 g/(Kg.d)	PN	CRC (CC = NR, RC = NR)	China	NR	②③④⑨

(continued)

10.3389/fnut.2022.945590

TABLE 1 (continued)

Study ID	Sample size (n)		Ages (year)		Dose of omega-3 PUFAs	Route of administration	Tumor types	Nationality	Type of omega 3 PUFAs administered	Outcomes
	Treatment	Control	Treatment	Control						
Hu et al. (22)	20 (M/F: 11/9)	20 (M/F: 12/8)	62.16 ± 5.77	59.13 ± 4.43	POD_{1-5}-AOD_{1-7}: 100 mL/d	PN	CRC (CC = 12, RC = 28)	China	EPA, DHA	①②③④⑤⑥⑦⑧⑨⑩
Song et al. (31)	34 (M/F: 20/14)	34 (M/F: 19/15)	60.84 ± 6.17	60.25 ± 5.46	AOD_{1-7}: 100 mL/d	EN	CC	China	ALA, EPA, DHA	①②④⑧⑨
Wang et al. (43)	50 (M/F: 27/23)	50 (M/F: 28/22)	60.12 ± 10.14	60.24 ± 10.09	AOD_{1-7}: 200 mL/d	PN	RC	China	EPA, DHA	②③④⑤⑥⑦⑨⑩
Jiang et al. (38)	50 (M/F: 25/25)	50 (M/F: 27/23)	61.83 ± 5.66	62.79 ± 4.87	POD_{1-7}-AOD_{1-7}: 2 mL/kg·d	PN	CRC (CC = 44, RC = 56)	China	ALA, EPA, DHA	⑧⑨⑩
Jiang and Xie (30)	41 (M/F:25/16)	36 (M/F: 24/12)	63.05 ± 5.27	63.05 ± 5.27	POD_{1-5}-AOD_{1-7}: 100 mL/d	EN	CRC (CC = 39, RC = 38)	China	EPA, DHA	⑤⑥⑦⑧⑨⑩
Yuan et al. (46)	60 (M/F: 37/23)	60 (M/F: 33/27)	55.3 ± 7.6	54.4 ± 7.0	$AOD_{24weeks}$: 10 mL/d	PN	CC	China	NR	①②③⑤⑥⑦⑧⑨
Wang and Li. (44)	27 (M/F: 15/12)	27 (M/F: 14/13)	69.69 ± 5.48		AOD_{1-7}: 0.2 g/kg·d	PN	CRC (CC = 21, RC = 33)	China	NR	⑧⑨
Liu et al. (24)	30 (M/F: 18/12)	30 (M/F: 14/16)	59 ± 11	62 ± 11	AOD_{1-5}: 100 mL/d	PN	RC	China	EPA, DHA	①②③④⑤⑥⑦

NR, not report; POD, pre-operation day; AOD, after-operation day; M, male; F, female; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CRC, colorectal cancer; RC, rectal cancer; CC, colon cancer; PN, parenteral nutrition; EN, enteral nutrition; ① CD3+; ② CD4+; ③ CD8+; ④ CD4+/CD8+; ⑤ IgA; ⑥ IgG; ⑦ IgM; ⑧ total protein (TP); ⑨ albumin (ALB); ⑩ prealbumin (PA).

10.3389/fnut.2022.945590

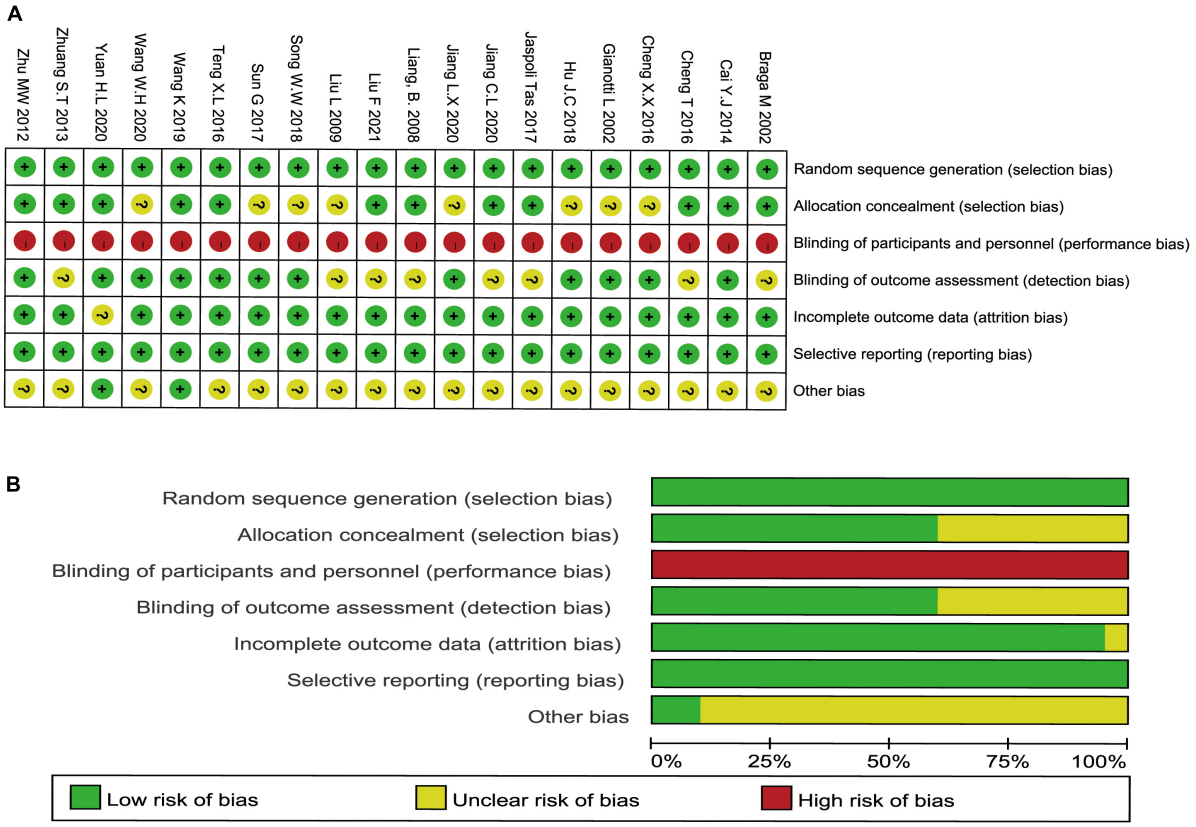

FIGURE 2
Methodological quality graph and summary of the included studies: **(A)** schematic diagram of the literature methodology quality assessment and **(B)** the ratio of literature methodology quality assessment.

Q-test $P = 0.000$, Figure 6B), and PA (I^2 test = 90.4% and *Q*-test $P = 0.000$, Figure 6C). Accordingly, we performed aggregated analysis using the random-effects model. We observed that the omega-3 PUFAs group had significantly higher TP index ($Z = 5.712$, $P = 0.000$; SMD = 0.53, 95% CI 0.17–0.88; Figure 6A), ALB index ($Z = 5.081$, $P = 0.000$; SMD = 0.43, 95% CI 0.15–0.70; Figure 6B), and PA index ($Z = 5.151$, $P = 0.000$; SMD = 0.46, 95% CI 0.01–0.90; Figure 6C) compared with the matching group in the aggregated analysis.

Sensitivity analysis for the robustness of the pooled analysis

Figure 7 shows the sensitivity analysis for the robustness of the pooled findings for $CD4^+$, ALB, and PA by excluding a study each time and considering significant heterogeneity in the included studies having a sample size ≥ 10. Sensitivity analysis of the $CD4^+$ results (Figure 7A) indicated that excluding any study did not obviously explain the heterogeneity, suggesting that the $CD4^+$ aggregated outcomes were moderately robust. In contrast, the sensitivity analyses of ALB (Figure 7B) and PA (Figure 7C) showed that missing none of the studies noticeably affected the robustness of the aggregated analysis. Based on the results of the sensitivity analyses, the aggregated outcomes have a degree of robustness.

Contour-enhanced funnel plot to detect possible publication bias

We distinguished the detailed causes of bias using the contour-enhanced funnel plot with the statistical significance level ($P < 0.01$, $P < 0.05$, $P \leq 0.1$, or $P > 0.1$) and incorporated standard milestones into the funnel charts. The results of $CD4^+$ (Figure 8A), ALB (Figure 8B), and PA (Figure 8C) showed that most of the missing studies occurred in higher statistically significant areas ($P < 0.01$), which suggested that the origin of asymmetry was probably due to undiscovered elements and not publication bias. Further, to explain the undetected bias, we traced back the primordial studies, speculating the small sample size, blinding missing, and intention-to-treat analysis of many studies; such factors would potentially affect our conclusions.

Meta-regression analysis

We performed meta-regression to assess the effect of potential confounding factors and their sources of heterogeneity on the aggregated effect estimates. These covariates are

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Treatment group	Control group	Relative (95% CI)	Absolute (95% CI)		
IgA												
7	randomised trials	serious[a]	not serious[b]	not serious	serious[c]	none	242	236	-	SMD **0.54 SD higher** (0.1 higher to 0.99 higher)	⨁⨁◯◯ Low	CRITICAL
IgM												
6	randomised trials	serious[a]	not serious[b]	not serious	serious[c]	none	221	216	-	SMD **0.52 SD more** (0.05 more to 0.99 more)	⨁⨁◯◯ Low	CRITICAL
IgG												
7	randomised trials	serious[a]	not serious	not serious	serious[c]	none	241	236	-	SMD **0.65 SD higher** (0.47 higher to 0.84 higher)	⨁⨁◯◯ Low	CRITICAL
CD3+												
7	randomised trials	serious[a]	not serious	not serious	serious[c]	none	224	225	-	SMD **0.73 SD higher** (0.54 higher to 0.92 higher)	⨁⨁◯◯ Low	CRITICAL
CD4+												
10	randomised trials	serious[a]	not serious	not serious	not serious[c]	none	373	373	-	SMD **0.76 SD higher** (0.53 higher to 0.98 higher)	⨁⨁⨁◯ Moderate	CRITICAL
CD8+												
9	randomised trials	serious[a]	serious	not serious	very serious[c,d]	publication bias strongly suspected all plausible residual confounding would reduce the demonstrated effect[a]	339	339	-	SMD **0.28 SD lower** (0.66 lower to 0.09 higher)	⨁◯◯◯ Very low	IMPORTANT
CD4+/CD8+												
9	randomised trials	serious[a]	not serious	not serious	serious[c]	none	313	313	-	SMD **0.66 SD higher** (0.39 higher to 0.92 higher)	⨁⨁◯◯ Low	CRITICAL
TP												
9	randomised trials	serious[a]	not serious[b]	not serious	not serious[c]	none	383	379	-	SMD **0.53 SD higher** (0.17 higher to 0.88 higher)	⨁⨁⨁◯ Moderate	CRITICAL
ALB												
15	randomised trials	serious[a]	not serious[b]	not serious	not serious	none	641	637	-	SMD **0.43 SD higher** (0.15 higher to 0.7 higher)	⨁⨁⨁◯ Moderate	CRITICAL
PA												
11	randomised trials	serious[a]	serious[b]	not serious	not serious	publication bias strongly suspected all plausible residual confounding would reduce the demonstrated effect[a]	469	464	-	SMD **0.46 SD higher** (0.01 higher to 0.9 higher)	⨁⨁◯◯ Low	CRITICAL

CI: confidence interval; **SMD:** standardised mean difference

Explanations

a. The included studies had large deviations in randomization, allocation concealment, and blinding
b. Significant heterogeneity
c. The sample size is small
d. The confidence interval is wider

FIGURE 3
GRADE evidence profile for all outcome measures. TP, total protein; ALB, albumin; PA, prealbumin.

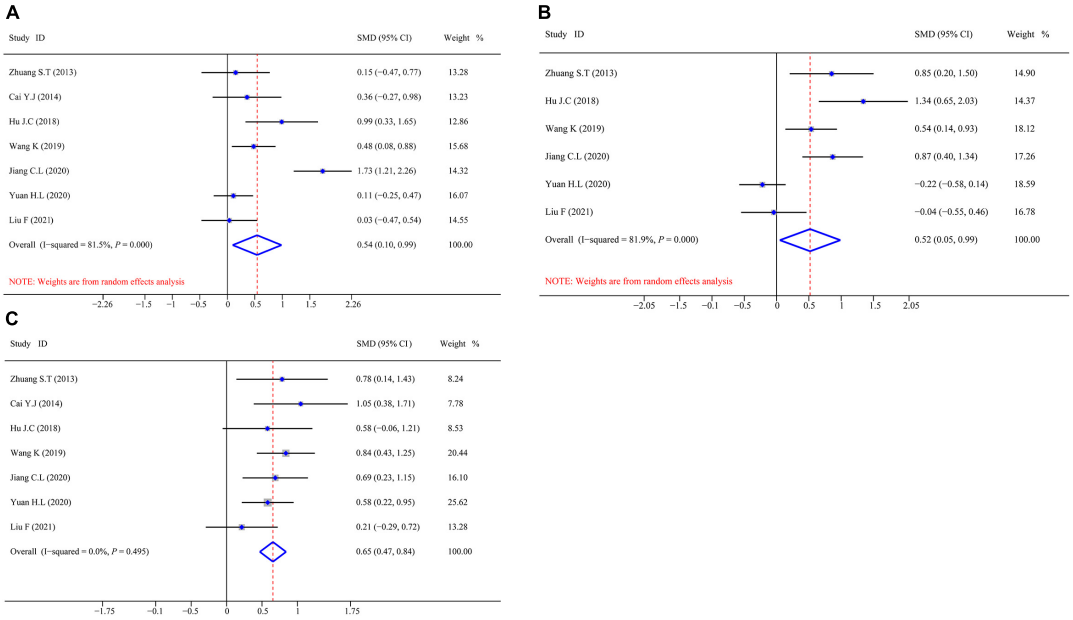

FIGURE 4
Forest plot and SMD presentation for IgA, IgM, and IgG. **(A)** Forest plot of IgA; **(B)** forest plot of IgM; and **(C)** forest plot of IgG. In all aggregated analyses, IgA and IgM used random-effects model, and IgG used fixed-effect model.

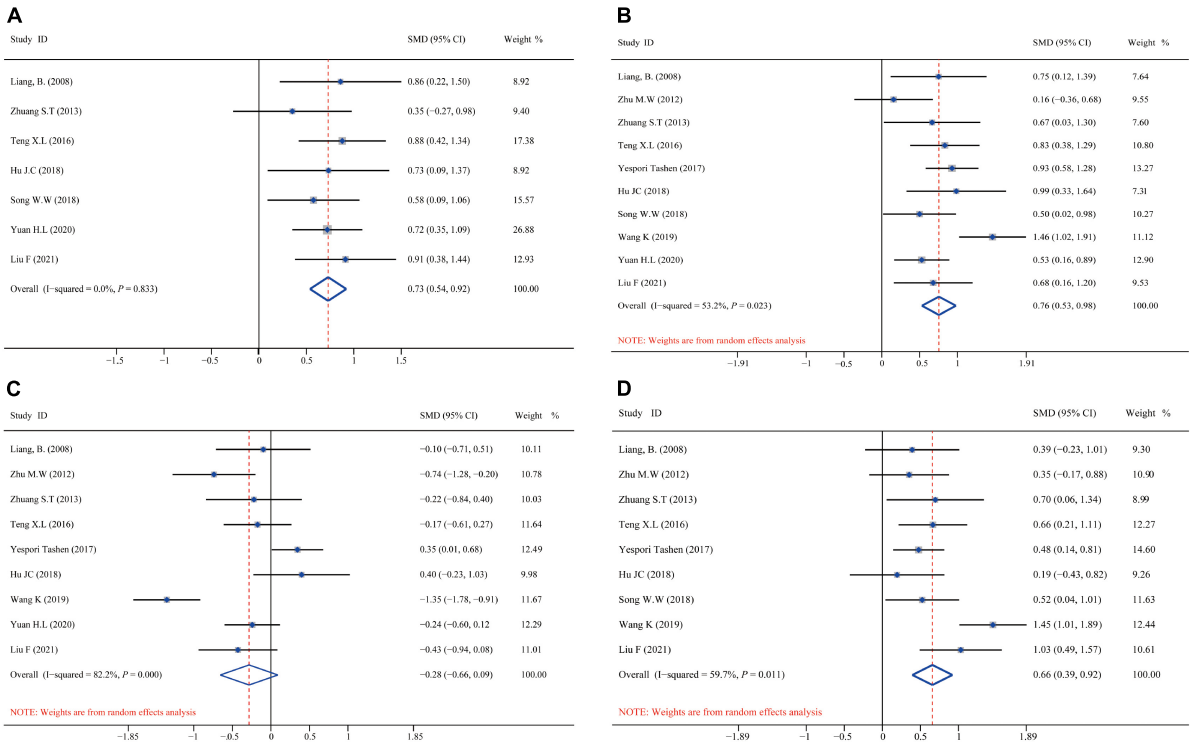

FIGURE 5
Forest plot and SMD presentation for $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$. **(A)** Forest plot of $CD3^+$; **(B)** forest plot of $CD4^+$; **(C)** forest plot of $CD8^+$; and **(D)** forest plot of $CD4^+/CD8^+$. In all aggregated analyses, $CD3^+$ used fixed-effect model, and $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ used random-effects model.

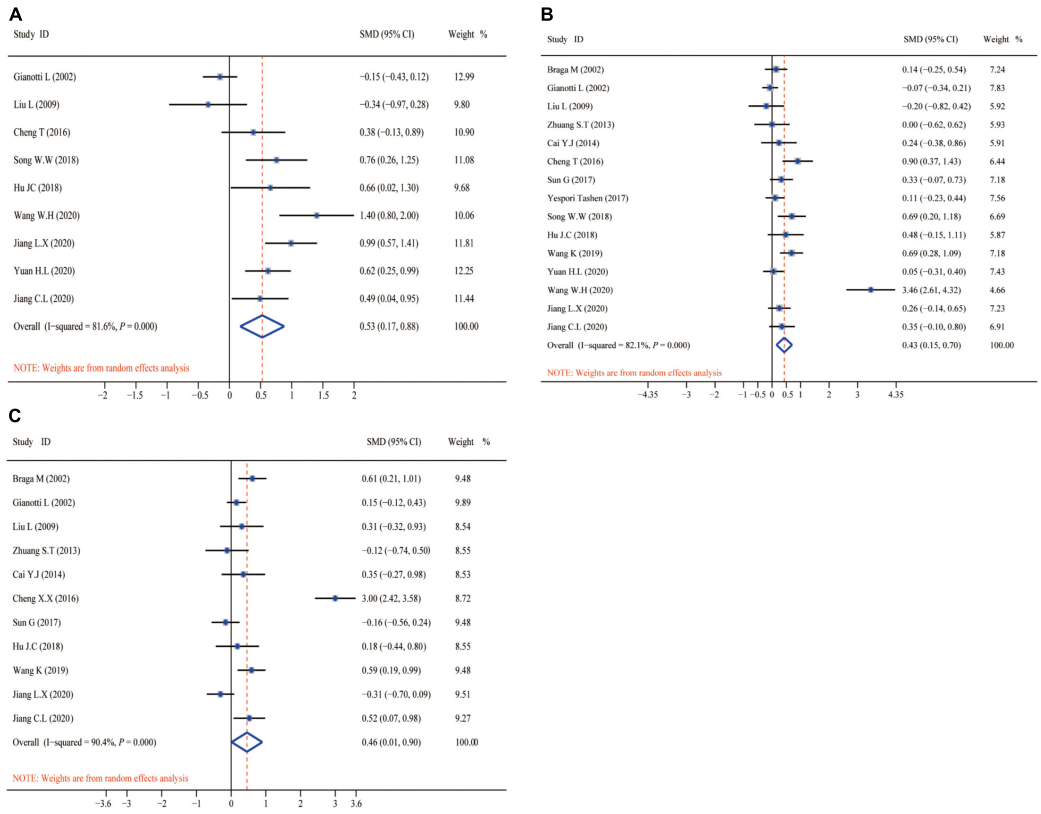

FIGURE 6
Forest plot and SMD presentation for TP, ALB, and PA. **(A)** Forest plot of TP; **(B)** forest plot of ALB; and **(C)** forest plot of PA. All aggregated analyses were used random-effect model. TP, total protein; ALB, albumin; PA, prealbumin.

speculated to be latent elements influencing heterogeneity of the combined outcomes: (1) route of administration of omega-3PUFAs (PN or EN); (2) tumor type (colon/rectal/colorectal cancer); and (3) total sample size (<100 or ≥100). According to univariate analysis, the administration route of omega-3 PUFAs and total sample size (Figure 9A and Table 2) had no remarkable effect on $CD4^+$ and ALB results ($P > 0.05$). Conversely, tumor type significantly affected the combined effect of PA ($P = 0.00$, Figure 9A and Table 2). Further, multivariate analysis was performed to assess the effect of these covariates on the combined effects. We identified that the three covariates neither affected the combined effects of $CD4^+$ and ALB nor the heterogeneity stem from this model ($P > 0.05$, Figure 9B and Table 2). In contrast, multivariate analysis demonstrated that the endpoint of PA was affected by the tumor type ($P = 0.00$, Figure 9B and Table 2), indicating that the heterogeneity may be due to this covariate.

Discussion

This study revealed that omega-3 PUFAs could significantly improve the immune functions, including humoral immune function and T-cell immune function, and nutritional status of patients suffering from CRC. We determined the levels of humoral immunity-related indicators (IgA, IgM, and IgG), T-cell immunity-related indicators ($CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$), and nutritional status-related indicators (TP, ALB, and PA) at baseline and after administering omega-3 PUFAs to the treatment group. The integrated analysis revealed that IgA (SMD = 0.54, 95% CI 0.10–0.99), IgM (SMD = 0.52, 95% CI 0.05–0.99), and IgG (SMD = 0.65, 95% CI 0.47–0.84) were significantly increased in the omega-3 PUFAs group compared with the control group. These findings demonstrate that omega-3 PUFAs effectively improve the humoral immune function of patients with CRC after surgery. In addition, the aggregated analysis revealed that $CD3^+$ (SMD = 0.73, 95% CI 0.54–0.92), $CD4^+$ (SMD = 0.76, 95% CI 0.53–0.98), and the ratio of $CD4^+/CD8^+$ (SMD = 0.66, 95% CI 0.39–0.92) were significantly higher in the omega-3 PUFAs group ($Z = 7.465$, $P = 0.833$; SMD = 0.73, 95% CI 0.54–0.92; Figure 5A) compared with the matching group. Contrarily, $CD8^+$ significantly decreased ($Z = -3.253$, $P = 0.000$; SMD = −0.28, 95% CI −0.66 to 0.09; Figure 5C) in the omega-3 PUFAs group compared with the control group. These outcomes insinuate that omega-3 PUFAs effectively

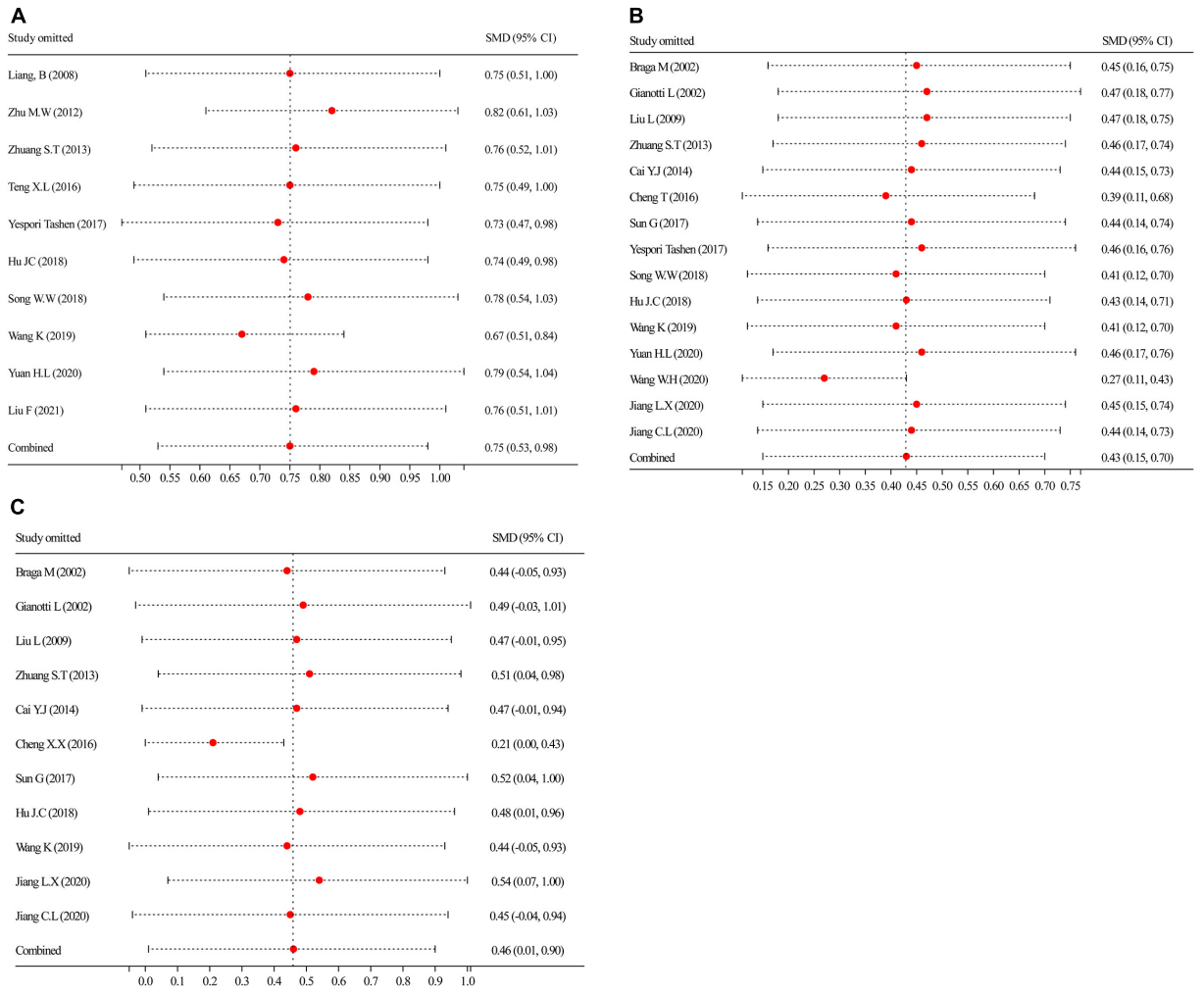

FIGURE 7
By leaving a procedure, each time was performed to carry out sensitivity analyses. **(A)** Sensitivity analysis of $CD4^+$; **(B)** sensitivity analysis of ALB; and **(C)** sensitivity analysis of PA. ALB, albumin; PA, prealbumin.

improve the T-cell immune functions of patients with CRC after surgery. Moreover, the results of comprehensive analysis showed that TP (SMD = 0.53, 95% CI 0.17–0.88), ALB (SMD = 0.43, 95% CI 0.15–0.70), and PA (SMD = 0.46, 95% CI 0.01–0.90) were significantly higher in the omega-3 PUFAs cohort compared with the matching cohort. These outcomes indicate that omega-3 PUFAs effectively enhanced the nutritional status of patients with CRC after surgery. The above supporting evidence indicates that omega-3 PUFAs are conducive to improving the immune functions and nutritional status of patients. Thus, omega-3 PUFAs are an effective immunonutritional therapy/support for treating patients with CRC after radical surgery.

The evaluation and application of immunonutritional therapy/support have largely been overlooked (49). So far, the ESPEN has recommended general immunonutrition support for malnourished patients with cancer (50), which coincides with the plan of Enhanced Recovery After Surgery (ERAS) (51). α-Linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the main components of omega-3 PUFAs (52). EPA and DHA are the main metabolites (53) and affect the structure and function of the cell membrane by competitively inhibiting the synthesis of arachidonic acid on cell membranes. Additionally, they alter the cell membrane surface receptors and regulate signal transduction, thereby modulating the inflammatory balance, inhibiting lipid peroxidation, regulating immune function, and even achieving auxiliary antitumor effects (54–57). Presently, omega-3 PUFAs are believed to play several crucial roles in the human body: (1) they inhibit arachidonic acid metabolism and thereby reduce the release of pro-inflammatory substances and thus the inflammatory response (58). They also inhibit inflammatory response by acting on cytokines related to enzymes or genes associated with the inflammatory response

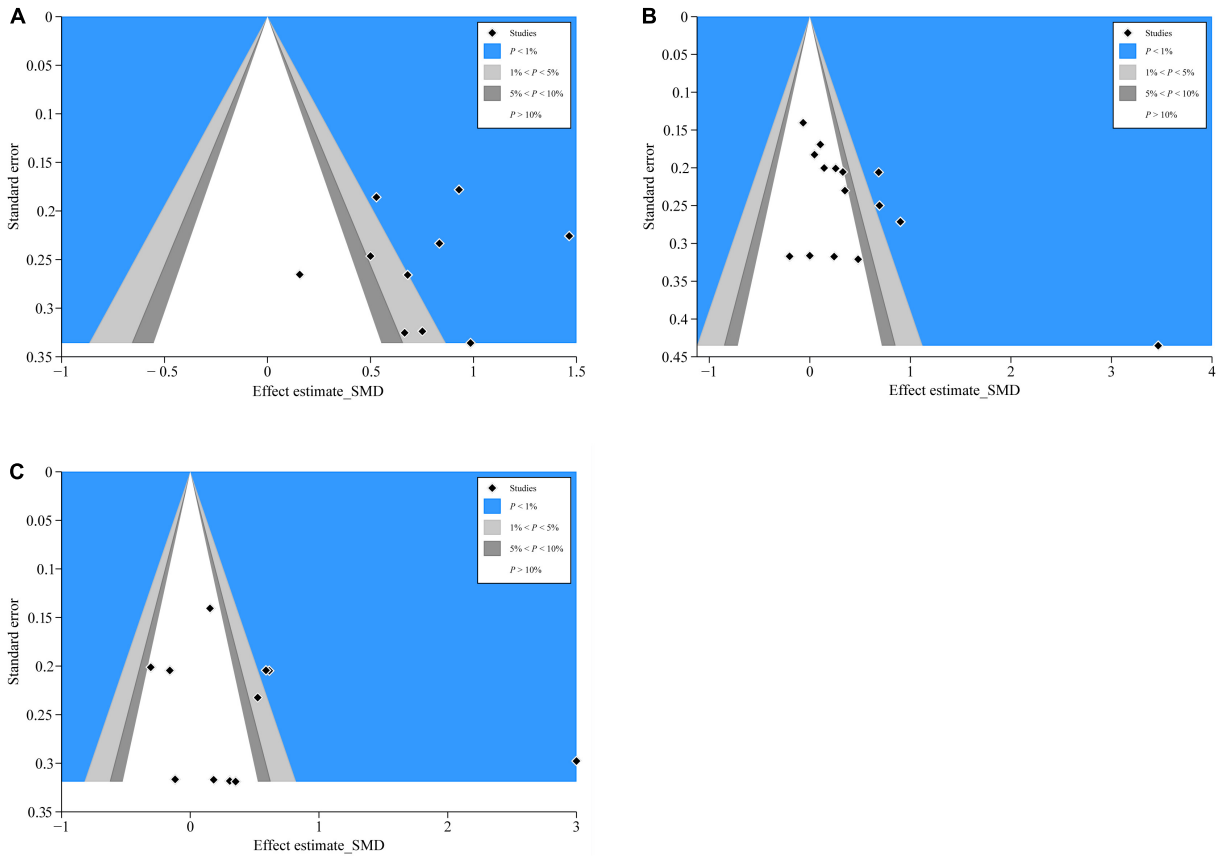

FIGURE 8
Contour-enhanced funnel plots of $CD4^+$, ALB, and PA. **(A)** $CD4^+$'s contour-enhanced funnel plot; **(B)** ALB's contour-enhanced funnel plot; and **(C)** PA's contour-enhanced funnel plot.

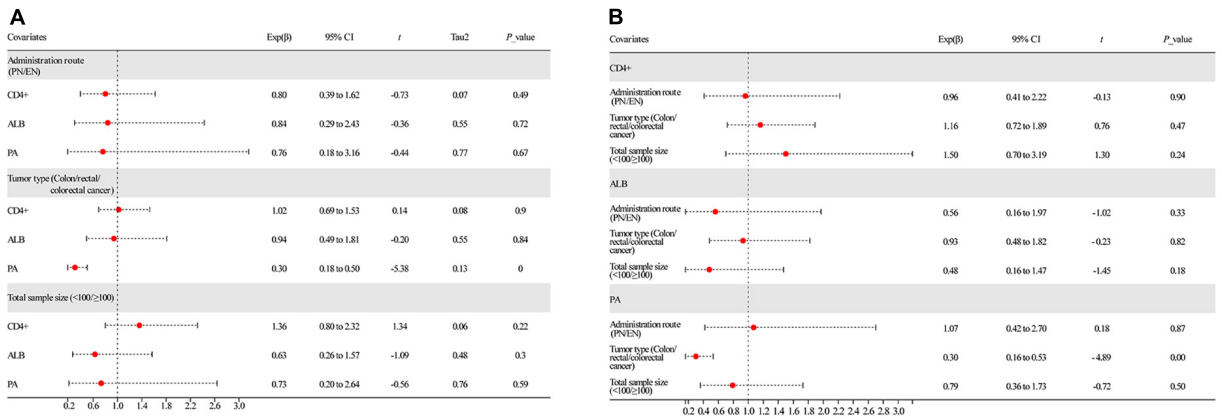

FIGURE 9
Results of meta-regression analysis. **(A)** Univariate analysis of all covariates and **(B)** multivariate analysis of all covariates. ALB, albumin; PA, prealbumin.

for decreasing the production of pro-inflammatory cytokines (59). They improve the nutritional level of patients, thereby improving the cachexia caused by cancer and the quality of life of patients (23). (2) Omega-3 PUFAs affect the integrity of specific cell membrane structures and thereon affect the normal movement of cells, receptor formation, binding of receptors and

TABLE 2 Results of meta-regression analysis.

Covariates	Univariate analysis				Multivariate analysis		
	Exponentiated coefficient	95% CI	*P*	Tau^2	Exponentiated coefficient	95% CI	*P*
Administration route (PN/EN)							
$CD4^+$ (10 studies)	0.80	0.39–1.62	0.49	0.07	0.96	0.41 to 2.22	0.90
ALB (15 studies)	0.84	0.29–2.43	0.72	0.55	0.56	0.16 to 1.97	0.33
PA (11 studies)	0.76	0.18–3.16	0.67	0.77	1.07	0.42 to 2.70	0.87
Tumor type (Colon/rectal/colorectal cancer)							
$CD4^+$ (10 studies)	1.02	0.69–1.53	0.90	0.08	1.16	0.72 to 1.89	0.47
ALB (15 studies)	0.94	0.49–1.81	0.84	0.55	0.93	0.48 to 1.82	0.82
PA (11 studies)	0.30	0.18–0.50	**0.00**	0.13	0.30	0.16 to 0.53	**0.00**
Total sample size (<100/ ≥100)							
$CD4^+$ (10 studies)	1.36	0.80–2.32	0.22	0.06	1.50	0.70 to 3.19	0.24
ALB (15 studies)	0.63	0.26–1.57	0.30	0.48	0.48	0.16 to 1.47	0.18
PA (11 studies)	0.73	0.20–2.64	0.59	0.76	0.79	0.36 to 1.73	0.50

NA, not applicable; ALB, albumin; PA, prealbumin. Significant results are in bold and underlined presentation.

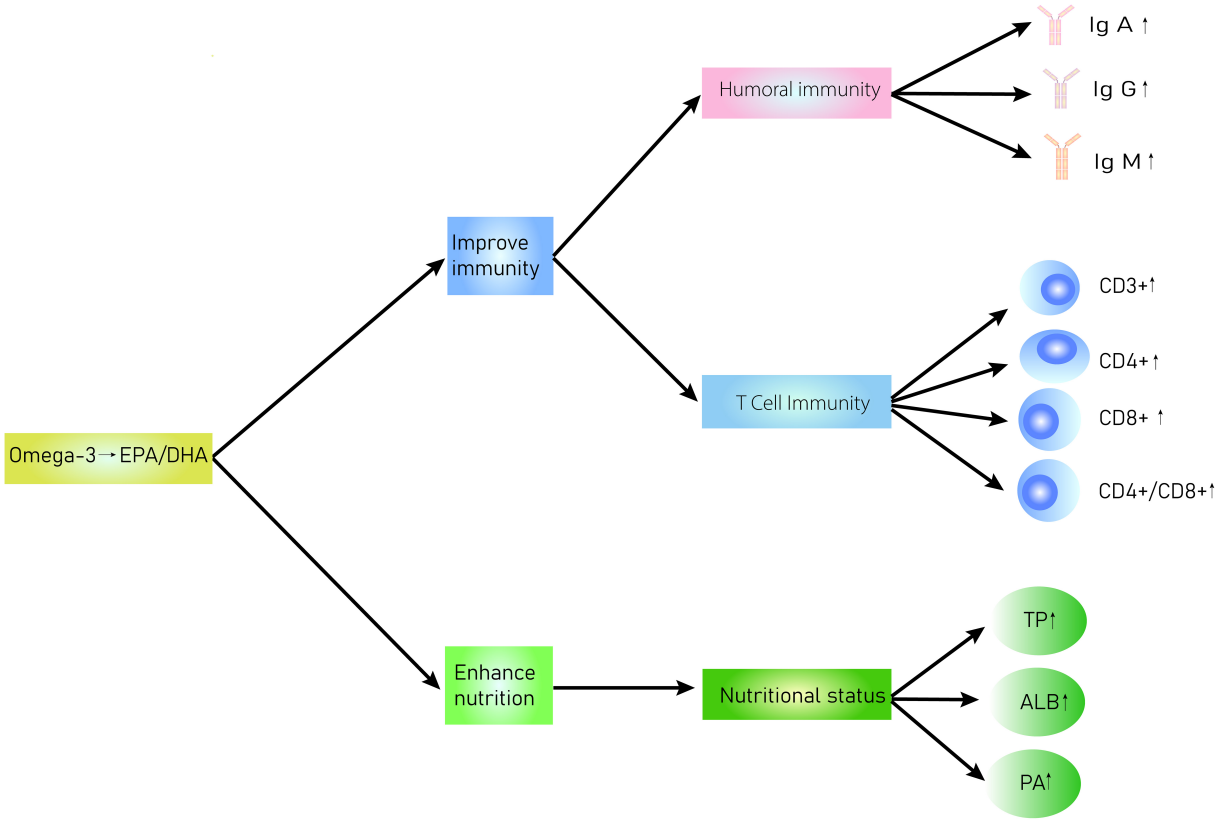

FIGURE 10
Interaction diagram of omega-3 PUFAs regulation of immune functions and nutritional status in patients with CRC.

ligands, signal transduction function of cell membranes, and ultimately disrupt the production and release of cytokines, such as inhibiting the degradation of NF-κB and COX-2 and release of inflammatory factors (60). (3) Deficiency of omega-3 PUFAs can induce the expression of prostaglandins, leukotrienes, and thromboxane A2, resulting in a severe stress response that leads to immunosuppression, platelet aggregation, and excessive inflammatory response (61). **Figure 10** shows the interaction of

omega-3 PUFAs in regulating immune functions and nutritional status. Interestingly, a clinical trial reported that omega-3 PUFAs and glutamine can improve the immune function of patients, including $CD4^+$, $CD8^+$, complement C3, IgG, and IgA, and reduce inflammatory indicators (62). Accordingly, we believe that a lack of omega-3 PUFAs may impair the immune function and nutritional status of patients with postoperative CRC. However, supplementation with omega-3 PUFAs has the potential to improve the immune functions and nutritional status of patients with postoperative CRC. Nonetheless, previous studies indicate that long-term supplementation with omega-3 PUFAs may lead to gastrointestinal disturbances, bleeding, and other adverse reactions. Thus, the justification and safety of long-term high doses of omega-3 PUFA supplementation are yet to be established. Nevertheless, the prospect of omega-3 PUFA therapy is promising. To strengthen recommendations of omega-3 PUFAs therapy for patients with CRC, further studies that focus on large-scale EN or PN omega-3 PUFAs administration and its long-term use are necessary.

This study mainly focused on the clinical benefits of omega-3 PUFAs in patients with CRC after radical surgery. However, the latent boundedness of this comprehensive analysis should be highlighted. Since the 20 studies included in this meta-analysis were neither single nor double-blind, the detection bias risk must be addressed. Meanwhile, the undiscovered bias predicted by the contour-enhanced funnel plot suggested that studies with small sample sizes and a lack of intention-to-treat analysis may explain the underlying bias. Such elements may produce a latent influence on the result. Meta-regression analysis identified the sample size of the primordial research as a latent covariate, which led to prominent heterogeneity and reduced the effectiveness of the outcomes of the aggregated analysis. Although we tried to maintain the homogeneity of included studies through strict inclusion and exclusion criteria, heterogeneity was inevitable among all the studies included in the meta-analysis. The protocol of "PICOS" was different for all included studies, which lead to clinical heterogeneity. Additionally, the original studies were conducted using different RCT methods, with large or small sample sizes, etc., which lead to methodological heterogeneity. Therefore, we attributed the significant heterogeneity between included studies to the clinical and methodological heterogeneities. Taken together, this meta-analysis of 1,613 patients from 20 RCTs provided key proof that replenishment of omega-3 PUFAs was significantly effective in improving the immune functions and nutritional status of patients with postoperative CRC. However, methodological boundedness should be noted when adopting the outcomes of this research. It is widely believed that CRC management in the preoperative or postoperative phases is in dire need of the involvement of multidisciplinary teams and long-term medication. Accordingly, there is an urgent need to increase RCTs with multidimensional efficacy and large-scale nutritional status assessments to assess the risk–benefit profile of omega-3 PUFAs in postoperative CRC management.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

THY performed the search and drafted the manuscript. THY and KX performed the data extraction and analyzed the data. TY and TX designed the study, drafted the manuscript preparation, and revision. JD, WH, TT, and SL were equally involved and contributed to the study conduction. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2021) 71:209–49. doi: 10.3322/caac.21660

2. American Cancer Society. *Colorectal Cancer Facts & **Figures 2020-2022***. Atlanta: American Cancer Society (2020).

3. Araghi M, Soerjomataram I, Jenkins M, Brierley J, Morris E, Bray F, et al. Global trends in colorectal cancer mortality: projections to the year 2035. *Int J Cancer.* (2019) 144:2992–3000. doi: 10.1002/ijc.32055

4. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. Colon cancer, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Netw.* (2021) 19:329–59. doi: 10.6004/jnccn.2021.0012

5. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. NCCN guidelines insights: Rectal Cancer, Version 6.2020. *J Natl Compr Cancer Netw.* (2020) 18:806–15. doi: 10.6004/jnccn.2020.0032

6. Vecchione L, Stintzing S, Pentheroudakis G, Douillard JY, Lordick F. ESMO management and treatment adapted recommendations in the COVID-19 era: colorectal cancer. *ESMO Open.* (2020) 5(Suppl 3):e000826. doi: 10.1136/esmoopen-2020-000826

7. Van Cutsem E, Nordlinger B, Cervantes A, Group EGW. Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. *Ann Oncol.* (2010) 21(Suppl 5):v93–7. doi: 10.1093/annonc/mdq222

8. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, et al. ESPEN guidelines on nutrition in cancer patients. *Clin Nutr.* (2017) 36:11–48. doi: 10.1016/j.clnu.2016.07.015

9. Klek S, Szybinski P, Szczepanek K. Perioperative immunonutrition in surgical cancer patients: a summary of a decade of research. *World J Surg.* (2014) 38:803–12. doi: 10.1007/s00268-013-2323-z

10. Hiramatsu K, Shindoh J, Hanaoka Y, Toda S, Ueno M, Matoba S, et al. Postoperative nutritional status is predictive of the survival outcomes in patients undergoing resection of stage iii colorectal cancer. *World J Surg.* (2021) 45:3198–205. doi: 10.1007/s00268-021-06202-4

11. Tanaka T, Sato T, Yamashita K, Hosoda K, Nakamura T, Watanabe M, et al. Effect of preoperative nutritional status on surgical site infection in colorectal cancer resection. *Dig Surg.* (2017) 34:68–77. doi: 10.1159/000448123

12. Gallardo-Valverde JM, Calañas-Continente A, Baena-Delgado E, Zurera-Tendero L, Vázquez-Martínez C, Membrives-Obrero A, et al. Obstruction in patients with colorectal cancer increases morbidity and mortality in association with altered nutritional status. *Nutr Cancer.* (2005) 53:169–76. doi: 10.1207/s15327914nc5302_6

13. Karin M, Bogut A, Hojsak I, Babić E, Volarić M, Bevanda M, et al. Nutritional status and its effect on complications in patients with colorectal cancer. *Wien Klin Wochenschr.* (2020) 132:431–7. doi: 10.1007/s00508-020-01671-4

14. Williams DGA, Ohnuma T, Krishnamoorthy V, Raghunathan K, Sulo S, Cassady BA, et al. Impact of early postoperative oral nutritional supplement utilization on clinical outcomes in colorectal surgery. *Perioper Med (Lond).* (2020) 9:29. doi: 10.1186/s13741-020-00160-6

15. Merkow RP, Hall BL, Cohen ME, Dimick JB, Wang E, Chow WB, et al. Relevance of the c-statistic when evaluating risk-adjustment models in surgery. *J Am Coll Surg.* (2012) 214:822–30. doi: 10.1016/j.jamcollsurg.2011.12.041

16. Vonlanthen R, Slankamenac K, Breitenstein S, Puhan MA, Muller MK, Hahnloser D, et al. The impact of complications on costs of major surgical procedures: a cost analysis of 1200 patients. *Ann Surg.* (2011) 254:907–13. doi: 10.1097/SLA.0b013e31821d4a43

17. Moya P, Miranda E, Soriano-Irigaray L, Arroyo A, Aguilar MD, Bellón M, et al. Perioperative immunonutrition in normo-nourished patients undergoing laparoscopic colorectal resection. *Surg Endosc.* (2016) 30:4946–53. doi: 10.1007/s00464-016-4836-7

18. Thornblade LW, Varghese TK Jr., Johnson Shi X, Ek Johnson EK, Bastawrous A, Billingham RP, et al. Preoperative immunonutrition and elective colorectal resection outcomes. *Dis Colon Rectum.* (2017) 60:68–75. doi: 10.1097/dcr.0000000000000740

19. Sanchez-Guillen L, Arroyo A. Immunonutrition in patients with colon cancer. *Immunotherapy.* (2020) 12:5–8. doi: 10.2217/imt-2019-0179

20. Horie H, Okada M, Kojima M, Nagai H. Favorable effects of preoperative enteral immunonutrition on a surgical site infection in patients with colorectal cancer without malnutrition. *Surg Today.* (2006) 36:1063–8. doi: 10.1007/s00595-006-3320-8

21. Volpato M, Hull MA. Omega-3 polyunsaturated fatty acids as adjuvant therapy of colorectal cancer. *Cancer Metastasis Rev.* (2018) 37:545–55. doi: 10.1007/s10555-018-9744-y

22. Hu JC, Yao ZW, Jiang LX, Zhang YF, Zhao DW, Zhang ZB, et al. Effects of ω-3 polyunsaturated fatty acids on perioperative nutritional and immune function in laparoscopic colorectal cancer patients with incomplete obstruction. *J Laparosc Surg.* (2018) 23:54–7. doi: 10.13499/j.cnki.fqjwkzz.2018.01.054

23. Chen GC, Qin LQ, Lu DB, Han TM, Zheng Y, Xu GZ, et al. N-3 polyunsaturated fatty acids intake and risk of colorectal cancer: meta-analysis of prospective studies. *Cancer Causes Control.* (2015) 26:133–41. doi: 10.1007/s10552-014-0492-1

24. Liu F, He G, Bai L. Effect of fish oil fat emulsion on the immune function of patients with rectal cancer after the surgery. *China Pharm.* (2021) 30:39–42. doi: 10.3969/j.issn.1006-4931.2021.08.011

25. Sorensen LS, Thorlacius-Ussing O, Schmidt EB, Rasmussen HH, Lundbye-Christensen S, Calder PC, et al. Randomized clinical trial of perioperative omega-3 fatty acid supplements in elective colorectal cancer surgery. *Br J Surg.* (2014) 101:33–42. doi: 10.1002/bjs.9361

26. Bakker N, van den Helder RS, Stoutjesdijk E, van Pelt J, Houdijk APJ. Effects of perioperative intravenous omega-3 fatty acids in colon cancer patients: a randomized, double-blind, placebo-controlled clinical trial. *Am J Clin Nutr.* (2020) 111:385–95. doi: 10.1093/ajcn/nqz281

27. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928

28. Mahat RK, Panda S, Rathore V, Swain S, Yadav L, Sah SP. The dynamics of inflammatory markers in coronavirus disease-2019 (COVID-19) patients: A systematic review and meta-analysis. *Clin Epidemiol Glob Health.* (2021) 11:100727. doi: 10.1016/j.cegh.2021.100727

29. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *Int J Infect Dis.* (2020) 96:467–74. doi: 10.1016/j.ijid.2020.05.055

30. Jiang, CL, Xie D. Effect of omega-3 polyunsaturated fatty acids on serum interleukin-8, superoxide dismutase and malondialdehyde levels in patients after laparoscopic colorectal cancer resection. *Chinese J Med.* (2020) 55:1031–3. doi: 10.3969/j.issn.1008-1070.2020.09.033

31. Song, WW, Li W, Chen ZH, Chen HS. Effect of Omega-3 polyunsaturated fatty acids on immune response and postoperative inflammatory response during perioperative period of radical resection of colon cancer. *China Med Pharmacy.* (2018) 8:19–22. doi: 10.3969/j.issn.2095-0616.2018.06.007

32. Cai, YJ, Zhuang ST, Li QZ, Wu SH, Weng ZS. Clinical observation and nursing care of colorectal cancer patients receiving early enteral nutrition immunization after surgery. *Nurs Stud.* (2014) 28:2876–7. doi: 10.3969/j.issn.10096493.2014.023.025

33. Zhuang, ST, Li QZ, Cai YJ. Effect of early enteral nutrition with ω-3 polyunsaturated fatty acid on nutritional status and body immunity of postoperative patients with colorectal cancer. *Chinese J Postgrad Med.* (2013) 36:27–30. doi: 10.3877/cma.j.issn.1674-0785.2013.16.055

34. Braga M, Gianotti L, Vignali A, Carlo VD. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery.* (2002) 132:805–14. doi: 10.1067/msy.2002.128350

35. Chen XX, Ma JC, Su H, Li Y, Cai H, Guo QJ, et al. Efficacy of omega-3 polyunsaturated fatty acids in the perioperative stage of radical colon cancer resection. *Chinese J Gen Surg.* (2016) 25:1785–91. doi: 10.3978/j.issn.1005-6947.2016.12.019

36. Cheng T, Zhu XY, Teng XL, Guo JQ, Zou WJ, Lu JY. Effect of omega-3 polyunsaturated fatty acids on nutrition and quality of life of patients undergoing chemotherapy after radical colon cancer resection. *China Mod Doctor.* (2016) 54:25–8.

37. Gianotti L, Braga M, Nespoli L, Radaelli G, Beneduce A, Di Carlo V, et al. A randomized controlled trial of preoperative oral supplementation with a specialized diet in patients with gastrointestinal cancer. *Gastroenterology.* (2002) 122:1763–70. doi: 10.1053/gast.2002.33587

38. Jiang LX, Yu XW, Song PP, Lin SB, Ye FF, Zhou SK. Omega-3 polyunsaturated fatty acids on the antitumor effect of colorectal cancer patients undergoing radical operation. *China Mod Doctor.* (2020) 58:56–9.

39. Liang B, Wang S, Ye YJ, Yang XD, Wang YL, Qu J, et al. Impact of postoperative omega-3 fatty acid-supplemented parenteral nutrition on clinical

outcomes and immunomodulations in colorectal cancer patients. *World J Gastroenterol.* (2008) 14:2434–9. doi: 10.3748/wjg.14.2434

40. Liu L, Zhuang W, Chen Z. Effect of ω-3 polyunsaturated fatty acid on inflammatory response and nutritional state of patients with gastrointestinal malignancies after operation. *Chinese J Bases Clin Gen Surg.* (2009) 16:964–7.

41. Sun, G, Xu C, Xie Q, Peng B. Effect of omega-3 polyunsaturated fatty acids on inflammatory reaction after laparoscopic surgery for colorectal cancer. *China J Mod Med.* (2017) 27:116–20. doi: 10.3969/j.issn.1005-8982.2017.12.24

42. Teng, XL, Guo JJ, Zou WJ. A study on the mechanism of the influence of omega-3 polyunsaturated fatty acids on inflammation, nutrition, immune function and prognosis in patients with radical resection of colorectal cancer. *China Mod Doctor.* (2016) 54:13–6.

43. Wang K, Zhang HH, Shi FY, Guo LL. Effect of parenteral nutrition containing fish oil fat milk on nutritional status and immune function of patients with rectal cancer after surgery. *Mod Digest Interv.* (2019) 24:885–8. doi: 10.3969/j.issn.1672-2159.2019.08.016

44. Wang WH, Li YN. Parenteral nutrition support efficacy of ω-3 poly unsaturated fatty acids on colorectal cancer patients with postoperative complication of septic shock. *Chinese J Coloproctol.* (2020) 40:17–9.

45. Yespoli T, Jayden K, Wang QS. Effects of omega-3 polyunsaturated fatty acids and glutamine on immune function and nutritional status of colorectal cancer surgery patients. *Front Med.* (2017) 7:362–3. doi: 10.3969/j.issn.2095-1752.2017.02.319

46. Yuan, HL, Ma CN, Yu YP. Effects of adding omega-3 polyunsaturated fatty acid on nutritional indicators and immune function of patients undergoing chemotherapy after colon cancer surgery. *Oncol Prog.* (2020) 18:1910–2. doi: 10.11877/j.issn.1672-1535.2020.18.18.21

47. Zhu MW, Tang DN, Hou J, Wei JM, Hua B, Sun JH, et al. Impact of fish oil enriched total parenteral nutrition on elderly patients after colorectal cancer surgery. *Chin Med J (Engl).* (2012) 125:178–81. doi: 10.3760/cma.j.issn.0366-6999.2012.02.003

48. Guyatt GH, Oxman AD, Kunz R, Woodcock J, Brozek J, Helfand M, et al. GRADE guidelines: 7. Rating the quality of evidence–inconsistency. *J Clin Epidemiol.* (2011) 64:1294–302. doi: 10.1016/j.jclinepi.2011.03.017

49. Lorenzon L, Brandl A, Guiral DC, Hoogwater F, Lundon D, Marano L, et al. Nutritional assessment in surgical oncology: An ESSO-EYSAC global survey. *Eur J Surg Oncol.* (2020) 46:2074–82. doi: 10.1016/j.ejso.2020.08.028

50. Arends J, Baracos V, Bertz H, Bozzetti F, Calder PC, Deutz NEP, et al. ESPEN expert group recommendations for action against cancer-related malnutrition. *Clin Nutr.* (2017) 36:1187–96. doi: 10.1016/j.clnu.2017.06.017

51. Ljungqvist O, Scott M, Fearon KC. Enhanced recovery after surgery: A review. *JAMA Surg.* (2017) 152:292–8. doi: 10.1001/jamasurg.2016.4952

52. Wang W, Yang J, Nimiya Y, Lee KSS, Sanidad K, Qi W, et al. ω-3 Polyunsaturated fatty acids and their cytochrome P450-derived metabolites suppress colorectal tumor development in mice. *J Nutr Biochem.* (2017) 48:29–35. doi: 10.1016/j.jnutbio.2017.06.006

53. Shityakov S, Sun C J P-C, Guu CF, Wei DT, Dandekar T. Supervised machine learning models and protein-protein interaction network analysis of gene expression profiles induced by omega-3 polyunsaturated fatty acids. *Curr Chinese Sci.* (2022) 2:118–28. doi: 10.2174/2210298102666220112114505

54. Gutiérrez S, Svahn SL, Johansson ME. Effects of Omega-3 Fatty Acids on Immune Cells. *Int J Mol Sci.* (2019) 20:5028. doi: 10.3390/ijms20205028

55. Liang P, Henning SM, Schokrpur S, Wu L, Doan N, Said J, et al. Effect of Dietary Omega-3 Fatty Acids on Tumor-Associated Macrophages and Prostate Cancer Progression. *Prostate.* (2016) 76:1293–302. doi: 10.1002/pros.23218

56. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta.* (2015) 1851:469–84. doi: 10.1016/j.bbalip.2014.08.010

57. Watson H, Mitra S, Croden FC, Taylor M, Wood HM, Perry SL, et al. A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. *Gut.* (2018) 67:1974–83. doi: 10.1136/gutjnl-2017-314968

58. Xu ZL, Shi Y, Liu JW, Zhao ZW, Pei LH. Effect and mechanism of Omega-3 polyunsaturated fatty acids in the treatment of ulcerative colitis. *Chinese J Gen Pract.* (2018) 16:561–96. doi: 10.16766/j.cnki.issn.1674-4152.000155

59. Neki K, Eto K, Kosuge M, Ohkuma M, Noaki R, Hashizume R, et al. Comparison of postoperative outcomes between laparoscopic and open surgery for colorectal cancer. *Anticancer Res.* (2017) 37:5173–7. doi: 10.21873/anticanres.11939

60. Chen YJ, Xie LJ, Zhuang YD, Guo SR. Effect of omega-3 polyunsaturated fatty acids on the inflammatory response and nerve damage in severe traumatic brain injury patients. *Chinese J Clin Nutr.* (2015) 23:224–8. doi: 10.3760/cma.j.issn.1674-635X.2015.04.006

61. Li QZ, Zhuang ST, Cai YJ, Zhang MX, Wu SH. Effects of ω-3 polyunsaturated fatty acid on nutrition status, inflammatory response and prognosis of postoperative patients with colorectal cancer. *Parent Enteral Nutr.* (2013) 20:196–204. doi: 10.16151/j.1007-810x.2013.04.020

62. Chuntrasakul C, Siltharm S, Sarasombath S, Sittapairochana C, Leowattana W, Chockvivatanavanit S, et al. Metabolic and immune effects of dietary arginine, glutamine and omega-3 fatty acids supplementation in immunocompromised patients. *J Med Assoc Thai.* (1998) 81:334–43.

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Effects of preoperative carbohydrate loading on recovery after elective surgery: A systematic review and Bayesian network meta-analysis of randomized controlled trials

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Background: Preoperative carbohydrate loading is an important element of the enhanced recovery after surgery (ERAS) paradigm in adult patients undergoing elective surgery. However, preoperative carbohydrate loading remains controversial in terms of improvement in postoperative outcomes and safety. We conducted a Bayesian network meta-analysis to evaluate the effects and safety of different doses of preoperative carbohydrates administrated in adult patients after elective surgery.

Methods: MEDLINE (PubMed), Web of Science, EMBASE, EBSCO, the Cochrane Central Register of Controlled Trials, and China National Knowledge Infrastructure (CNKI) were searched to identify eligible trials until 16 September 2022. Outcomes included postoperative insulin resistance, residual gastric volume (RGV) during the surgery, insulin sensitivity, fasting plasma glucose (FPG), fasting serum insulin (Fin) level, the serum levels of C-reactive protein (CRP), postoperative scores of pain, patients' satisfaction, thirst, hunger, anxiety, nausea and vomit, fatigue, and weakness within the first 24 h after surgery and the occurrences of postoperative infection. The effect sizes were estimated using posterior mean difference (continuous variables) or odds ratios (dichotomous variables) and 95 credible intervals (CrIs) with the change from baseline in a Bayesian network meta-analysis with random effect.

Results: Fifty-eight articles (N = 4936 patients) fulfilled the eligibility criteria and were included in the meta-analysis. Both preoperative oral low-dose carbohydrate loading (MD: −3.25, 95% CrI: −5.27 to −1.24) and oral high-dose carbohydrate loading (MD: −2.57, 95% CrI: −4.33 to −0.78) were associated with postoperative insulin resistance compared to placebo/water. When trials at high risk of bias were excluded, association with insulin resistance was

found for oral low-dose carbohydrate loading compared with placebo/water (MD: −1.29, 95%CrI: −2.26 to −0.27) and overnight fasting (MD: −1.17, 95%CrI: −1.88 to −0.43). So, there was large uncertainty for all estimates vs. control groups. In terms of safety, oral low-dose carbohydrate administration was associated with the occurrences of postoperative infection compared with fasting by 0.42 (95%CrI: 0.20–0.81). In the other outcomes, there was no significant difference between the carbohydrate and control groups.

Conclusion: Although preoperative carbohydrate loading was associated with postoperative insulin resistance and the occurrences of postoperative infection, there is no evidence that preoperative carbohydrate administration alleviates patients' discomfort.

Systematic review registration: [https://www.crd.york.ac.uk/PROSPERO/], identifier [CRD42022312944].

KEYWORDS

preoperative carbohydrate loading, insulin resistance, postoperative comfort and safety, elective surgery, adults, Bayesian network meta-analysis

Introduction

Surgery, as a form of stress, induces peripheral insulin resistance, which can result in hyperglycemia, which, in turn, may have potentially adverse effects on postoperative patients (1, 2). Efficient management of preoperative interventions could reduce postoperative complications and facilitate recovery.

Enhanced recovery after surgery (ERAS) is a multimodal, multidisciplinary project aimed at improving the recovery of patients undergoing surgery during the entire perioperative period (3). The overall complication occurrences were reduced by up to 50% when the ERAS protocols were used compared with traditional perioperative patient management (4, 5).

The preoperative administration of carbohydrate loading as a part of ERAS protocols reduces insulin resistance and tissue glycosylation, improves postoperative glucose control, and enhances postoperative comfort (6). Several randomized controlled trials (RCTs) and meta-analysis have shown that preoperative carbohydrate loading decreased postoperative insulin resistance and side effects compared with those consuming placebo/water or in a fasted state (7, 8). Other RCTs, however, have shown that perioperative carbohydrate administration had no effect on postoperative insulin resistance (9, 10). Thus, the administration of preoperative carbohydrates remains somewhat controversial.

The conventional pairwise meta-analysis has its limitations. First, the previous meta-analysis cannot compare different controls (such as fasting, placebo, or water) simultaneously, so these meta-analyses need to combine these groups into one treatment arm, thus limited interpretability (8). Second, because of the scarcity of direct head-to-head comparisons of interventions in trials, it is unable to assess the comparative effects of interventions (11).

Therefore, to overcome this limitation, we conducted an updated systematic review and network meta-analysis (NMA) to pool and analyze data comparing different preoperative drinks used for clinical and metabolic postoperative outcomes in adult patients undergoing elective surgery (12).

Materials and methods

Protocol registration

This is a systematic review and NMA of preoperative carbohydrate intervention trials in adult patients undergoing elective surgery. The Preferred Reporting Items for Systematic Reviews (PRISMA) and Meta-analyses for RCTs were used to organize the reporting (13). The study protocol was registered (registration number: CRD42022312944) with the International Prospective Register of Systematic Reviews (PROSPERO) following the standard reporting method.

Data sources

MEDLINE (PubMed), Web of Science, EMBASE, EBSCO, the Cochrane Central Register of Controlled Trials, and China National Knowledge Infrastructure (CNKI) were searched to identify eligible trials. We updated the literature search weekly,

and the search was performed from database inception until 16 September 2022 (details are shown in **Supplementary Table 1**).

Trial selection criteria

Eligible trials included the preoperative administration of at least 10 g carbohydrate loading (orally or intravenously) before 4 h of the surgery started, and with fasting, placebo, or water, undergoing any type of elective surgery in adults. Studies also included carbohydrate-based solutions containing other compounds (such as glutamine and whey protein). Patients with diabetes mellitus or those who were receiving emergency surgery were also excluded.

Trial identification

Two investigators independently screened articles by title, abstract, and full text using the inclusion criteria. The inclusion of a study was decided by consensus between the two investigators. When differences occurred, investigators consulted or discussed with a third one to solve them.

Intervention categories

Five categories were used to classify the preoperative administration for the included RCTs:

(1) Low-dose carbohydrate: The dose of oral carbohydrate is between 10 and 50 g before surgery (10–50 g);
(2) High-dose carbohydrate: The dose of oral carbohydrate is greater than 50 g before surgery (>50 g);
(3) Carbohydrate, iv: preoperative carbohydrate by intravenous perfusion;
(4) Placebo/water (control group);
(5) Fasting (control group).

Outcome measures

The primary outcome was mean change from baseline to the end point (within the first 24 h after surgery) in insulin resistance, as measured by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) method according to the following equation: HOMA-IR = [fasting insulin(μU/mL) × fasting glucose (mmol/L)]/22.5)]. Secondary outcomes were included: residual gastric volume (RGV) during the operation; insulin sensitivity (measured by the hyperinsulinemic glucose clamp method) within the first 24 h after surgery; fasting plasma glucose (FPG) within the first 24 h after surgery; fasting serum insulin (Fin) level within the first 24 h after surgery; the serum levels of C-reactive protein (CRP) within the first 24 h after surgery; postoperative pain, patients' satisfaction, thirst, hunger, anxiety, postoperative nausea and vomit (PONV), fatigue, weakness (all measured on a visual analog scale [VAS]), and the occurrences of postoperative infection.

Data extraction

The following study characteristics were extracted for each eligible study: (1) trial information: the first author, study year, the study country, and trial name; (2) patient characteristics: sample size in each treatment, the type of surgery, and American Society of Anesthesiologists (ASA) grade; (3) intervention details: the type, total dose, administrate route, and timing of each treatment; (4) outcome measures: the primary or secondary outcomes including insulin resistance, RGV, insulin sensitivity, FPG, Fin level, the serum levels of CRP, pain, thirst, hunger, anxiety, nausea and vomit, fatigue, weakness within the first 24 h after surgery, and the occurrences of postoperative infection.

Quality and risk of bias assessment

The quality of every eligible trial was assessed independently by two researchers based on the Cochrane risk of bias 2.0 tool in RCTs in a blind fashion (14), which contains five domains: randomization process, deviations from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Disagreements were discussed and resolved through consensus.

Data synthesis and analysis

We estimated the effect sizes for group differences with respect to baseline changes. We used the imputation of correlation when standard deviations were not available for the mean change value, but were available for baseline and endpoint values (15). Arithmetic difference between baseline and end point was used when the study did not report mean change. Meta-analytic calculations were conducted using R Version 4.1.2 (RStudio, Boston, MA, USA) (16). We performed a Bayesian network model and all analyses were conducted using the "gemtc" package version 1.0-1 (17) and jagsUI packages version 1.5.2 (18). Network plot command of Stata version 16.0 (StataCorp, College Station, TX 77845, USA) was used to draw the comparison-adjusted funnel (19).

Mean difference (MD) was used to model continuous variables, whereas dichotomous outcomes were modeled using a binomial likelihood and logit link (20). The outcomes were converted to standard units. Additionally, missing standard deviations were calculated from standard errors, ranges, or

interquartile ranges as described in the Cochrane Handbook (21). In this study, a NMA was conducted within a Bayesian framework to assess the relative effectiveness of preoperative carbohydrate loading for recovery after elective surgery.

The consistency model and the inconsistency model were used to analyze all outcomes, and the difference in deviance information criterion (DIC) and I^2 was used to compare the overall findings. If the difference in DIC between the two models was ≥ 5, we used the inconsistency model. Both a fixed-effect model and a random model were run for each result, and a lower DIC value indicated a greater model fit.

The Markov chain Monte Carlo (MCMC) algorithm was used to estimate the posterior densities of all unknown parameters in each model. It was based on simulations of 200,000 iterations in each of four chains and provided evidence for confirming the convergence of the models.

The trials we included were tested for consistency and inconsistency. We used the node splitting method to perform to compare the treatment effect direct and indirect comparisons of multiple interventions, and $P > 0.05$ was considered to indicate good consistency (22, 23).

Probability values were summarized and are reported as the surface under the cumulative ranking (SUCRA) curve. When the intervention was certain to be the worst, the SUCRA value would be 0, and when it was certain to be the best, the SUCRA value would be 1 (24).

To investigate the source of heterogeneity, meta-regression was used to explore and account for the heterogeneity with the risk of bias, the category of surgery, and the blinding of these studies' designs.

The planned sensitivity analyses of the outcomes were conducted to evaluate the robustness of the model. First, in addition to the Bayesian random effect network, sensitivity analyses were performed using a fixed-effect network. Second, the transitivity assumption was tested by splitting the "water or placebo" group within the network. Third, all analyses were repeated after excluding high-risk trials and data from imputation methods. In addition, for the primary outcome, we planned to add subgroup analyses conducted for different surgical categories, and a comparison-adjusted funnel plot was used to assess the presence of small-study effects bias.

The Confidence in Network Meta-Analysis (CINeMA) methodological framework and application were used to evaluate confidence in NMA effect estimates for all outcomes and treatment comparisons (25, 26).

Results

Study selection

A total of 9411 records were retrieved, of which 58 articles (N = 4936 patients) fulfilled the eligibility criteria and were included in the meta-analysis, the retrieval process is shown in Figure 1. A total of five interventions were included in this meta-analysis: oral low-dose carbohydrate (10–50 g) loading, oral high-dose carbohydrate (more than 50 g) loading, carbohydrate by intravenous perfusion (Carbohydrate, iv), placebo/water, and fasting. Detailed trial and patient characteristics are shown in Table 1.

Risk of bias and quality of evidence

The overall quality of RCTs included in the network was high and moderate. The risk of bias of 58 studies included in the meta-analysis is shown in Figure 2 (details of the risk of bias 2.0 assessment in each trial are shown in Supplementary Figure 1). According to the risk of bias 2.0 tool of Cochrane Collaboration, 25 (43%) studies were high-quality across all domains and 12 RCTs (21%) were at high risk of bias.

Primary outcome

The primary outcome of this study was postoperative insulin resistance, and it was measured by the homeostasis model assessment-insulin resistance (HOMA-IR) method. The network plot for the primary outcome is shown in Figure 3. Each circle represented an intervention, and the area of each circle was proportional to the number of patients for which the intervention was accepted and indicated the sample size, and the width of the line was proportional to the number of trials that directly compared the two interventions.

Twenty-four studies with 1,390 patients reported insulin resistance during the study period (27–51). Both interventions associated insulin resistance compared with placebo/water, with MD ranging from −3.25 (95%CrI, −5.27 to −1.24) for administrated oral low-dose carbohydrate to −2.57 (95%CrI, −4.33 to −0.78) for oral high-dose carbohydrate loading before surgery. The subgroup analysis based on the category of surgery revealed that the association of oral low-dose carbohydrate compared to placebo/water would correlate with insulin resistance (MD, −4.37 [95%CrI, −8.42 to −0.47]) for patients undergoing major abdominal surgery. Figure 4 shows the results. The result of CineMA represents the confidence in this estimate was low (Supplementary Table 2).

Among all trials included, oral low-dose carbohydrate loading had the highest probability of being the best intervention (SUCRA value of 0.74 compared with other interventions). The corresponding results of SUCRA values are shown in Figure 5. Inconsistency analysis calculated by the node split method showed no significant difference between direct and indirect evidence of this network model, with P-value ranging from 0.05 to 0.32 (Supplementary Table 3). The result of the network meta-regression shows that the covariates we included may not affect the value of insulin resistance (Supplementary Table 4).

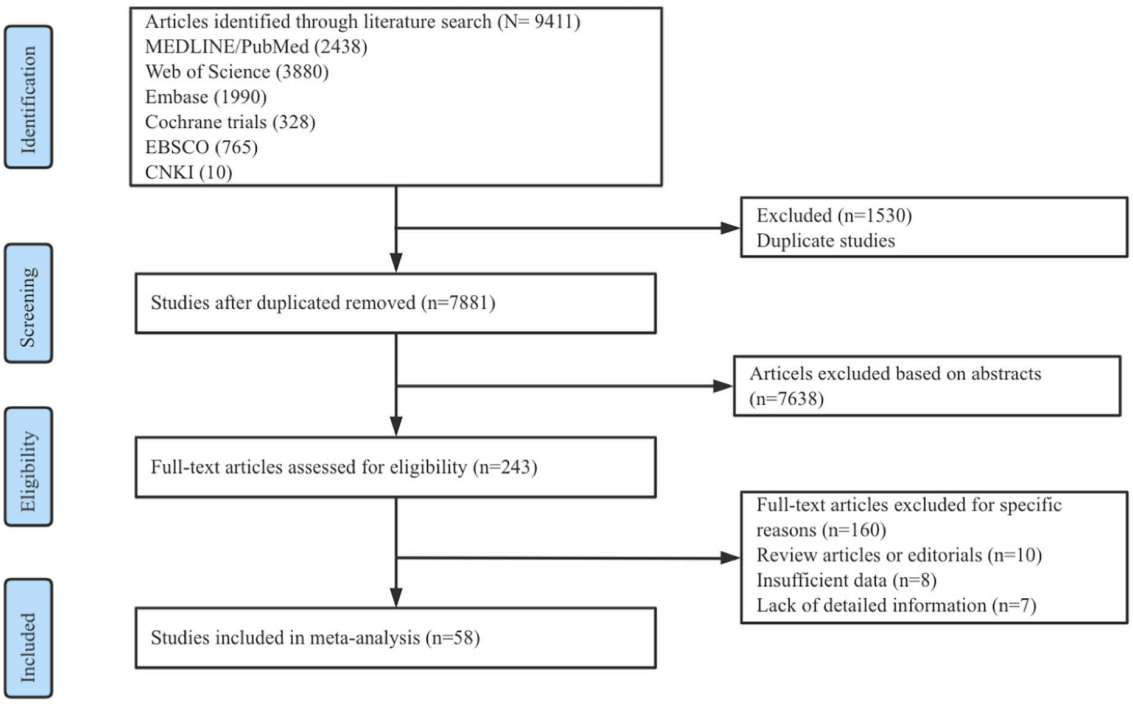

FIGURE 1
Flow diagram of study identification, screening, eligibility assessment, and inclusion.

After excluding studies with a high risk of bias and data of trials with imputation methods (network plot is shown in Supplementary Figure 2), there was an association of oral low-dose carbohydrate loading (MD, −1.29 [95%CrI, −2.26 to −0.27]) with insulin resistance for postoperative patients compared with placebo/water remained. Oral low-dose carbohydrate loading (MD, −1.17 [95% CrI, −1.88 to −0.43]) administration was associated with insulin resistance compared with fasting. The subgroup analysis showed that when patients undergoing major abdominal surgery, administrated oral low-dose carbohydrates before surgery was associated with insulin resistance (MD, −1.35 [95% CrI, −2.64 to −0.01]) compared with fasting. Figure 6 shows the forest plot results. And the SUCRA followed a similar pattern, with oral low-dose carbohydrates having the highest probability of being the best intervention when compared with other interventions; the SUCRA value is 0.88 (Supplementary Table 5).

A comparison-adjusted funnel plot for postoperative insulin resistance implies the presence of publication bias between the carbohydrate groups and controls (Supplementary Figure 3).

Secondary outcomes

Supplementary Figure 4 represents network plots for each secondary outcome. The confidence in these estimates was generally moderate to very low (Supplementary Table 6).

Residual gastric volume during the surgery (mL)

Four studies reported RGV of intraoperative, involving 1,062 participants (52–55). The multiple-treatments meta-analysis results are shown in Table 2. There was no statistically significant difference between the groups in the network.

Postoperative insulin sensitivity (mg/kg/min)

Seven trials measured insulin sensitivity by hyperinsulinaemic–euglycaemic clamp method, involving 170 participants. The results showed carbohydrate loading dose had no significant differences in any of the comparisons (Table 2).

Postoperative fasting plasma glucose (mmol/L)

Twenty-seven trials reported the FPG of patients after surgery, involving 1886 participants (30–37, 40–50, 56–65). Compared with the control groups, preoperative carbohydrate loading had no significant effect on postoperative FPG. Table 2 shows the results.

Postoperative Fin level (μU/mL)

Twenty-two studies were included, with data available for 1,379 participants (9, 29, 30, 33, 34, 36–46, 48–50, 61, 64, 65). Compared with placebo or water, high-dose carbohydrate loading before surgery was associated with a decrease in Fin level (MD, −5.53 [95%Crl, −10.61 to −0.62]). However, because

TABLE 1 Characteristics of the included studies.

References	Country	Sample size (I/C)	Type of surgery	ASA	Type of study	Type of intervention			
						Type	Specification, %, and route	Dose, ml	Comparator	Outcomes
Ajuzieogu et al. (52)	Nigeria	30/30/30	Abdominal myomectomy	I–II	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo; fasting	① ③
Bisgaard et al. (67)	Denmark	43/43	Laparoscopic cholecystectomy	I–II	RCT	High-dose carbohydrate	150 g, oral	‡1200	Water	② ⑬
Braga et al. (53)	Italy	18/18	Pancreaticoduodenectomy	N.S	RCT	Low-dose carbohydrate	50 g, oral	*250	Placebo	① ⑧
Breuer et al. (74)	Germany	56/60/44	Cardiac surgery	III–IV	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo; fasting	⑮
Canbay et al. (27)	Turkey	25/25	Pancreaticoduodenectomy	I–II	RCT	High-dose carbohydrate	150 g, oral	‡1200	Fasting	⑦
Chaudhary et al. (70)	Nepal	33/33	Femur fracture surgery	N.S	RCT	High-dose carbohydrate	150 g, oral	‡1200	Fasting	②
Chen et al. (28)	China	12/12/12	Open gastrectomy for cancer	I–II	RCT	Low-dose carbohydrate	50 g, oral	*500	Water; fasting	① ⑦
Cho et al. (29)	Korea	44/44	Laparoscopic Gynecologic Surgery	I–II	RCT	High-dose carbohydrate	76.8 g, oral	**600	Fasting	② ⑤ ⑥ ⑦ ⑮
Borges Dock-Nascimento et al. (56)	Brazil	12/12/12	Laparoscopic cholecystectomy	I–II	RCT	High-dose carbohydrate	75 g, oral	**600	Water; fasting	⑤
Doo et al. (57)	Korea	25/25	Thyroidectomy	I–II	RCT	High-dose carbohydrate	51.2 g, oral	*400	Fasting	③ ⑤ ⑨ ⑩ ⑪ ⑫ ⑬
Faria et al. (30)	Brazil	11/10	Laparoscopic cholecystectomy	I–II	RCT	Low-dose carbohydrate	25 g, oral	*200	Fasting	⑤ ⑥ ⑦
Feguri et al. (31)	Brazil	20/20	CABG	N.S	RCT	High-dose carbohydrate	75 g, oral	**600	Water	⑦
Feguri et al. (75)	Brazil	14/14	CABG	N.S	RCT	Low-dose carbohydrate	25 g, oral	*200	Fasting	⑮
Gianotti et al. (54)	Italy	331/331	Major abdominal surgery	I–III	RCT	High-dose carbohydrate	100 g, oral	†††800	Water	① ⑮
Gümüs et al. (32)	Turkey	35/33	Laparoscopic cholecystectomy	N.S	RCT	Low-dose carbohydrate	50 g, oral	*400	Fasting	⑤ ⑦
Harsten et al. (72)	Sweden	30/30	Hip replacement	I–III	RCT	High-dose carbohydrate	100 g, oral	¶800	Placebo	⑫
He et al. (48)	China	30/29/29	Elective cesarean delivery	N.S	RCT	Low-dose carbohydrate	50 g, oral	*400	Placebo; fasting	⑤ ⑥ ⑦

(Continued)

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TABLE 1 (Continued)

References	Country	Sample size (I/C)	Type of surgery	ASA	Type of study	Type of intervention			Outcomes
						Type	Specification, %, and route	Dose, ml	Comparator	
Helminen et al. (68)	Finland	57/56	Laparoscopic cholecystectomy	I–II	RCT	High-dose carbohydrate	67 g, oral	*200	Fasting	② ⑩ ⑪ ⑫ ⑬
Hosny et al. (65)	UK	21/21	CABG	II–III	RCT	Low-dose carbohydrate	50 g, iv	500	Water	⑤ ⑥
Itou et al. (55)	Japan	135/139	Mixed#	I–II	RCT	Low-dose carbohydrate	25 g, oral	¶ ¶ ¶ 1000	Fasting	①
Järvelä et al. (79)	Finland	50/51	CABG	N.S	RCT	Low-dose carbohydrate	50 g, oral	*400	Fasting	⑥
Kaska et al. (58)	Czech Republic	75/72/74	Colorectal surgery	I–II	RCT	High-dose carbohydrate; Carbohydrate, iv	100.8 g, oral; 50 g, iv	¶ 800; *500	Fasting	⑤ ⑮
Kweon et al. (33)	Korea	43/45	Orthopedic surgery	I–III	RCT	High-dose carbohydrate	102 g, oral	¶ 800	Fasting	⑤ ⑥ ⑦
Lauwick et al. (69)	Belgium	100/100	Thyroidectomy	I–II	RCT	Low-dose carbohydrate	50 g, oral	*400	Placebo	② ⑨ ⑩ ⑪ ⑬
Lee et al. (80)	Republic of Korea	28/29	CABG	N.S	RCT	High-dose carbohydrate	102 g, oral	¶ 800	Fasting	⑤
Ljungqvist et al. (81)	Sweden	6/6	Open cholecystectomy	I–III	RCT	High-dose carbohydrate, iv	250 g, iv	N.S	Fasting	④
Ljunggren and Hahn (63)	Sweden	19/18/20	Hip replacement surgery	I–III	RCT	High-dose carbohydrate	150 g, oral	‡1200	Water; fasting	④ ⑤
Ljunggren et al. (64)	Sweden	10/12	Hip replacement surgery	I–III	RCT	High-dose carbohydrate	150 g, oral	‡1200	Flavored water	④
Liu et al. (59)	China	58/62	Craniotomy	I–II	RCT	Low-dose carbohydrate	50 g, oral	*400	Fasting	⑤ ⑮
Liu et al. (73)	China	60/60	Open gastrointestinal surgery	II–IV	RCT	Low-dose carbohydrate	25 g, oral	*200	Fasting	⑮
Mathur et al. (9)	New Zealand	69/73	Abdominal surgery	I–III	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo	⑤ ⑥ ⑧
Marquini et al. (34)	Brazil	34/40	Gynecologic surgery	I–II	RCT	High-dose carbohydrate	178 g, oral	¶ ¶ 200	Placebo	⑤ ⑥ ⑦

(Continued)

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TABLE 1 (Continued)

References	Country	Sample size (I/C)	Type of surgery	ASA	Type of study	Type of intervention: Type	Type of intervention: Specification, %, and route	Type of intervention: Dose, ml	Type of intervention: Comparator	Outcomes
Mousavie et al. (62)	Iran	26/26/26	Laparoscopic cholecystectomy	I–II	RCT	Low-dose carbohydrate; Carbohydrate, iv	25 g, oral; 25 g, i.v	*200; *250	Fasting	② ⑤ ⑫
Nygren et al. (60)	Sweden	7/7	Colorectal surgery	N.S	RCT	High-dose carbohydrate	150 g, oral	‡1200	Fasting	⑤
Onalan et al. (35)	Turkey	25/25	Laparoscopic cholecystectomy	N.S	RCT	High-dose carbohydrate	150 g, oral	‡1200	Fasting	② ⑤ ⑦ ⑨ ⑩ ⑪
Pexe-Machado et al. (38)	Brazil	10/12	Laparotomy for gastrointestinal malignancy##	I–III	RCT	High-dose carbohydrate	66 g, oral	**600	Fasting	⑤ ⑥ ⑦ ⑧
Pędziwiatr et al. (36)	Cracow	20/20	Laparoscopic cholecystectomy	I–III	RCT	High-dose carbohydrate	50.4 g, oral	*400	Water	⑤ ⑥ ⑦ ⑮
Perrone et al. (37)	Brazil	8/9	Cholecystectomy^ or inguinal hernia repair	I–II	RCT	High-dose carbohydrate	54 g, oral	††711	Water	⑤ ⑥ ⑦ ⑧
Rapp-Kesek et al. (39)	Sweden	9/9	CABG	N.S	RCT	High-dose carbohydrate	100 g, oral	†800	Fasting	⑤ ⑥ ⑦
Qin et al. (49)	China	111/112	Elective gastrectomy, colorectal resection, or duodenopancreatectomy	N.S	RCT	High-dose carbohydrate	150 g, oral	‡1200	Water	⑤ ⑥ ⑦ ⑮
de Andrade Gagheggi Ravanini et al. (40)	Brazil	21/17	Cholecystectomy	I–II	RCT	High-dose carbohydrate	67 g, oral	*200	Fasting	⑥ ⑦ ⑫
Rizvanović et al. (41)	Croatia	25/25	Colorectal surgery	I–III	RCT	High-dose carbohydrate	75 g, oral	**600	Fasting	⑤ ⑥ ⑦ ⑧ ⑨
Sada et al. (71)	Kosovo	22/23/26	Abdominal surgery	I–II	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo; fasting	⑨ ⑩ ⑪ ⑫ ⑭
Awad et al. (82)	UK	20/20	Laparoscopic cholecystectomy	N.S	RCT	Low-dose carbohydrate	45 g, oral	***900	Placebo	⑤
Singh et al. (46)	India	40/40/40	Laparoscopic cholecystectomy	N.S	RCT	High-dose carbohydrate	75 g, oral	**600	Placebo; fasting	⑤ ⑥ ⑦
Shi et al. (43)	China	25/25/25	Cesarean section	I–II	RCT	Low-dose carbohydrate	42.6 g, oral	*300	Water; fasting	⑤ ⑥ ⑦

(Continued)

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TABLE 1 (Continued)

References	Country	Sample size (I/C)	Type of surgery	ASA	Type of study	Type of intervention			Outcomes
						Type	Specification, %, and route	Dose, ml	Comparator	
Soop et al. (7)	Sweden	8/7	Hip replacement surgery	N.S	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo	④ ⑤ ⑥
Soop et al. (83)	Sweden	8/6	Hip replacement surgery	I–II	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo	④ ⑤
van Stijn et al. (84)	Netherlands	10/8	Rectal cancer surgery	N.S	RCT	Low-dose carbohydrate	42 g, oral	‡‡‡750	Placebo	④ ⑤ ⑧
Suh et al. (85)	USA	70/64	Mixed^^	II–IV	RCT	High-dose carbohydrate	100 g, oral	†††592	Fasting	⑤
Tewari et al. (86)	UK	16/16	Elective major open abdominal surgery	N.S	RCT	High-dose carbohydrate	150 g, oral	‡1200	Placebo	④
Tran et al. (47)	Canada	19/19	Mixed###	N.S	RCT	Low-dose carbohydrate	50 g, oral	§§400	Fasting	⑦ ⑮
Wang et al. (87)	China	36/37	Endoscopic submucosal dissection	I–II	RCT	Carbohydrate	42.6 g, oral	§§§1065	Fasting	⑨ ⑩ ⑫ ⑬ ⑮
Wu et al. (50)	China	43/43	Free flap surgery for oral cancer	I–III	RCT	Low-dose carbohydrate	48 g, oral	*400	Fasting	⑤ ⑥ ⑦ ⑮
Yi et al. (66)	Malaysia	62/56	Mixed^^^	I–III	RCT	Low-dose carbohydrate	27 g, oral	††711	Fasting	⑧ ⑮
Yu et al. (42)	China	24/24	Radical distal subtotal gastrectomy	I–III	RCT	Low-dose carbohydrate	50 g, oral	§§500	Placebo	⑤ ⑥ ⑦
Yuill et al. (61)	UK	31/34	Abdominal surgery	N.S	RCT	High-dose carbohydrate	151.2 g, oral	‡1200	§Placebo	⑤ ⑥
Zhang and Min (44)	China	29/29	Gynecological surgery	I–II	RCT	High-dose carbohydrate	150 g, oral	‡1200	Fasting	② ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑭
Zhou (45)	China	29/30	Gastrectomy	N.S	RCT	Low-dose carbohydrate	50 g, oral	*500	Fasting	⑤ ⑥ ⑦ ⑮

Outcomes: ①: residual gastric volume (RGV) during the surgery; ②: postoperative pain; ③: postoperative patient satisfaction; ④: insulin sensitivity (measured by hyperinsulinemic glucose clamp); ⑤: postoperative fasting plasma glucose (FPG); ⑥: postoperative fasting insulin level (Fins); ⑦: insulin resistance [measured by postoperative homeostasis model assessment-insulin resistance (HOMA-IR)]; ⑧: the serum levels of C-reactive protein (CRP) within the first 24 h after surgery; ⑨: postoperative scores of thirst; ⑩: postoperative scores of hunger; ⑪: postoperative scores of anxiety; ⑫: postoperative scores of nausea and vomit; ⑬: postoperative scores of fatigue; ⑭: postoperative scores of weakness; ⑮ the occurrence of postoperative infection. *: 2 h before the surgery; †: 400 mL—between 9:00 and 11:00 p.m. before the surgery, and 400 mL—2–3 h before the surgery; ‡: 800 mL—8 h before the surgery, and 400 mL—2 h before the surgery; §: 1000 ml—8 h before the surgery and 500 mL—2 h before the surgery; ¶: 400 mL in the evening before surgery and 400 mL in the morning on the day of surgery; **: 400 mL—8 h before the surgery and 200 mL—2 h before the surgery; ††: 474 mL —at the evening drinking and 237 mL — 3 h before the operation; §§: 3 h before the surgery; ¶¶: 4 h before the surgery; ***: 600 mL—8:00 p.m. before the surgery and 300 mL—2–3 h before the surgery; †††: oral from 8 PM before the operation and stop consumption 2 h before the planned time of operation; ‡‡‡: 250 ml-given 15, 11, and 4 h before surgery; §§§: 710 mL —in the evening and 355 mL—2 h before surgery; ¶¶¶: 500 mL—between 9:00 and 11:00 p.m. before the surgery, and 500 mL—2 h before the surgery. #: Procedures included otorhinolaryngological surgery, orthopedic/plastic surgery, gynecological surgery, breast and thyroid surgery, or thoracic surgery. ^: Open or laparoscopic. ##: Procedures included subtotal gastrectomy, hemicolectomy, and anterior resection. ^^: Procedures included laparoscopic Roux-en-Y gastric bypass, Laparoscopic sleeve gastrectomy. ###: Procedures included CABG and spinal surgical; ^^^: Procedures included total abdominal hysterectomy bilateral salpingo-oophorectomy, salpingo-oophorectomy, radical hysterectomy, and debulking tumor; N.S, not stated; ASA, American Society of Anesthesiologists; VAS, visual analog scale; CABG, coronary artery bypass grafting; iv, intravenous perfusion.

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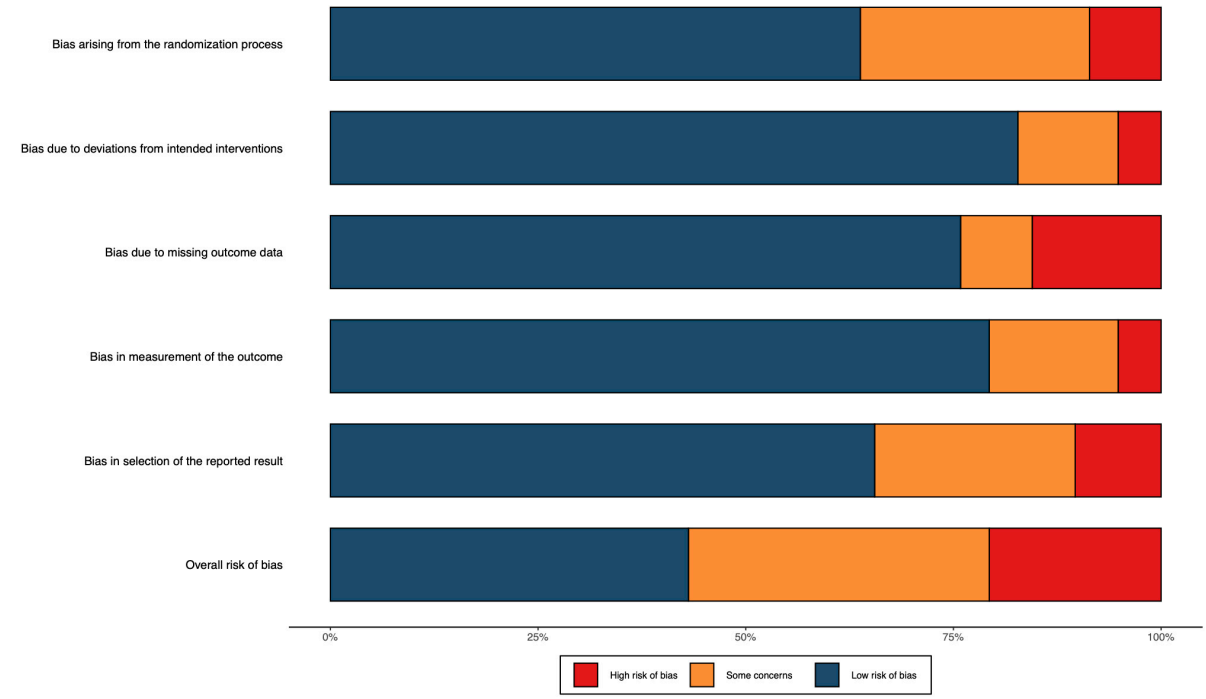

FIGURE 2
Risk of bias included RCTs. The colors in the bar next to each row/criteria represent the percentage of total studies falling within the high risk of bias/some concerns/low risk of bias.

the confidence interval was wide and close to insignificance, the results should be interpreted with caution. Table 2 displays the results of the multiple-treatments meta-analysis.

The serum levels of C-reactive protein within the first 24 h after surgery

Seven studies collected blood samples to assess the serum levels of CRP, with data available for 443 participants (9, 37, 38, 41, 44, 53, 66). Multiple-treatments meta-analysis shows no significant difference in any of the companions (Table 2).

Postoperative scores of pain

Eight studies reported postoperative scores of pain scores using a VAS, with data available on 739 participants (29, 35, 44, 62, 67–70). The results found no statistically significant difference after surgery (Table 2).

Postoperative scores of patients' satisfaction

This was reported by two studies using a VAS, with data available on 140 participants (52, 57). Multiple-treatments meta-analysis found no significant difference in any of the treatments within the network (Table 2).

Postoperative scores of thirst

Six studies reported postoperative thirst scores using a VAS, with data available on 539 participants (35, 44, 57, 68, 69, 71). The results found no statistically significant difference after surgery (Table 2).

Postoperative scores of hungry

This was reported by six studies using a VAS, with data available on 539 participants (35, 44, 57, 68, 69, 71). Multiple-treatments meta-analysis found no significant difference in any of the treatments within the network (Table 2).

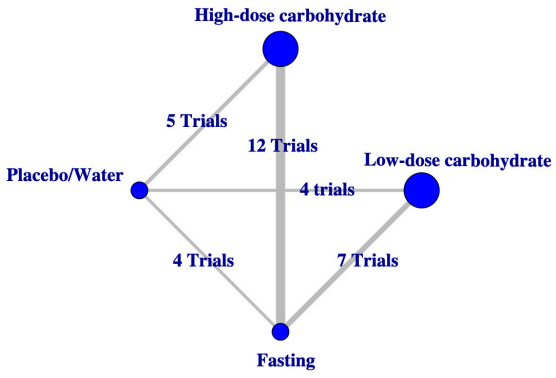

FIGURE 3
Network plot of evidence comparing different interventions for the primary outcome.

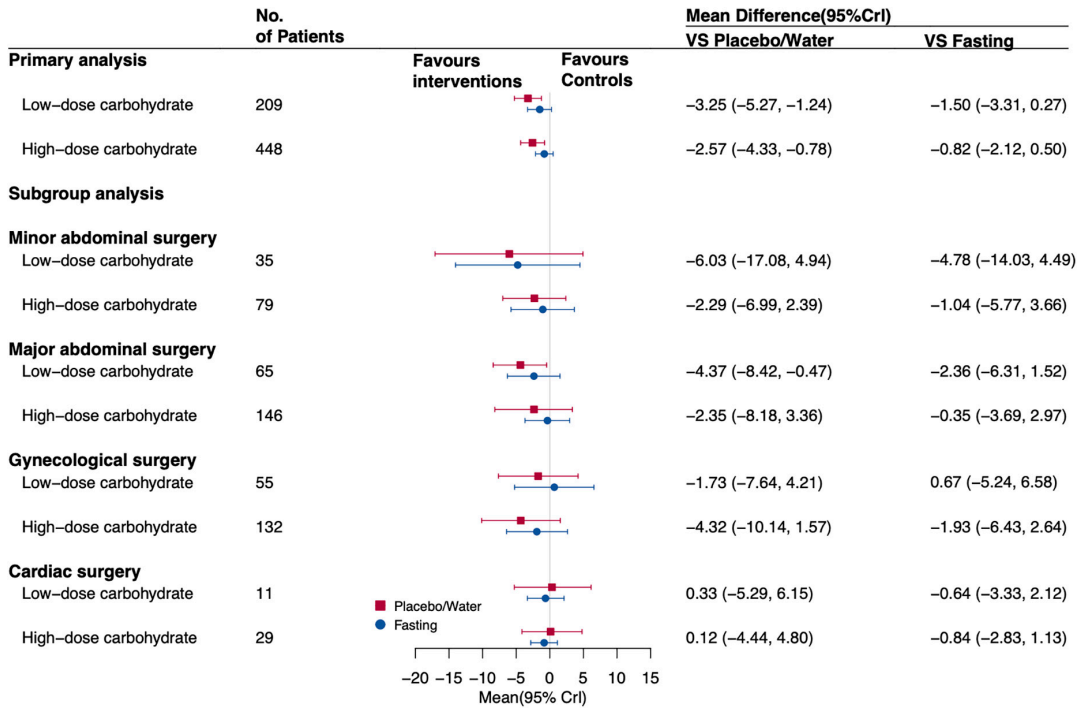

FIGURE 4
Forest plot for the estimates of different interventions on IR of postoperative patients. Values are mean differences (MDs) with 95% confidence intervals (CrIs).

Postoperative scores of anxiety

Three studies reported postoperative anxiety scores; all trials used a VAS, with data available on 318 participants (35, 69, 71). The results found no statistically significant difference after surgery (Table 2).

Postoperative scores of nausea and vomit

Seven studies reported postoperative nausea and vomiting scores; all trials used a VAS, and data on 527 participants were available (40, 46, 57, 62, 68, 71, 72). Multiple-treatments meta-analysis found no significant difference in any of the treatments within the network (Table 2).

Postoperative scores of fatigue

This was reported by four studies using a VAS, with data available on 449 participants (57, 67–69). Multiple-treatments meta-analysis found no significant difference in any of the treatments within the network (Table 2).

Postoperative scores of weakness

Two studies reported postoperative weakness scores using a VAS, with data available on 126 participants (44, 71). The results found no statistically significant difference after surgery (Table 2).

The occurrences of postoperative infection

Eleven studies reported the occurrences of postoperative infection, with data available on 1,765 participants (36, 45, 49, 50, 54, 58, 59, 66, 73–75) (Table 2). The NMA result revealed that compared with fasting, low-dose carbohydrate could reduce the occurrences of postoperative infection with statistical significance (odds ratio, 0.42 [95%CrI: 0.20–0.81]). The results of the network meta-regression shows that the covariates we included may not affect the value of secondary outcomes, except the postoperative FPG (Supplementary Table 7).

The value of SUCRA represented that oral low-dose carbohydrate loading had the highest probability of being the best intervention relative to other interventions in patients' postoperative comfort except for postoperative insulin sensitivity (mg/kg/min), fasting insulin levels (μU/mL), postoperative satisfaction, and weakness (Supplementary Table 8).

Network meta-regression showed that the covariates did not, indeed, influence the value of primary and secondary outcomes (Supplementary Table 9). When trials with a high risk of bias and imputed data were excluded, the results for the secondary outcomes were similar (Supplementary Table 11).

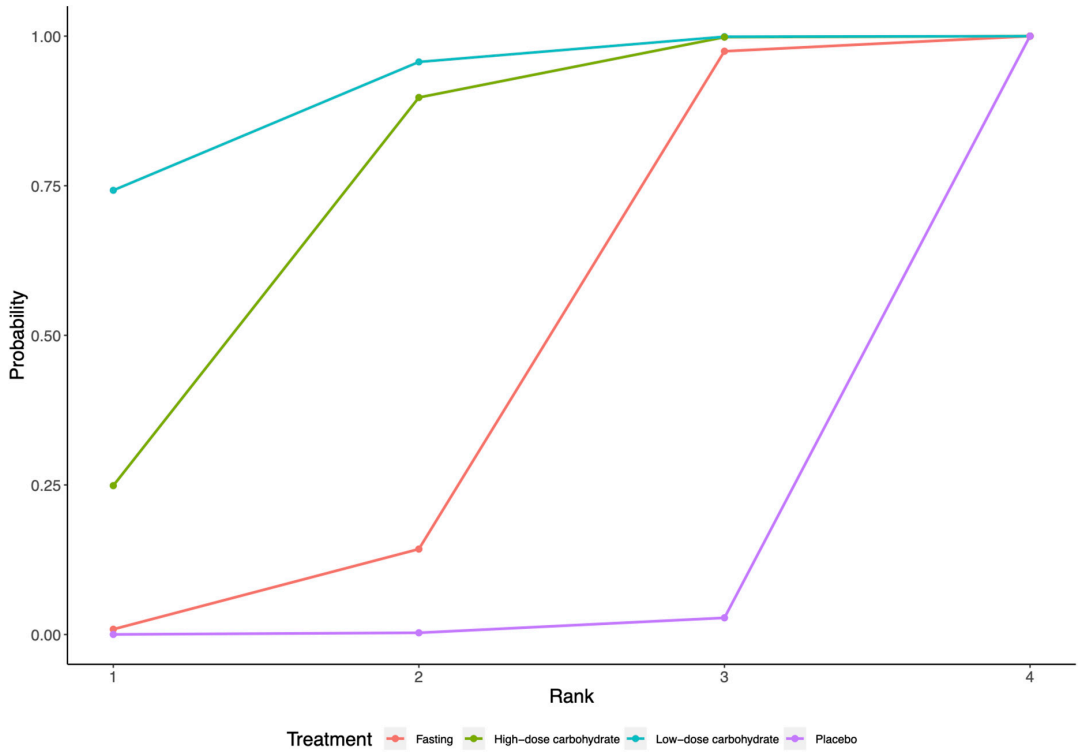

FIGURE 5
Surface under the cumulative ranking curve (SUCRA) for HOMA-IR.

Sensitivity analyses

A summary of clinical and statistical sensitivity analyses is given in **Supplementary Tables 10, 11**. In the clinical sensitivity, after splitting the "water/placebo" group into two separate arms, postoperative insulin resistance reported a significant MD of −4.02 (95% CrI [−6.46, −1.63]) for low-dose carbohydrate vs. placebo, and MD of −3.65 (95% CrI [−6.24, −1.06]) for high-dose carbohydrate vs. placebo, and the sensitivity analyses were consistent with the main analysis of the secondary outcomes. In the statistical sensitivity analyses, when excluding trials at high risk of bias and data for the imputation methods, oral low-dose carbohydrate loading compared to placebo/water associated with postoperative insulin resistance (MD, −1.29 [95% CrI, −2.26 to −0.27]) for postoperative patients, and compared with fasting, insulin resistance was correlated with oral low-dose carbohydrate (MD, −1.17 [95% CrI, −1.88 to −0.43]). The other results did not differ significantly.

Discussion

Summary of findings

The latest practice guidelines for preoperative fasting recommend that clear liquids may be ingested for up 2 h before an operation; however, it reported less thirst and hunger for fasting time of 2–4 h compared to more than 4 h of fasting, however, it reported equivocal findings for RGV, blood glucose values, hunger, and thirst of nutritional or carbohydrate drinks at 2–4 h relative to more than 4 h of fasting (1).

This NMA represents the most comprehensive analysis of currently available data regarding preoperative carbohydrate loading for patients undergoing elective surgery. We combined direct and indirect evidence from 58 trials comparing four different intervention arms in 4,936 patients undergoing elective surgery. The study that included sufficient numbers of patients to prove a potential association in clinical outcomes was of patients undergoing elective surgery, and it included the most patients available in the current literature. To maintain the homogeneity of interventions, our research divided the dose of carbohydrate loading into low dose (10–50 g) and high dose (>50 g). Our main findings indicate that among patients undergoing elective surgery, preoperative low-dose carbohydrate loading has been found to be associated with insulin resistance and postoperative infection rates.

Three published meta-analyses explored the influence of low-carbohydrate loading on postoperative outcomes (2, 8, 12). However, reports of the effects of carbohydrate loading on insulin sensitivity remain inconsistent. Smith et al. (8) conducted that no significant association was between carbohydrate loading and insulin resistance An earlier NMA of

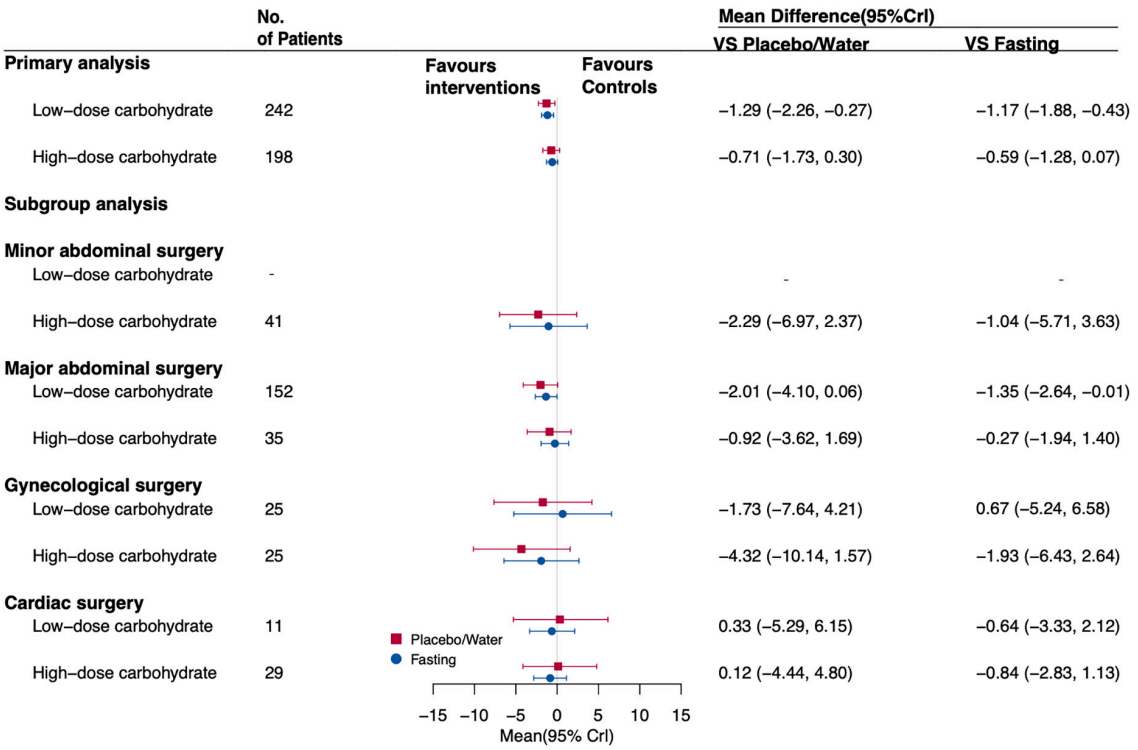

FIGURE 6
Forest plot for the estimates of different interventions on IR of postoperative patients that excluded trials at high risk of bias and data for the imputation methods. Values are mean differences (MDs) with 95% confidence intervals (CrIs).

43 RCTs found that only high-dose carbohydrate administration resulted in a statistically significant associated with insulin resistance compared with fasting, and water or placebo, but with wide confidence intervals so the results are not credible (12). A recent meta-analysis has investigated that compared with fasting, preoperative administration of carbohydrate associated with insulin resistance (2). In our study, we found that oral carbohydrate loading was associated with insulin resistance compared with placebo or water, and the association was still observed in an analysis of excluded high-risk trials and data for the imputation methods. A separate subgroup analysis based on the surgical categories identified the true effect of low-dose carbohydrate loading on insulin resistance, especially those undergoing major abdominal surgery that would otherwise be confounded by other surgical categories. This effect might be due to the preoperative carbohydrate loading, which stimulates an endogenous insulin release and switches off the overnight fasting metabolic state, toward anabolism (63). It should be mentioned that the confidence effect estimate is low or very low, and the significant heterogeneity among studies (different categories of surgery, different types of carbohydrates, and different populations); therefore, the result regarding the effect of carbohydrate loading on insulin resistance must be interpreted with caution.

The present meta-analysis found that oral high-dose carbohydrate (>50 g) was more effective in postoperative outcomes than relative to low-dose carbohydrate, and there is no dose–response relationship between carbohydrate and postoperative outcomes. This may be related to the fact that there is less data available in the network for low-dose carbohydrate comparisons, so some results have wider confidence intervals than in high-dose comparisons.

The gold standard of insulin sensitivity is measured by the hyperinsulinemic–euglycemic clamp method in humans (76). However, we found a small number of studies (n = 7) for this outcome, which could be due to the fact that it is a time-consuming, labor-intensive, and invasive procedure. The multiple-treatments meta-analysis found no evidence that carbohydrate loading was more or less effective in reducing insulin sensitivity compared with placebo/water or fasting. Therefore, more randomized controlled trials need to be included in future analyses to further confirm this outcome.

A recent meta-analysis has investigated that compared with fasting, preoperative administration of carbohydrates decreased patients' thirst, hungry, and pain (2). Meanwhile, in our study, there was no difference in postoperative patients' comfort between the administration of preoperative carbohydrates and control groups, and no other significant

TABLE 2 Network meta-analysis matrix of secondary outcomes.

Outcomes	Treatment estimates are MDs/ORs and 95% CrIs of the column-defining intervention compared with the row-defining intervention for different outcomes				
Residual gastric volume during the surgery ¶ (mL)	Low-dose carbohydrate				
	−2.51 (−16.31, 11.61)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	−0.81 (−14.99, 13.18)	1.66 (−6.74, 9.82)	–	Placebo/Water	
	−2.39 (−9.71, 4.99)	0.07 (−12.04, 12.11)	–	−1.62 (−13.76, 10.96)	Fasting
Postoperative insulin sensitivity ¶ (mg/kg/min)	Low-dose carbohydrate				
	0.28 (−1.62, 2.14)	High-dose carbohydrate			
	−0.75 (−3.55, 2.06)	−1.02 (−3.15, 1.13)	Carbohydrate, iv		
	0.30 (−1.49, 2.09)	0.02 (−0.56, 0.66)	1.05 (−1.08, 3.20)	Placebo/Water	
	0.45 (−1.65, 2.52)	0.16 (−0.91, 1.28)	1.20 (−0.66, 3.05)	0.15 (−0.96, 1.23)	Fasting
Postoperative fasting plasma glucose ¶ (mmol/L)	Low-dose carbohydrate				
	−0.28 (−0.86, 0.3)	High-dose carbohydrate			
	−0.81 (−1.67, 0.07)	−0.53 (−1.33, 0.29)	Carbohydrate, iv		
	−0.11 (−0.67, 0.47)	0.17 (−0.25, 0.60)	0.70 (−0.12, 1.52)	Placebo/Water	
	−0.28 (−0.78, 0.23)	0.00 (−0.38, 0.37)	0.53 (−0.27, 1.32)	−0.17 (−0.62, 0.27)	Fasting
Postoperative fasting insulin level ¶ (μU/mL)	Low-dose carbohydrate				
	−0.12 (−6.98, 6.99)	High-dose carbohydrate			
	−18.67 (−34.96, −2.31)	−18.58 (−34.29, −2.99)	Carbohydrate, iv^		
	−5.65 (−12.39, 1.21)	−5.53 (−10.61, −0.62)	13.03 (−1.79, 27.85)	Placebo/Water	
	−3.34 (−9.44, 2.75)	−3.23 (−7.96, 1.34)	15.35 (−0.54, 31.08)	2.31 (−3.32, 7.87)	Fasting
The serum levels of C-reactive protein within the first 24 h after surgery ¶ (mg/L)	Low-dose carbohydrate				
	7.12 (−30.65, 46.93)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	5.83 (−31.11, 45.84)	−1.42 (−28.28, 27.30)	–	Placebo/Water	
	−14.25 (−50.60, 22.64)	−21.28 (−46.71, 1.84)	–	−19.88 (−56.13, 12.37)	Fasting

(Continued)

TABLE 2 (Continued)

Outcomes	Treatment estimates are MDs/ORs and 95% CrIs of the column-defining intervention compared with the row-defining intervention for different outcomes				
Postoperative scores of pain¶	Low-dose carbohydrate				
	−0.35 (−5.33, 4.63)	High-dose carbohydrate			
	−1.26 (−6.83, 4.26)	−0.91 (−6.88, 5.02)	Carbohydrate, iv^		
	−0.77 (−5.53, 4.02)	−0.41 (−5.20, 4.34)	0.50 (−6.08, 7.13)	Placebo/Water	
	−1.52 (−6.32, 3.25)	−1.16 (−3.68, 1.29)	−0.25 (−5.84, 5.34)	−0.75 (−5.75, 4.22)	Fasting
Postoperative scores of patients' satisfaction¶	Low-dose carbohydrate				
	1.26 (−6.00, 8.49)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	5.25 (−2.00, 12.50)	4.00 (−1.02, 9.01)	–	Placebo/Water	
	3.26 (−1.95, 8.46)	2.00 (−2.99, 7.04)	–	−2.00 (−7.00, 3.03)	Fasting
Postoperative scores of thirst¶	Low-dose carbohydrate				
	−1.49 (−12.63, 9.56)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	−0.90 (−9.14, 7.43)	0.59 (−6.78, 8.04)	–	Placebo/Water	
	−3.35 (−14.46, 7.68)	−1.87 (−5.61, 1.85)	–	−2.48 (−9.92, 4.92)	Fasting
Postoperative scores of hungry¶	Low-dose carbohydrate				
	−1.12 (−11.51, 9.34)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	−0.69 (−8.46, 7.07)	0.43 (−6.52, 7.35)	–	Placebo/Water	
	−2.24 (−12.64, 8.2)	−1.13 (−4.64, 2.34)	–	−1.57 (−8.50, 5.37)	Fasting
Postoperative scores of anxiety¶	Low-dose carbohydrate				
	0.20 (−11.76, 12.13)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	0.09 (−8.59, 8.80)	−0.11 (−8.25, 8.02)	–	Placebo/Water	
	−2.52 (−14.48, 9.46)	−2.72 (−8.88, 3.45)	–	−2.61 (−10.74, 5.56)	Fasting
Postoperative scores of nausea and vomit¶	Low-dose carbohydrate				
	−1.01 (−3.23, 1.22)	High-dose carbohydrate			
	−0.26 (−2.04, 1.51)	0.75 (−1.54, 3.04)	Carbohydrate, iv		

(Continued)

TABLE 2 (Continued)

Outcomes	Treatment estimates are MDs/ORs and 95% CrIs of the column-defining intervention compared with the row-defining intervention for different outcomes				
	−1.78 (−4.12, 0.53)	−0.76 (−1.76, 0.16)	−1.52 (−3.92, 0.84)	Placebo/Water	
	−1.36 (−3.43, 0.72)	−0.35 (−1.16, 0.46)	−1.10 (−3.24, 1.05)	0.42 (−0.60, 1.5)	Fasting
Postoperative scores of fatigue¶	Low-dose carbohydrate				
	−0.70 (−4.96, 3.57)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	−0.70 (−3.65, 2.26)	0.00 (−3.06, 3.08)	–	Placebo/Water	
	−1.49 (−6.53, 3.12)	−0.81 (−3.23, 1.25)	–	−0.81 (−4.81, 2.80)	Fasting
Postoperative scores of weakness¶	Low-dose carbohydrate^				
	–	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	–	0.68 (−0.69, 2.12)	–	Placebo/Water	
	–	0.37 (−0.56, 1.47)	–	−0.31 (−1.67, 1.13)	Fasting
Occurrences of Postoperative infection#	Low-dose carbohydrate				
	0.63 (0.21, 2.00)	High-dose carbohydrate			
	–	–	Carbohydrate, iv^		
	−0.54 (−1.78, 0.66)	0.93 (0.42,1.70)	–	Placebo/Water	
	0.42 (0.20,0.81)	0.71 (0.37,1.30)	–	0.72 (0.37,1.40)	Fasting

Postoperative insulin sensitivity: measured by hyperinsulinemic glucose clamp; comparisons between treatments read from left to right: a network estimate less than 0 (continuous variables) or 1 (dichotomous variables) indicates that the treatment reported in the column is more effective than the corresponding treatment reported in row. ¶ : Mean difference (MD) and 95% confidence intervals (CrIs); #: odds ratios (ORs) and 95% confidence intervals (CrIs); ^: No data available for this outcome. Significant results are in bold. Low-dose carbohydrate: The dose of oral carbohydrate is between 10 and 50 g before surgery (10–50 g); High-dose carbohydrate: The dose of oral carbohydrate is greater than 50 g before surgery (>50 g); Carbohydrate, iv: preoperative carbohydrate by intravenous perfusion; Placebo/Water: flavored sweetened drink/purified water; fasting: overnight fasting before the day of surgery.

differences were found in any of the other secondary outcomes. However, some of these results had wide confidence intervals, indicating that data availability is limited. Future well-designed randomized studies will need to examine the biochemical effects and recovery of preoperative carbohydrate loading in elective surgery.

Strengths and limitations

This review has some strengths: First, a comprehensive search was conducted to identify eligible trials; independent study selection, data extraction, and risk of bias assessment were performed by two reviewers; and the CINeMA was used to assess confidence in the NMA results. Second, we also conducted a network meta-regression to evaluate which variables might influence the postoperative outcomes. This review used a Bayesian framework to overcame the tendency of the frequentist approach to be unstable in parameter estimation and obtain biased results (77). Third, we tested different model assumptions to verify the reliability of outcomes in this NMA. Fourth, a NMA is performed to analyze the effect of preoperative carbohydrate loading on various postoperative recovery indicators among elective surgery patients, compensating for the lack of direct comparison between them.

This study has several limitations. First, the results of this meta-analysis are highly dependent on the quality of the trials

included. According to the CINeMA results, the evaluation of the credibility of results was from moderate to very low, and there was large uncertainty regarding all the estimates. Second, although 58 RCTs were retrieved, only 21 trials reported postoperative low-dose carbohydrate administration in the network, two studies reported preoperative carbohydrate by intravenous perfusion, and there were relatively few direct comparisons. Third, this may, however, be a type II error (false-negative findings), as only a few trials are available to assess postoperative outcome indicators in many second outcomes. Fourth, small trials tend to report larger beneficial effects than large trials; however, only three trials in our review included more than 100 patients per arm, which may introduce bias due to small-study effects (78). Fifth, the SUCRA value was used to estimate a ranking probability of comparative effectiveness between the different interventions. Sixth, many trials, lack good design, resulting in combining different types of carbohydrates into one group and placebo and water into one group for the main analysis. Finally, double-blinding was not applied in many trials designs included, which may affect the results, but this is also difficult to resolve because fasting and drinking are easily known by the participants, and subsequent experiments need to be further refined.

Conclusion

In summary, when compared with fasting and placebo/water, preoperative carbohydrate appears to be associated with some postoperative outcomes; however, more research into these drinks, preferably multi-types carbohydrate trials are required to improve the strength of the evidence and inform clinical practice.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

ET, YC, YR, and YYZ designed and conducted the research. ET completed the first draft of the manuscript. YZ, SS, and SQ analyzed the data and performed the statistical analyses. CD, YH, and LY substantively revised it. XT critically reviewed the manuscript. All authors contributed to the design of the research (project conception, development of the overall research plan) and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. American Society of Anesthesiologists Committee. Practice guidelines for preoperative fasting and the use of pharmacologic agents to reduce the risk of pulmonary aspiration: application to healthy patients undergoing elective procedures: an updated report by the American society of anesthesiologists task force on preoperative fasting and the use of pharmacologic agents to reduce the risk of pulmonary aspiration. *Anesthesiology*. (2017) 126:376–93. doi: 10.1097/aln.0000000000001452

2. Cheng P-L, Loh E-W, Chen J-T, Tam K-W. Effects of preoperative oral carbohydrate on postoperative discomfort in patients undergoing elective surgery: a meta-analysis of randomized controlled trials. *Langenbecks Arch Surg*. (2021) 406:993–1005. doi: 10.1007/s00423-021-02110-2

3. Engelman DT, Ben Ali W, Williams JB, Perrault LP, Reddy VS, Arora RC, et al. Guidelines for perioperative care in cardiac

surgery: enhanced recovery after surgery society recommendations. *JAMA Surg.* (2019) 154:755–66. doi: 10.1001/jamasurg.2019.1153

4. Thiele RH, Rea KM, Turrentine FE, Friel CM, Hassinger TE, McMurry TL, et al. Standardization of care: impact of an enhanced recovery protocol on length of stay, complications, and direct costs after colorectal surgery. *J Am Coll Surg.* (2015) 220:430–43. doi: 10.1016/j.jamcollsurg.2014.12.042

5. Ljungqvist O, Scott M, Fearon KC. Enhanced recovery after surgery: a review. *JAMA Surg.* (2017) 152:292–8. doi: 10.1001/jamasurg.2016.4952

6. Ljungqvist O. Modulating postoperative insulin resistance by preoperative carbohydrate loading. *Best Pract Res Clin Anaesthesiol.* (2009) 23:401–9.

7. Soop M, Nygren J, Myrenfors P, Thorell A, Ljungqvist O. Preoperative oral carbohydrate treatment attenuates immediate postoperative insulin resistance. *Am J Physiol Endocrinol Metab.* (2001) 280:E576–83. doi: 10.1152/ajpendo.2001.280.4.E576

8. Smith MD, McCall J, Plank L, Herbison GP, Soop M, Nygren J. Preoperative carbohydrate treatment for enhancing recovery after elective surgery. *Cochrane Database Syst Rev* (2014) 8:CD009161.

9. Mathur S, Plank LD, McCall JL, Shapkov P, McIlroy K, Gillanders LK, et al. Randomized controlled trial of preoperative oral carbohydrate treatment in major abdominal surgery. *Br J Surg.* (2010) 97:485–94. doi: 10.1002/bjs.7026

10. Brady MC, Kinn S, Stuart P, Ness V. Preoperative fasting for adults to prevent perioperative complications. *Cochrane Database Syst Rev.* (2003) 4:CD004423.

11. Higgins JP, Welton NJ. Network meta-analysis: a norm for comparative effectiveness? *Lancet.* (2015) 386:628–30. doi: 10.1016/s0140-6736(15)61478-7

12. Amer MA, Smith MD, Herbison GP, Plank LD, McCall JL. Network meta-analysis of the effect of preoperative carbohydrate loading on recovery after elective surgery. *Br J Surg.* (2016) 104:187–97. doi: 10.1002/bjs.10408

13. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, et al. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med.* (2015) 162:777–84. doi: 10.7326/m14-2385

14. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ.* (2019) 366:l4898. doi: 10.1136/bmj.l4898

15. Abrams KR, Gillies CL, Lambert PC. Meta-analysis of heterogeneously reported trials assessing change from baseline. *Stat Med.* (2005) 24:3823–44. doi: 10.1002/sim.2423

16. RStudio Team,. *RStudio Team RStudio Integrated Development for R*. Boston, MA: RStudio Team (2020).

17. van Valkenhoef G. *Gemtc: Network Meta-Analysis Using Bayesian Methods: R Package Version 1.0-1*. (2021).

18. Kellner K. *JagsUI: A Wrapper Around 'rjags' to Streamline 'JAGS' Analyses*. (2021).

19. Chaimani A, Higgins JP, Mavridis D, Spyridonos P, Salanti G. Graphical tools for network meta-analysis in STATA. *PLoS One.* (2013) 8:e76654. doi: 10.1371/journal.pone.0076654

20. Doi SAR, Barendregt JJ. A generalized pairwise modelling framework for network meta-analysis. *Int J Evid Based Healthc.* (2018) 16:187–94. doi: 10.1097/xeb.0000000000000140

21. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. *Cochrane Handbook for Systematic Reviews of Interventions*. New York, NY: John Wiley & Sons (2019).

22. van Valkenhoef G, Dias S, Ades AE, Welton NJ. Automated generation of node-splitting models for assessment of inconsistency in network meta-analysis. *Res Synth Methods.* (2016) 7:80–93. doi: 10.1002/jrsm.1167

23. Ades AE, Sculpher M, Sutton A, Abrams K, Cooper N, Welton N, et al. Bayesian methods for evidence synthesis in cost-effectiveness analysis. *Pharmacoeconomics.* (2006) 24:1–19. doi: 10.2165/00019053-200624010-00001

24. Salanti G, Ades AE, Ioannidis JP. Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. *J Clin Epidemiol.* (2011) 64:163–71. doi: 10.1016/j.jclinepi.2010.03.016

25. Nikolakopoulou A, Higgins JPT, Papakonstantinou T, Chaimani A, Del Giovane C, Egger M, et al. CINeMA: an approach for assessing confidence in the results of a network meta-analysis. *PLoS Med.* (2020) 17:e1003082. doi: 10.1371/journal.pmed.1003082

26. Papakonstantinou T, Nikolakopoulou A, Higgins JP, Egger M, Salanti G. Cinema: software for semiautomated assessment of the confidence in the results of network meta-analysis. *Campbell Syst Rev.* (2020) 16:e1080.

27. Canbay Ö, Adar S, Karagöz AH, Çelebi N, Bilen CY. Effect of preoperative consumption of high carbohydrate drink (Pre-Op) on postoperative metabolic stress reaction in patients undergoing radical prostatectomy. *Int Urol Nephrol.* (2014) 46:1329–33. doi: 10.1007/s11255-013-0612-y

28. Chen J, Cheng L, Xie Z, Li Z. The effect of the preoperative oral intake of 10% glucose solution on postoperative insulin resistance in patients undergoing gastric cancer resection. *J Perianesth Nurs.* (2014) 34:1562–5.

29. Cho EA, Lee NH, Ahn JH, Choi WJ, Byun JH, Song T. Preoperative oral carbohydrate loading in laparoscopic gynecologic surgery: a randomized controlled trial. *J Minim Invasive Gynecol.* (2021) 28:1086–94.e1. doi: 10.1016/j.jmig.2020.12.002

30. Faria MS, de Aguilar-Nascimento JE, Pimenta OS, Alvarenga LC Jr, Dock-Nascimento DB, Slhessarenko N. Preoperative fasting of 2 hours minimizes insulin resistance and organic response to trauma after video-cholecystectomy: a randomized, controlled, clinical trial. *World J Surg.* (2009) 33:1158–64. doi: 10.1007/s00268-009-0010-x

31. Feguri GR, Lima PR, Lopes AM, Roledo A, Marchese M, Trevisan M, et al. Clinical and metabolic results of fasting abbreviation with carbohydrates in coronary artery bypass graft surgery. *Rev Bras Cir Cardiovasc.* (2012) 27:7–17. doi: 10.5935/1678-9741.20120004

32. Gümüs K, Pirhan Y, Aydın G, Keloglan S, Tasova V, Kahveci M. The effect of preoperative oral intake of liquid carbohydrate on postoperative stress parameters in patients undergoing laparoscopic cholecystectomy: an experimental study. *J Perianesth Nurs.* (2021) 36:526–31. doi: 10.1016/j.jopan.2020.10.012

33. Kweon S-H, Park JS, Lee YC. Oral carbohydrate administration in patients undergoing cephalomedullary nailing for proximal femur fractures: an analysis of clinical outcomes and patient satisfaction. *Geriatr Orthop Surg Rehabil.* (2020) 11:2151459320958609. doi: 10.1177/2151459320958609

34. Marquini GV, da Silva Pinheiro FE, da Costa Vieira AU, da Costa Pinto RM, Kuster Uyeda MGB, Girão M, et al. Preoperative fasting abbreviation (Enhanced Recovery After Surgery protocol) and effects on the metabolism of patients undergoing gynecological surgeries under spinal anesthesia: a randomized clinical trial. *Nutrition.* (2020) 77:110790. doi: 10.1016/j.nut.2020.110790

35. Onalan E, Andsoy II, Ersoy OF. The effect of preoperative oral carbohydrate administration on insulin resistance and comfort level in patients undergoing surgery. *J Perianesth Nurs.* (2019) 34:539–50. doi: 10.1016/j.jopan.2018.07.007

36. Pêdziwiatr M, Pisarska M, Matłok M, Major P, Kisielewski M, Wierdak M, et al. Randomized clinical trial to compare the effects of preoperative oral carbohydrate loading versus placebo on insulin resistance and cortisol level after laparoscopic cholecystectomy. *Pol Przegl Chir.* (2015) 87:402–8. doi: 10.1515/pjs-2015-0079

37. Perrone F, da-Silva-Filho AC, Adôrno IF, Anabuki NT, Leal FS, Colombo T, et al. Effects of preoperative feeding with a whey protein plus carbohydrate drink on the acute phase response and insulin resistance. A randomized trial. *Nutr J.* (2011) 10:66. doi: 10.1186/1475-2891-10-66

38. Pexe-Machado PA, de Oliveira BD, Dock-Nascimento DB, de Aguilar-Nascimento JE. Shrinking preoperative fast time with maltodextrin and protein hydrolysate in gastrointestinal resections due to cancer. *Nutrition.* (2013) 29:1054–9. doi: 10.1016/j.nut.2013.02.003

39. Rapp-Kesek D, Stridsberg M, Andersson LG, Berne C, Karlsson T. Insulin resistance after cardiopulmonary bypass in the elderly patient. *Scand Cardiovasc J.* (2007) 41:102–8. doi: 10.1080/14017430601050355

40. de Andrade Gagheggi Ravanini G, Portari Filho PE, Abrantes Luna R, Almeida de Oliveira V. Organic inflammatory response to reduced preoperative fasting time, with a carbohydrate and protein enriched solution; A randomized trial. *Nutr Hosp.* (2015) 32:953–7. doi: 10.3305/nh.2015.32.2.8944

41. Rizvanović N, Nesek Adam V, Čaušević S, Dervišević S, Delibegović S. A randomised controlled study of preoperative oral carbohydrate loading versus fasting in patients undergoing colorectal surgery. *Int J Colorectal Dis.* (2019) 34:1551–61. doi: 10.1007/s00384-019-03349-4

42. Yu Y, Zhou YB, Liu HC, Cao SG, Zahng J, Wang ZH. Effects of preoperative oral carbohydrate on postoperative insulin resistance in radical gastrectomy patients. *Zhonghua Wai Ke Za Zhi.* (2013) 51:696–700.

43. Shi Y, Dong B, Dong Q, Zhao Z, Yu Y. Effect of preoperative oral carbohydrate administration on patients undergoing cesarean section with epidural anesthesia: a pilot study. *J Perianesth Nurs.* (2021) 36:30–5. doi: 10.1016/j.jopan.2020.05.006

44. Zhang Y, Min J. Preoperative carbohydrate loading in gynecological patients undergoing combined spinal and epidural anesthesia. *J Invest Surg.* (2020) 33:587–95. doi: 10.1080/08941939.2018.1546352

45. Zhou H. *Effect of Oral Glucose Solution Two Hours before Operation on Patients with Gastric Cance*. Jiangsu: Yangzhou University (2018).

46. Singh BN, Dahiya D, Bagaria D, Saini V, Kaman L, Kaje V, et al. Effects of preoperative carbohydrates drinks on immediate postoperative outcome after day care laparoscopic cholecystectomy. *Surg Endosc.* (2015) 29:3267–72. doi: 10.1007/s00464-015-4071-7

47. Tran S, Wolever TM, Errett LE, Ahn H, Mazer CD, Keith M. Preoperative carbohydrate loading in patients undergoing coronary artery bypass or spinal surgery. *Anesth Analg.* (2013) 117:305–13. doi: 10.1213/ANE.0b013e318295e8d1

48. He Y, Liu C, Han Y, Huang Y, Zhou J, Xie Q. The impact of oral carbohydrate-rich supplement taken two hours before caesarean delivery on maternal and neonatal perioperative outcomes – a randomized clinical trial. *BMC Pregnancy Childbirth.* (2021) 21:682. doi: 10.1186/s12884-021-04155-z

49. Qin H, Ji J, Miao Y, Liu T, Zhao D, Jia Z, et al. Efficacy of the oral administration of maltodextrin fructose before major abdominal surgery: a prospective, multicenter clinical study. *World J Surg.* (2022) 46:2132–40. doi: 10.1007/s00268-022-06455-7

50. Wu HY, Yang XD, Yang GY, Cai ZG, Shan XF, Yang Y. Preoperative oral carbohydrates in elderly patients undergoing free flap surgery for oral cancer: randomized controlled trial. *Int J Oral Maxillofac Surg.* (2022) 51:1010–5. doi: 10.1016/j.ijom.2022.02.014

51. Castela I, Rodrigues C, Ismael S, Barreiros-Mota I, Morais J, Araújo JR, et al. Intermittent energy restriction ameliorates adipose tissue-associated inflammation in adults with obesity: a randomised controlled trial. *Clin Nutr.* (2022) 41:1660–6. doi: 10.1016/j.clnu.2022.06.021

52. Ajuzieogu OV, Amucheazi AO, Nwagha UI, Ezike HA, Luka SK, Abam DS. Effect of routine preoperative fasting on residual gastric volume and acid in patients undergoing myomectomy. *Niger J Clin Pract.* (2016) 19:816–20. doi: 10.4103/1119-3077.180049

53. Braga M, Bissolati M, Rocchetti S, Beneduce A, Pecorelli N, Di Carlo V. Oral preoperative antioxidants in pancreatic surgery: a double-blind, randomized, clinical trial. *Nutrition.* (2012) 28:160–4. doi: 10.1016/j.nut.2011.05.014

54. Gianotti L, Biffi R, Sandini M, Marrelli D, Vignali A, Caccialanza R, et al. Preoperative oral carbohydrate load versus placebo in major elective abdominal surgery (PROCY): a randomized, placebo-controlled, multicenter, phase III trial. *Ann Surg.* (2018) 267:623–30. doi: 10.1097/sla.0000000000002325

55. Itou K, Fukuyama T, Sasabuchi Y, Yasuda H, Suzuki N, Hinenoya H, et al. Safety and efficacy of oral rehydration therapy until 2 h before surgery: a multicenter randomized controlled trial. *J Anesth.* (2012) 26:20–7. doi: 10.1007/s00540-011-1261-x

56. Borges Dock-Nascimento D, Aguilar-Nascimento JE, Caporossi C, Sepulveda Magalhães Faria M, Bragagnolo R, Caporossi FS, et al. Safety of oral glutamine in the abbreviation of preoperative fasting: a double-blind, controlled, randomized clinical trial. *Nutr Hosp.* (2011) 26:86–90.

57. Doo AR, Hwang H, Ki M-J, Lee J-R, Kim D-C. Effects of preoperative oral carbohydrate administration on patient well-being and satisfaction in thyroid surgery. *Korean J Anesthesiol.* (2018) 71:394–400. doi: 10.4097/kja.d.18.27143

58. Kaska M, Grosmanová T, Havel E, Hyspler R, Petrová Z, Brtko M, et al. The impact and safety of preoperative oral or intravenous carbohydrate administration versus fasting in colorectal surgery–a randomized controlled trial. *Wien Klin Wochenschr.* (2010) 122:23–30. doi: 10.1007/s00508-009-1291-7

59. Liu B, Wang Y, Liu S, Zhao T, Zhao B, Jiang X, et al. A randomized controlled study of preoperative oral carbohydrate loading versus fasting in patients undergoing elective craniotomy. *Clin Nutr.* (2019) 38:2106–12. doi: 10.1016/j.clnu.2018.11.008

60. Nygren J, Soop M, Thorell A, Sree Nair K, Ljungqvist O. Preoperative oral carbohydrates and postoperative insulin resistance. *Clin Nutr.* (1999) 18:117–20. doi: 10.1054/clnu.1998.0019

61. Yuill KA, Richardson RA, Davidson HI, Garden OJ, Parks RW. The administration of an oral carbohydrate-containing fluid prior to major elective upper-gastrointestinal surgery preserves skeletal muscle mass postoperatively–a randomised clinical trial. *Clin Nutr.* (2005) 24:32–7. doi: 10.1016/j.clnu.2004.06.009

62. Mousavie SH, Negahi A, Hosseinpour P, Mohseni M, Movassaghi S. The effect of preoperative oral versus parenteral dextrose supplementation on pain, nausea, and quality of recovery after laparoscopic cholecystectomy. *J Perianesth Nurs.* (2021) 36:153–6. doi: 10.1016/j.jopan.2020.07.002

63. Ljunggren S, Hahn RG. Oral nutrition or water loading before hip replacement surgery; a randomized clinical trial. *Trials.* (2012) 13:97. doi: 10.1186/1745-6215-13-97

64. Ljunggren S, Hahn RG, Nyström T. Insulin sensitivity and beta-cell function after carbohydrate oral loading in hip replacement surgery: a double-blind, randomised controlled clinical trial. *Clin Nutr.* (2014) 33:392–8. doi: 10.1016/j.clnu.2013.08.003

65. Hosny H, Ibrahim M, El-Siory W, Abdel-Monem A. Comparative study between conventional fasting versus overnight infusion of lipid or carbohydrate on insulin and free fatty acids in obese patients undergoing elective on-pump coronary artery bypass grafting. A prospective randomized trial. *J Cardiothorac Vasc Anesth.* (2018) 32:1248–53. doi: 10.1053/j.jvca.2017.11.020

66. Yi HC, Ibrahim Z, Abu Zaid Z, Mat Daud Z, Md Yusop NB, Omar J, et al. Impact of enhanced recovery after surgery with preoperative whey protein-infused carbohydrate loading and postoperative early oral feeding among surgical gynecologic cancer patients: an open-labelled randomized controlled trial. *Nutrients.* (2020) 12:264. doi: 10.3390/nu12010264

67. Bisgaard T, Kristiansen VB, Hjortsø NC, Jacobsen LS, Rosenberg J, Kehlet H. Randomized clinical trial comparing an oral carbohydrate beverage with placebo before laparoscopic cholecystectomy. *Br J Surg.* (2004) 91:151–8. doi: 10.1002/bjs.4412

68. Helminen H, Branders H, Ohtonen P, Saarnio J. Effect of pre-operative oral carbohydrate loading on recovery after day-case cholecystectomy: a randomised controlled trial. *Eur J Anaesthesiol.* (2019) 36:605–11. doi: 10.1097/eja.0000000000001002

69. Lauwick SM, Kaba A, Maweja S, Hamoir EE, Joris JL. Effects of oral preoperative carbohydrate on early postoperative outcome after thyroidectomy. *Acta Anaesthesiol Belg.* (2009) 60:67–73.

70. Chaudhary NK, Sunuwar DR, Sharma R, Karki M, Timilsena MN, Gurung A, et al. The effect of pre-operative carbohydrate loading in femur fracture: a randomized controlled trial. *BMC Musculoskelet Disord.* (2022) 23:819. doi: 10.1186/s12891-022-05766-z

71. Sada F, Krasniqi A, Hamza A, Gecaj-Gashi A, Bicaj B, Kavaja F. A randomized trial of preoperative oral carbohydrates in abdominal surgery. *BMC Anesthesiol.* (2014) 14:93. doi: 10.1186/1471-2253-14-93

72. Harsten A, Hjartarson H, Toksvig-Larsen S. Total hip arthroplasty and perioperative oral carbohydrate treatment: a randomised, double-blind, controlled trial. *Eur J Anaesthesiol.* (2012) 29:271–4. doi: 10.1097/EJA.0b013e3283525ba9

73. Liu X, Zhang P, Liu MX, Ma JL, Wei XC, Fan D. Preoperative carbohydrate loading and intraoperative goal-directed fluid therapy for elderly patients undergoing open gastrointestinal surgery: a prospective randomized controlled trial. *BMC Anesthesiol.* (2021) 21:157. doi: 10.1186/s12871-021-01377-8

74. Breuer JP, von Dossow V, von Heymann C, Griesbach M, von Schickfus M, Mackh E, et al. Preoperative oral carbohydrate administration to ASA III-IV patients undergoing elective cardiac surgery. *Anesth Analg.* (2006) 103:1099–108. doi: 10.1213/01.ane.0000237415.18715.1d

75. Feguri GR, Lima PRL, Franco AC, Cruz FRH, Borges DC, Toledo LR, et al. Benefits of fasting abbreviation with carbohydrates and omega-3 infusion during CABG: a double-blind controlled randomized trial. *Braz J Cardiovasc Surg.* (2019) 34:125–35. doi: 10.21470/1678-9741-2018-0336

76. Ljunggren S, Nyström T, Hahn RG. Accuracy and precision of commonly used methods for quantifying surgery-induced insulin resistance: prospective observational study. *Eur J Anaesthesiol.* (2014) 31:110–6. doi: 10.1097/eja.0000000000000017

77. Marsman M, Schönbrodt FD, Morey RD, Yao Y, Gelman A, Wagenmakers EJA. Bayesian bird's eye view of 'Replications of important results in social psychology'. *R Soc Open Sci.* (2017) 4:160426. doi: 10.1098/rsos.160426

78. Zhang Z, Xu X, Ni H. Small studies may overestimate the effect sizes in critical care meta-analyses: a meta-epidemiological study. *Crit Care.* (2013) 17:R2. doi: 10.1186/cc11919

79. Järvelä K, Maaranen P, Sisto T. Pre-operative oral carbohydrate treatment before coronary artery bypass surgery. *Acta Anaesthesiol Scand.* (2008) 52:793–7. doi: 10.1111/j.1399-6576.2008.01660.x

80. Lee B, Soh S, Shim JK, Kim HY, Lee H, Kwak YL. Evaluation of preoperative oral carbohydrate administration on insulin resistance in off-pump coronary artery bypass patients: a randomised trial. *Eur J Anaesthesiol.* (2017) 34:740–7. doi: 10.1097/eja.0000000000000637

81. Ljungqvist O, Thorell A, Gutniak M, Häggmark T, Efendic S. Glucose infusion instead of preoperative fasting reduces postoperative insulin resistance. *J Am Coll Surg.* (1994) 178:329–36.

82. Awad S, Constantin-Teodosiu D, Constantin D, Rowlands BJ, Fearon KC, Macdonald IA, et al. Cellular mechanisms underlying the protective effects of preoperative feeding: a randomized study investigating muscle and liver glycogen content, mitochondrial function, gene and protein expression. *Ann Surg.* (2010) 252:247–53. doi: 10.1097/SLA.0b013e3181e8fbe6

83. Soop M, Nygren J, Thorell A, Weidenhielm L, Lundberg M, Hammarqvist F, et al. Preoperative oral carbohydrate treatment attenuates endogenous glucose release 3 days after surgery. *Clin Nutr.* (2004) 23:733–41. doi: 10.1016/j.clnu.2003.12.007

84. van Stijn MFM, Soeters MR, van Leeuwen PAM, Schreurs WH, Schoorl MG, Twisk JWR, et al. Effects of a carbohydrate-, glutamine-, and antioxidant-enriched oral nutrition supplement on major surgery-induced insulin resistance: a randomized pilot study. *JPEN J Parenter Enteral Nutr.* (2018) 42:719–29. doi: 10.1177/0148607117711691

85. Suh S, Hetzel E, Alter-Troilo K, Lak K, Gould JC, Kindel TL, et al. The influence of preoperative carbohydrate loading on postoperative outcomes in bariatric surgery patients: a randomized, controlled trial. *Surg Obes Relat Dis.* (2021) 17:1480–8. doi: 10.1016/j.soard.2021.04.014

86. Tewari N, Awad S, Duška F, Williams JP, Bennett A, Macdonald IA, et al. Postoperative inflammation and insulin resistance in relation to body composition, adiposity and carbohydrate treatment: a randomised controlled study. *Clin Nutr.* (2019) 38:204–12. doi: 10.1016/j.clnu.2018.01.032

87. Wang Y, Zhu Z, Li H, Sun Y, Xie G, Cheng B, et al. Effects of preoperative oral carbohydrates on patients undergoing ESD surgery under general anesthesia: a randomized control study. *Medicine.* (2019) 98:e15669. doi: 10.1097/md.0000000000015669

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Early lean mass sparing effect of high-protein diet with excess leucine during long-term bed rest in women

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Muscle inactivity leads to muscle atrophy. Leucine is known to inhibit protein degradation and to promote protein synthesis in skeletal muscle. We tested the ability of a high-protein diet enriched with branched-chain amino acids (BCAAs) to prevent muscle atrophy during long-term bed rest (BR). We determined body composition (using dual energy x-ray absorptiometry) at baseline and every 2-weeks during 60 days of BR in 16 healthy young women. Nitrogen (N) balance was assessed daily as the difference between N intake and N urinary excretion. The subjects were randomized into two groups: one received a conventional diet (1.1 ± 0.03 g protein/kg, 4.9 ± 0.3 g leucine per day) and the other a high protein, BCAA-enriched regimen (1.6 ± 0.03 g protein-amino acid/kg, 11.4 ± 0.6 g leucine per day). There were significant BR and BR × diet interaction effects on changes in lean body mass (LBM) and N balance throughout the experimental period (repeated measures ANCOVA). During the first 15 days of BR, lean mass decreased by 4.1 ± 0.9 and 2.4 ± 2.1% ($p < 0.05$) in the conventional and high protein-BCAA diet groups, respectively, while at the end of the 60-day BR, LBM decreased similarly in the two groups by 7.4 ± 0.7 and 6.8 ± 2.4%. During the first 15 days of BR, mean N balance was 2.5 times greater ($p < 0.05$) in subjects on the high protein-BCAA diet than in those on the conventional diet, while we did not find significant differences during the following time intervals. In conclusion,

during 60 days of BR in females, a high protein-BCAA diet was associated with an early protein-LBM sparing effect, which ceased in the medium and long term.

KEYWORDS

lean body mass, high-protein diet, branched-chain amino acids, leucine, long-term bed rest

Introduction

Physical inactivity or bed rest (BR) frequently occurs in patients affected by several diseases, but also during physiologic aging (1–3). Muscle inactivity leads to muscle atrophy, loss of bone mineral density and decreased insulin sensitivity (2, 4–10). Beyond its well-known functional-motor structural role, skeletal muscle represents a homeostatic "organ," fundamental for the maintenance of whole-body metabolic control (11). Indeed, skeletal muscle mass regulates the amino acid balance of organs and tissues, as well as the availability of nutrients and metabolic precursors (11–13). Moreover, it has a central role in maintaining the general energy balance, through complex interplay mechanisms, and contributes to preserve the individual's state of health and quality of life (11).

Together, with physical activity, nutrition has a fundamental role in homeostatic regulation and maintenance of muscle mass and function (14–16). Among nutrients, essential amino acids, occupy a central place, linked not only to the formation of proteins, but also to the control of numerous and complex subcellular pathways, fundamental for maintaining cellular energy balance and, ultimately, for survival itself (17–19). The action of essential amino acids on muscle, in addition to being of a direct type on specific regulatory factors (i.e., mammalian target of rapamycin, mTOR, complex 1), is also mediated by hormonal control (i.e., insulin, glucagon, cortisol), involved in anabolic and catabolic processes, through the regulation of transcriptional sequences (19, 20). Evidence indicates that high-protein diets and essential amino acid supplementation may ameliorate muscle protein loss in healthy volunteers during experimental BR (21, 22). In addition, protein/amino acid intake has been reported to modulate insulin signaling and β-cell function in "*in vivo*" experiments (23, 24). Branched-chain amino acids (BCAAs), i.e., leucine, valine, and isoleucine are essential amino acids, exhibiting selective effects not only on stimulation of muscle protein synthesis, but also on insulin mediated glucose uptake, moreover they play an important role in several metabolic and signaling functions, particularly *via* activation of the mTOR signaling pathway (25, 26). Among BCAAs, the most noteworthy effects have been observed with leucine (26). In particular, leucine: (a) is a constituent of proteins (27); (b) regulates protein synthesis translation initiation in skeletal muscle (28–30); (c) modulates insulin/phosphoinositide 3-kinase (PI3K) signal cascade (31); (d) is a fuel for skeletal muscle cells (32); (e) is a primary nitrogen donor for the production of alanine and glutamine in skeletal muscle (33); (f) modulates the pancreatic β-cell insulin release (34). All these diverse metabolic roles allow leucine to influence the rate of muscle protein synthesis, insulin secretion and glucose homeostasis (25, 35). Evidence indicates that leucine alone may exert and anabolic response (36), while no such data exists for isoleucine or valine, although isoleucine could potentially increase muscle growth by up-regulating the protein expression of GLUT1 and GLUT4 in muscle (26).

The aim of the present study was to assess the effects of a high-protein diet enriched with BCAAs on progression of lean body mass (LBM) atrophy over 2-months experimental BR in healthy young female volunteers as an experimental model of long-term physical inactivity. LBM was assessed approximately every 2 weeks by dual-energy X-ray absorptiometry (DXA). Whole-body protein kinetics were evaluated by nitrogen (N) balance before and during BR. Healthy volunteers who underwent a prolonged BR represent a good model to investigate the effects of muscle unloading on physiological functions (37).

Materials and methods

Subjects and study design

Sixteen medically and psychologically healthy females, aged 25–40 years (32 ± 4 years), participated in the Women's International Space Simulation for Exploration (WISE)—2005 BR study. The study was completed in two campaigns (February 2005–May 2005, September 2005–December 2005). Non-smoking volunteers were recreationally active but athletically untrained, thus competitive athletes were excluded from the study. Included volunteers met the following criteria: a body mass index between 20 and 25 kg/m^2, regular menstrual cycles, no intake of oral contraceptives in the 2 months before the study, no family history of chronic diseases

or psychiatric disorders, and absence of musculoskeletal, orthopedic, blood clotting, and cardiovascular disorders. Mean weight and height of the subjects were 59 ± 4 kg and 166 ± 7 cm, respectively.

The procedures were conducted in accordance with the ethical principles stated in the Declaration of Helsinki 1964. The protocol was reviewed and approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, CCPPRB—Toulouse 1: dossier n°1-04-16: 20/07/04—avis n°2: p.1), by the University of California, San Diego Institutional Review Board and by the Institutional Review Boards of the National Aeronautics and Space Administration, Johnson Space Center Committee for the Protection of Human Subjects. The whole study procedures was explained to the volunteers both verbally and in writing. All participants provided a written informed consent. The study was performed at the Institute for Space Medicine and Physiology in Toulouse, France. The whole study protocol is discussed in detail elsewhere (38, 39), briefly, it consisted in a long-term BR which included a 20-day ambulatory control period followed by 60 days of strict 6° head-down-tilt (HDT) BR (40). A 20-day ambulatory recovery period followed the BR period.

After a 20-day ambulatory adaptation to a controlled diet (i.e., conventional diet), subjects were randomized into two eucaloric diet groups (n = 8, each): a control group who continued the conventional diet and a protein group on a high protein diet enriched with BCAA. During the 20 days pre-BR (ambulatory period, AMB), resting metabolic rate was determined by indirect calorimetry using Deltatrac II (General Electric, Indianapolis, IN) according to the manufacturer. The prescribed caloric intake for the two groups was 140% of their resting metabolic rate during the pre-BR. During BR, energy intake was adjusted downward to 110% of resting metabolic rate both in the control (conventional diet) and protein (high protein-BCAA diet) groups. In the control group on the conventional diet, the prescribed daily protein intake was 1.1 ± 0.03 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ of body weight; while, in the group on the high protein-BCAA diet, dietary protein content was increased to about 1.45 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ and enriched with 0.15 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ of BCAAs (leucine/valine/isoleucine = 2/1/1). Free BCAAs (3.6 g/day free leucine, 1.8 g/day free isoleucine, and 1.8 g/day free valine) were added as a supplement (Friliver, Bracco, Italy) during the three main meals. Thus, the total protein/amino acid daily intake for this group was of 1.6 ± 0.03 $g{\cdot}kg^{-1}$ body weight. To compensate for the additional increase in energy intake from protein in this group, carbohydrate content was reduced during the BR period. Fat mass was monitored every 15 days and maintained at basal levels by changing energy intake, if necessary. All subjects were always restricted to the HDT position except for mealtime when they were allowed to elevate on one elbow. Body weight, urine production, intake of fluids and body temperature were regularly monitored.

Body mass and composition

Body weight was measured daily during pre-BR and during BR. Lean and fat mass were determined by DXA with a Hologic QDR 4,500 W, Software Version 11.1 (Hologic, Bedford, MA). At baseline, measurements were obtained twice, respectively, on days −14 and −2 before the start of BR in each group. In the ambulatory condition DXA measurements obtained at days −14 and −2 were averaged. DXA scans were performed four times during the 60-day BR, respectively, on days 15, 31, 43, and 60 (days BR 15, BR 31, BR 43, and BR 60). DXA scans were executed in the morning in the fasting state. The bladder was emptied before the scan. The subjects had nothing to eat or drink after dinner the night before.

Nitrogen balance during the bed rest

Nitrogen (N) balance reflects the equilibrium between protein N intake and N losses to define anabolic and catabolic conditions of whole-body protein kinetics (i.e., difference between rates of synthesis and degradation). N balance is calculated as the difference between N intake and N losses. In this study, N balance was estimated from the difference between N protein/amino acid intake and urinary N excretion (41), with the addition of a constant value of 4 g/day to account for N losses from the skin and feces (41, 42). A total of 24-h urine samples were collected, aliquoted in 10 mL samples and at −20°C. Urinary N from these aliquots was measured by chemo-luminescence (Antek 7000, Antek Instruments, U.S.) in an accredited laboratory (Central Biochemical Laboratory, Université de Lyon, Lyon, France). Protein intake was assessed by entering all food eaten including all ingredients used to prepare complex recipes, into the Nutrilog Edition Expert software, version 2.0 (Marans, France). All food and leftovers were weighed individually. N balance [g] was then calculated daily according to the following equation: N balance = (24-h protein/BCAA intake / 6.25) – (24-h urinary urea N + 4). In the ambulatory condition N balance of days −1 and −2 were averaged. During the BR period the average daily N balance values were calculated in the time intervals between consecutive LBM measurements. Values of N balance were expressed as mg/kg LBM per day.

Data and statistical analysis

The numerical data are presented as means ± standard deviation (SD). The differences between pre-BR and during 60-days BR were analyzed by repeated-measures ANCOVA with time (AMB or BR days) and diet (conventional or high protein-BCAA diet) as the two factors using ambulatory values as covariates. *Post hoc* analysis was performed, when appropriate, by using paired t-test or Mann-Whitney test with Bonferroni's

adjustments. Levene's test showed normal distribution and equal variance of ambulatory values of LBM, N intake, N excretion and N balance in the two groups. All p-values were considered significant when < 0.05. All analyses were performed using the IBM SPSS statistic 21 software (Version 21.0, SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows mean values of nutrient intakes in the AMB and BR periods in the conventional and high protein-BCAA diet groups. During BR, energy intake decreased by about 8% in both groups. Protein intake increased during BR by about 11 and 53% in the conventional and high protein-BCAA diet groups, respectively. Leucine intake increased by about 100% during the BR period in the high protein-BCAA diet group.

Changes in body weight and composition during the 60-day BR are shown in Table 2. Body weight significantly decreased following BR. At the end of the BR period body weight decreased by 5.9 ± 1.1% in the conventional diet group and by 4.1 ± 1.7% in the high protein-BCAA diet group ($p = 0.01$). Fat mass did not change significantly during the BR period in both groups. Lean mass significantly decreased during the first 31 days of BR and did not change during the following 29 days of BR, both in the high protein-BCAA and the conventional diet groups. There were significant BR effect and BR × diet interaction on changes in LBM.

After 15 days from the beginning of the BR, the decrease in lean mass was about twice greater in the conventional diet group than that in the high protein-BCAA diet group (Figure 1). Nonetheless, at the end of the 60 days of BR the total changes in LBM were similar in the two groups.

As shown in Table 3, during the BR period N intake and urine N excretion were 55 ± 8 and 52 ± 1% greater in subjects on the high protein-BCAA diet than in those on the conventional diet. There were significant BR effect and BR × diet interaction on N balance in both groups. Between the 1st and the 15th day of BR, N balance was significantly greater in the high protein-BCAA diet group than in the conventional diet group, while we did not find significant differences during the following time intervals.

Discussion

We have studied healthy young women during 60 days of BR in eucaloric conditions at different levels of protein intake. In the high protein-BCAA and conventional diet groups protein/amino acid intakes were about 1.6 and 1.1 $g \cdot kg^{-1} \cdot day^{-1}$, respectively. The high protein diet was enriched with 0.15 $g \cdot kg^{-1} \cdot day^{-1}$ of BCAAs with the following BCAA proportions, leucine/valine/isoleucine: 2/1/1. The diet high in protein and BCAA caused a slowing down of the loss of lean mass secondary to BR of about 42%, only in the first 15 days of inactivity. This lean mass saving effect was abolished in the later stages of BR. In fact, at the end of long-BR the total changes in LBM were similar in the two groups. The results of N balance agreed with changes in LBM.

The subjects were in energy balance during the whole study period as demonstrated by the absence of significant changes in fat mass. This finding is important as our previous studies showed that a positive or negative energy balance accelerated BR-induced muscle atrophy (43, 44). In fact, we have demonstrated that during 5 weeks of BR a positive energy balance was associated with greater loss of LBM and activation

TABLE 1 Summary of dietary intake in ambulatory condition and during 60-days of bed rest.

			AMB	BR	p BR effect	p BR × diet
Energy intake	($kcal \cdot kg^{-1} \cdot day^{-1}$)	Conventional diet	32.3 ± 3.1	29.3 ± 2.0*	<0.001	0.12
		High protein-BCAA diet	31.7 ± 1.1	29.9 ± 1.1*		
Carbohydrate intake	(% energy intake)	Conventional diet	58 ± 0.6	56 ± 0.8*	<0.001	<0.001
		High protein-BCAA diet	57 ± 0.6	50 ± 1.1*§		
Lipid intake	(% energy intake)	Conventional diet	30 ± 0.3	30 ± 0.6*	<0.001	<0.001
		High protein-BCAA diet	30 ± 0.3	28 ± 0.3*§		
Protein/amino acid intake	(% energy intake)	Conventional diet	13 ± 0.6	14 ± 0.6*	<0.001	<0.001
		High protein-BCAA diet	13 ± 0.6	21 ± 1.1*§		
	($g \cdot kg^{-1} \cdot day^{-1}$)	Conventional diet	1.0 ± 0.03	1.1 ± 0.03*	<0.001	< 0.001
		High protein-BCAA diet	1.0 ± 0.03	1.6 ± 0.03*§		
Leucine intake	($g \cdot day^{-1}$)	Conventional diet	5.2 ± 0.4	4.9 ± 0.3	<0.001	<0.001
		High protein-BCAA diet	5.7 ± 0.4	11.4 ± 0.6*§		

AMB, ambulatory; BR, bed rest. All data were expressed as means ± SD. All data were analyzed by repeated-measures ANCOVA with time (AMB or BR days) and diet (conventional or high protein-BCAA diet) as the two factors using AMB values as covariates. *Post hoc* analysis was performed, when appropriate, by using paired t-test with Bonferroni's adjustment. *$p < 0.05$, BR vs. AMB. §$p < 0.05$, high protein-BCAA vs. normal protein, conventional diet); SD, standard deviation.

TABLE 2 Body composition in ambulatory condition and during 60-days of bed rest.

		AMB	BR 15 days	BR 31 days	BR 43 days	BR 60 days	*p* BR effect	*p* BR × diet
Total mass	Conventional diet	55.6 ± 3.9	54.1 ± 4.0*	52.9 ± 4.1*	52.6 ± 4.0*	52.3 ± 3.8	<0.001	0.02
	High protein-BCAA diet	61.0 ± 4.4	60.1 ± 3.9*	59.1 ± 4.0*	58.8 ± 4.0	58.5 ± 4.1		
Lean mass	Conventional diet	38.8 ± 3.0	37.2 ± 3.2*	36.1 ± 3.1*	36.1 ± 2.9	35.9 ± 2.9	0.008	0.012
	High protein-BCAA diet	42.8 ± 5.2	41.7 ± 4.2*	40.3 ± 4.6*	40.6 ± 4.3	39.8 ± 4.1		
Fat mass	Conventional diet	14.7 ± 3.8	14.7 ± 3.9	14.7 ± 3.8	14.3 ± 3.6	14.3 ± 3.5	0.57	0.08
	High protein-BCAA diet	15.9 ± 2.1	16.1 ± 2.0	16.5 ± 2.1	15.9 ± 2.0	16.4 ± 2.0		

Body composition measurement with dual-energy X-ray absorptiometry (DXA). Units are kg. Data are means ± SD. AMB, ambulatory; BR, bed rest. AMB values are means of measurements at –14 and –2 pre-bed rest days. All data were expressed as means ± SD. All data were analyzed by repeated-measures ANCOVA with time (AMB or BR days) and diet (conventional or high protein-BCAA diets) as the two factors using AMB values as covariates. *Post hoc* analysis was performed, when appropriate, by using paired *t*-test with Bonferroni's adjustment. *$p < 0.05$, vs. the preceding AMB or BR day of assessment; SD, standard deviation.

of the systemic inflammatory response and antioxidant defenses (44–48). Several pieces of literature provide evidence to support the role of inflammation in impairing muscle homeostasis with a consequent loss of skeletal muscle (49–51). We have also shown that a negative energy balance (hypocaloric nutrition) during 2 weeks of BR leads to a greatest wasting of LBM by increasing protein catabolism in the postabsorptive period and by impairing postprandial anabolic utilization of free amino acids (43).

Muscle atrophy depends on an imbalance between muscle protein synthesis and breakdown. It has been suggested that disuse atrophy in humans is caused mainly by a decreased basal protein synthesis, with no changes in protein degradation, at least in the early stages of BR or immobilization (52–54). In fact, the rate of basal protein synthesis declines immediately after unloading and stays at a suppressed level for the duration

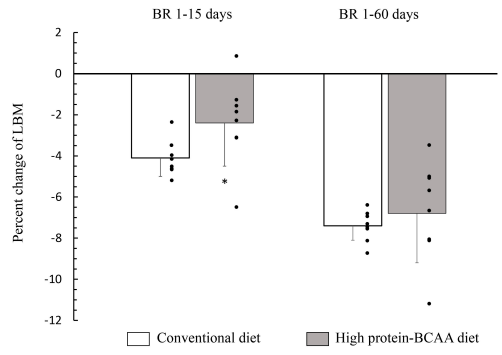

FIGURE 1
Effects of high protein-BCAA diet on lean body mass (LBM) during the first 15 days and during entire bed rest (BR) period of 60 days. All data were expressed as means ± SD. Individual data are additionally shown. All data were analyzed by repeated-measures ANCOVA with time (BR 1–15 days or BR 1–60 days) and diet (conventional or high protein-BCAA diet) as the two factors. BR effect, $p < 0.001$; BR × diet interaction, $p = 0.036$. *Post hoc* analysis was performed by using Mann-Whitney test with Bonferroni's adjustment. *$p < 0.05$, high protein-BCAA vs. normal protein (conventional diet); SD, standard deviation.

of the disuse (52–59). The most important role in the loss of muscle mass during a period of disuse has been explained elsewhere (60, 61) as a decline in both post-absorptive and postprandial muscle protein synthesis rates. This response now called anabolic resistance, is a state of diminished muscle protein synthesis, despite provision of an adequate amount of essential amino acids to elicit an appropriate synthesis response (54, 60–66). In addition, during limb immobilization and BR, a decreased post-absorptive muscle protein synthesis has been reported (55, 60, 62, 67, 68).

In our study, we speculated that the beneficial effect of a high protein-BCAA diet on LBM in the early phase of BR was due to leucine supplementation while maintaining an adequate intake of protein and of the other BCAAs, valine and isoleucine. In fact, several studies have shown that leucine, considered a strong stimulator of protein synthesis (30, 69, 70), has anabolic effects on protein metabolism by increasing the rate of protein synthesis and by decreasing the rate of protein degradation in resting human muscle (26, 30, 35, 71–73). On one hand, leucine increases muscle protein synthesis by modulating the activation of mTOR signaling pathway (25, 74, 75), on the other it reduces protein degradation by regulating autophagy through the acetyl-coenzyme on mTOR complex 1 and by diminishing oxidative stress (76, 77).

In the current study, we have demonstrated that an anti-catabolic action of the high protein-BCAA diet ceases after 15 days (**Figure 1**). In agreement with our study, English et al. have demonstrated that a diet with leucine supplementation (0.06 g/kg per meal) protected against muscle loss after 7-day BR but not after 14 days in middle-aged males; showing that the beneficial effects of leucine supplementation may not be maintained through a prolonged muscle disuse (78). It is not clear why the anabolic action on protein metabolism by chronic leucine supplementation, is not maintained for prolonged periods, despite its powerful effect on acute muscle protein synthesis.

Among several potential mechanisms, including a leucine "desynchronization effect" (63) one possibility is that beyond a certain level, excess of leucine may stimulate key enzymes in

TABLE 3 Nitrogen (N) intake, loss and balance.

		AMB	BR 1–15 days	BR 16–31 days	BR 32–43 days	BR 44–60 days	*p* BR effect	*p* BR × diet
N intake	Conventional diet	241 ± 17	234 ± 20	239 ± 20	240 ± 17	233 ± 11	<0.001	< 0.001
	High protein-BCAA diet	268 ± 31	359 ± 23*	370 ± 20*	373 ± 20*	363 ± 23*		
N loss	Conventional diet	276 ± 43	329 ± 19	327 ± 15	321 ± 16	322 ± 24	<0.001	< 0.001
	High protein-BCAA diet	309 ± 38	416 ± 30*	427 ± 21*	441 ± 21*	421 ± 28*		
N balance	Conventional diet	−35 ± 44	−95 ± 14	−88 ± 17	−81 ± 17	−89 ± 15	<0.001	0.01
	High protein-BCAA diet	−41 ± 44	−57 ± 19*	−56 ± 27	−68 ± 30	−58 ± 28		

AMB, ambulatory; BR, bed rest. Units are mg N × kg LBM^{-1} × d^{-1}. All data were expressed as means ± SD. Values of N balance were not significantly different from zero ($p > 0.15$) in AMB conditions but were significantly negative ($p < 0.01$) in all BR conditions (paired *t*-test with Bonferroni's adjustment). All data were analyzed by repeated-measures ANCOVA with time (AMB or BR days) and diet (conventional or high protein-BCAA diet) as the two factors using AMB values as covariates. *Post hoc* analysis was performed by using unpaired *t*-test with Bonferroni's adjustment. *$p < 0.05$, high protein-BCAA vs. conventional diet. LBM, lean body mass; SD, standard deviation.

BCAA catabolism (i.e., BCAA aminotransferase and branched-chain alpha-keto acid dehydrogenase) thus increasing the oxidation of serum leucine (79, 80). Moreover, a surplus in leucine intake can decrease the plasma concentration of the other EAAs, an effect called BCAA antagonism (81–83). Further investigations are needed to confirm the "desynchronization effect" hypothesis. During immobilization/BR or aging, another important mechanism explaining a reduced muscle protein synthesis, and subsequent smaller increases in lean mass in response to protein feeding, may be due to a decreased amino acid transporter expression. Among them, the large neutral amino acid transporter 1 (LAT1), which preferentially transports leucine and the other BCAAs into the cells, together with the sodium-coupled neutral amino acid transporter 2 (SNAT2), have been shown to activate mTOR signaling (84). In physiological condition, these important amino acid transporters (LAT1 and SNAT2) are sensitive to changes in nutrient status and are associated with activation of mTOR signaling and muscle protein synthesis (85). In fact, mRNA expression and protein content of LAT1 and SNAT2 are increased by EAAs and resistance exercise (86, 87). However, Drummond et al. also found that the EAA-induced increase in LAT1 and SNAT2 proteins was abolished by 7 days of BR (88). Therefore, we hypothesized that the loss of effectiveness of the high protein-BCAA diet could be associated with a decreased muscle protein synthesis by a mechanism involving reduced mTOR signaling pathway, and amino acid transporter expression, thus causing a "desynchronization effect" of leucine in the first 15 days of inactivity. Taken together, these findings suggest limited effect of increasing protein supplementation with EAAs, leucine or BCAAs in offsetting muscle loss in states of long-term BR or leg immobilization, as disuse models.

N balance has been traditionally used to estimate whole body protein balance in response to nutritional interventions (41). In this study, we have shown a consistency of the results obtained every 15 days with DXA scan measurements with those obtained with daily monitoring of the N balance. In order to compare N balance and DXA results, the average daily N balance values were calculated in the time intervals between consecutive LBM measurements. Results of N balance were consistent with those of DXA. During the first 15 days of BR, as compared with the conventional diet group, the high protein-BCAA group exhibited 42% lower LBM loss and 2.5 times greater N balance. During the remaining part of the study, no significant differences were observed between the two groups in both lean mass loss and N balance. Koyama et al. have previously compared changes in lean tissue mass measured by DXA with N balance studies in obese women, studied over two periods of treatment with a very low-energy diet (89). There was a moderate correlation between the changes in lean mass measured by the two methods ($r = 0.40$, $p < 0.05$) (89).

Limitations of our study are that only women were investigated and that sample size for each group was small ($n = 8$ per group, conventional vs. high protein-BCAA diets). Therefore, further studies should have a cross design and include larger sample sizes to investigate if there is a difference between sex that could affect the potential ergogenic benefit provided by leucine supplementation during BR. The results of the WISE—2005 study in women, were compared to those derived from many studies on long-term BR (55 days and more) in males (90–95). This is important to evaluate any sex differences in muscle atrophy and in the effectiveness of countermeasures. In a recent review, Gao and Chilibeck examined the results of previous nutritional interventions during BR for prevention of muscle loss (96). Findings were mixed, among the 11 protein/amino acid supplementation studies included in the review, 4 failed to find any beneficial effects on muscle mass (96). These discrepancies have been attributed to differences in dietary protein quantity and quality (96). Therefore, the difference between diet groups in our study may be due to differences in leucine content or in protein intake between groups (1.1 g·kg^{-1}·day^{-1} vs. 1.45 g·kg^{-1}·day^{-1}). In fact, we believe that the apparent heterogeneity between studies (especially in short-term BR) could be the consequence of differences in the diet composition (quantity of protein), experimental models (i.e., leg immobilization vs. bed rest) and bed rest duration rather that to sex-related factors and age (**Table 4**) (38, 41, 78, 97–106). In addition, as referred by Stein

TABLE 4 Effects of amino acid and protein supplements on muscle mass and function in different models of muscle unloading in healthy volunteers of different ages and sex.

References	Subjects	Nutritional intervention	Muscle unloading	Time	Results	Outcome[a]
Kilroe et al. (97)	Young males	High-protein intake, 1.6 g/kg/day	Leg immobilization	3 days	No effects on muscle mass (MRI) and protein synthesis (stable isotopes)	–
Dirks et al. (98)	Older males	Protein supplement, 40 g/day	Leg immobilization	5 days	No effects on muscle mass (CT) and strength	–
Backx et al. (99)	Young males	Leucine supplement, 7.5 g/day	Leg immobilization	7 days	No effects on muscle mass (CT) and strength	–
Edwards et al. (100)	Young males	Leucine supplement, 7 g/day	Leg immobilization	7 days	No effects on muscle mass (DXA), strength and protein synthesis (stable isotopes)	–
Holloway et al. (101)	Young males	Essential AA supplement, 70 g/day (BCAA 24 g/day, 50% leucine)	Leg immobilization	7 days	Preservation of muscle mass (MRI). No effects on muscle strength	+
Reidy et al. (102)	Older adults	Protein supplement, 17 g; neuromuscular electrical stimulation	Bed rest	5 days	Preservation of muscle mass (DXA); no effects on muscle strength	+
Stein et al. (41)	Young males	BCAA supplement, 12 g/day (33% leucine)	Bed rest	6 days	Preservation of nitrogen balance	+
English et al. (78)	Middle-aged male adults	Leucine supplement, 13 g/day	Bed rest	7 days	Preservation of muscle mass (DXA)	+
Arentson-Lantz et al. (103)	Older men and women	Leucine supplement, 15 g/day	Bed rest	7 days	Preservation of muscle mass (DXA); no effects on muscle strength	+
Present study	Young females	High-protein-BCAA intake, 1.6 g/kg/day (BCAA 22 g/day, 50% leucine)	Bed rest	7 days	Preservation of muscle mass (DXA) and of nitrogen balance	+
Deutz et al. (104)	Older men and women	β-hydroxy-β-methylbutyrate supplement, 3 g/day	Bed rest	10 days	Preservation of muscle mass (DXA)	+
English et al. (78)	Middle-aged male adults	Leucine, 13 g/day	Bed rest	14 days	No effects on muscle mass (DXA)	–
Rudwill et al. (105)	Young males	High-protein intake, 1.8 g/kg/day (33% whey protein)	Bed rest	21 days	No effects on muscle mass (DXA)	–
Present study	Young females	High-protein-BCAA intake, 1.6 g/kg/day (BCAA 22 g/day, 50% leucine)	Bed rest	60 days	No effects on muscle mass (DXA) or nitrogen balance	–
Owen et al. (106)	Young males	High-protein intake, 1.8 g/kg/day (33% whey protein); resistive vibration exercise	Bed rest	21 days	Preservation of muscle mass (MRI)	+
Dorfman et al. (38)	Young females	High-protein-BCAA intake, 1.6 g/kg/day (BCAA 22 g/day, 50% leucine)	Bed rest	60 days	Preservation of myocardial mass (MRI)	+

This table reports selected studies describing the effects of protein and amino acid supplements on muscle mass and function in experimental models of muscle inactivity, in young and elderly male and female subjects. The various experimental models of physical inactivity are not physiologically equivalent. Unilateral immobilization of the lower limb is associated to complete mechanical unloading of the affected muscles. In contrast, bedridden subjects maintain residual movement and muscle contraction while they are carrying out all the daily activities lying in bed. Previous observations clearly demonstrate that the anabolic effects of proteins and amino acids on muscle mass and function are directly proportional to the level of contractile activity (59, 66). It is therefore predictable that the anabolic action of amino acid and protein supplements is reduced in conditions of complete muscle unloading as in the leg immobilization model. The amino acid leucine has a particular direct effect of stimulating muscle protein synthesis compared to other amino acids (30). However, the activation of protein synthesis requires in addition the presence of all amino acids in optimal proportions. The administration of leucine causes the induction of the branched-chain α-ketoacid dehydrogenase enzyme, which irreversibly catabolizes all three BCAAs (79, 80). An excess of leucine can therefore determine a relative reduction of the other BCAAs, valine and isoleucine, resulting in a lack of stimulation of protein synthesis. The optimal action of a leucine supplementation is achieved when it is administered in addition to the other BCAAs and in combination with an adequate protein/amino acid intake (65, 83). These considerations may explain the muscle mass saving effect obtained during 7 days of leg immobilization by administering high doses of leucine in combination with the other essential amino acids including the other BCCAs. In contrast to leg immobilization studies, short-term bed rest (BR) studies (i.e., 5–10 days), which also include our present observation, consistently demonstrate a muscle-sparing effect associated with leucine, BCAA, β-hydroxy-β-methylbutyrate (a leucine metabolite) or protein supplementation. In contrast, leucine, BCAA, or protein supplementation was not associated with skeletal muscle-saving effects in middle- (i.e., 14–21 days) or long-term (i.e., 60 days) BR studies. Except for the conditions in which the nutritional supplement were associated with resistive vibration exercise or with the myocardium contractile activity. The effects of leucine, amino acid and protein supplementation on muscle mass during muscle unloading do not appear to depend on age and sex. AA, amino acids; BCAA, branched-chain amino acids; MRI, magnetic resonance imaging; CT, computed tomography; [a], + outcome: preservation of muscle mass and/or nitrogen balance; –outcome: no effects on muscle mass and/or nitrogen balance.

and Blanc, baseline protein intake was different between studies failing to report a beneficial effect and those finding a positive effect (107).

From the results of our study, it can be assumed that it is useful to administer a high-protein diet with BCAAs in the short-term BR, such as in acute illness of short duration, whereas in long-lasting pathological conditions, this nutritional approach appears useless. Nevertheless, a limitation of this study is that it has been carried out in healthy participants free of medical conditions that may exacerbate muscle loss. Prolonged immobility is harmful with rapid reductions in muscle mass, bone mineral density and impairment in other body systems. These effects are further exacerbated in individuals with critical illness. From the results of our study, we believe that if BR is absolutely recommended, as clinical intervention for a variety of health problems, a high-protein diet strategy could be helpful in mitigating short-term disuse muscle atrophy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Boards of National Aeronautics and Space Administration, Johnson Space Center Committee for the Protection of Human Subjects, the University of California-San Diego Institutional Review Board, and Comité consultatif de Protection des Personnes dans la Recherche Biomédicale of Toulouse, France. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GB, FDG, and AM designed the study, analyzed the results, and wrote the manuscript. GB, PV, NF, and M-PB provided resources, expertise, and critically reviewed the manuscript. FM, AN, and MG prepared the figures and tables and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Biolo G, De Cicco M, Lorenzon S, Dal Mas V, Fantin D, Paroni R, et al. Treating hyperglycemia improves skeletal muscle protein metabolism in cancer patients after major surgery. *Crit Care Med.* (2008) 36:1768–75. doi: 10.1097/CCM.0b013e318174de32

2. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc.* (2013) 14:542–59. doi: 10.1016/j.jamda.2013.05.021

3. Di Girolamo FG, Fiotti N, Milanovic Z, Situlin R, Mearelli F, Vinci P, et al. The aging muscle in experimental bed rest: a systematic review and meta-analysis. *Front Nutr.* (2021) 8:633987. doi: 10.3389/fnut.2021.633987

4. Biolo G, Antonione R, Barazzoni R, Zanetti M, Guarnieri G. Mechanisms of altered protein turnover in chronic diseases: a review of human kinetic studies. *Curr Opin Clin Nutr Metab Care.* (2003) 6:55–63. doi: 10.1097/00075197-200301000-00009

5. Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, et al. Physical inactivity rapidly induces insulin resistance and

microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol.* (2007) 27:2650–6. doi: 10.1161/ATVBAHA.107.153288

6. Ferrucci L, Baroni M, Ranchelli A, Lauretani F, Maggio M, Mecocci P, et al. Interaction between bone and muscle in older persons with mobility limitations. *Curr Pharm Des.* (2014) 20:3178–97. doi: 10.2174/13816128113196660690

7. Biolo G, Cederholm T, Muscaritoli M. Muscle contractile and metabolic dysfunction is a common feature of sarcopenia of aging and chronic diseases: from sarcopenic obesity to cachexia. *Clin Nutr.* (2014) 33:737–48. doi: 10.1016/j.clnu.2014.03.007

8. Barazzoni R, Deutz NEP, Biolo G, Bischoff S, Boirie Y, Cederholm T, et al. Carbohydrates and insulin resistance in clinical nutrition: recommendations from the ESPEN expert group. *Clin Nutr.* (2017) 36:355–63. doi: 10.1016/j.clnu.2016.09.010

9. Di Girolamo FG, Situlin R, Biolo G. What factors influence protein synthesis and degradation in critical illness? *Curr Opin Clin Nutr Metab Care.* (2017) 20:124–30. doi: 10.1097/MCO.0000000000000347

10. Di Girolamo FG, Fiotti N, Sisto UG, Nunnari A, Colla S, Mearelli F, et al. Skeletal muscle in hypoxia and inflammation: insights on the covid-19 pandemic. *Front Nutr.* (2022) 9:865402. doi: 10.3389/fnut.2022.865402

11. Mukund K, Subramaniam S. Skeletal muscle: a review of molecular structure and function, in health and disease. *Wiley Interdiscip Rev Syst Biol Med.* (2020) 12:e1462. doi: 10.1002/wsbm.1462

12. Kim KM, Jang HC, Lim S. Differences among skeletal muscle mass indices derived from height-, weight-, and body mass index-adjusted models in assessing sarcopenia. *Korean J Intern Med.* (2016) 31:643–50. doi: 10.3904/kjim.2016.015

13. Biolo G, Pisot R, Mazzucco S, Di Girolamo FG, Situlin R, Lazzer S, et al. Anabolic resistance assessed by oral stable isotope ingestion following bed rest in young and older adult volunteers: relationships with changes in muscle mass. *Clin Nutr.* (2017) 36:1420–6. doi: 10.1016/j.clnu.2016.09.019

14. Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. *Clin Nutr.* (2014) 33:929–36. doi: 10.1016/j.clnu.2014.04.007

15. Argiles JM, Campos N, Lopez-Pedrosa JM, Rueda R, Rodriguez-Manas L. Skeletal muscle regulates metabolism via interorgan crosstalk: roles in health and disease. *J Am Med Dir Assoc.* (2016) 17:789–96. doi: 10.1016/j.jamda.2016.04.019

16. Di Girolamo FG, Guadagni M, Fiotti N, Situlin R, Biolo G. Contraction and nutrition interaction promotes anabolism in cachectic muscle. *Curr Opin Clin Nutr Metab Care.* (2019) 22:60–7. doi: 10.1097/MCO.0000000000000527

17. Broer S, Broer A. Amino acid homeostasis and signalling in mammalian cells and organisms. *Biochem J.* (2017) 474:1935–63. doi: 10.1042/BCJ20160822

18. Di Girolamo FG, Situlin R, Fiotti N, Tence M, De Colle P, Mearelli F, et al. Higher protein intake is associated with improved muscle strength in elite senior athletes. *Nutrition.* (2017) 42:82–6. doi: 10.1016/j.nut.2017.05.003

19. Takahara T, Amemiya Y, Sugiyama R, Maki M, Shibata H. Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *J Biomed Sci.* (2020) 27:87. doi: 10.1186/s12929-020-00679-2

20. Cucca A, Mazzucco S, Bursomanno A, Antonutti L, Di Girolamo FG, Pizzolato G, et al. Amino acid supplementation in l-dopa treated Parkinson's disease patients. *Clin Nutr.* (2015) 34:1189–94. doi: 10.1016/j.clnu.2014.12.007

21. Stein TP, Donaldson MR, Leskiw MJ, Schluter MD, Baggett DW, Boden G. Branched-chain amino acid supplementation during bed rest: effect on recovery. *J Appl Physiol.* (2003) 94:1345–52. doi: 10.1152/japplphysiol.00481.2002

22. Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab.* (2004) 89:4351–8. doi: 10.1210/jc.2003-032159

23. Newsholme P, Krause M. Nutritional regulation of insulin secretion: implications for diabetes. *Clin Biochem Rev.* (2012) 33:35–47.

24. Newsholme P, Cruzat V, Arfuso F, Keane K. Nutrient regulation of insulin secretion and action. *J Endocrinol.* (2014) 221:R105–20. doi: 10.1530/JOE-13-0616

25. Holecek M. Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements. *Nutr Metab.* (2018) 15:33. doi: 10.1186/s12986-018-0271-1

26. Zhang S, Zeng X, Ren M, Mao X, Qiao S. Novel metabolic and physiological functions of branched chain amino acids: a review. *J Anim Sci Biotechnol.* (2017) 8:10. doi: 10.1186/s40104-016-0139-z

27. Nie C, He T, Zhang W, Zhang G, Ma X. Branched chain amino acids: beyond nutrition metabolism. *Int J Mol Sci.* (2018) 19:954. doi: 10.3390/ijms19040954

28. Kimball SR, Jefferson LS. New functions for amino acids: effects on gene transcription and translation. *Am J Clin Nutr.* (2006) 83:500S–7S. doi: 10.1093/ajcn/83.2.500S

29. Boutry C, El-Kadi SW, Suryawan A, Wheatley SM, Orellana RA, Kimball SR, et al. Leucine pulses enhance skeletal muscle protein synthesis during continuous feeding in neonatal pigs. *Am J Physiol Endocrinol Metab.* (2013) 305:E620–31. doi: 10.1152/ajpendo.00135.2013

30. Ham DJ, Caldow MK, Lynch GS, Koopman R. Leucine as a treatment for muscle wasting: a critical review. *Clin Nutr.* (2014) 33:937–45. doi: 10.1016/j.clnu.2014.09.016

31. Peyrollier K, Hajduch E, Blair AS, Hyde R, Hundal HS. L-leucine availability regulates phosphatidylinositol 3-kinase, p70 S6 kinase and glycogen synthase kinase-3 activity in L6 muscle cells: evidence for the involvement of the mammalian target of rapamycin (mTOR) pathway in the L-leucine-induced up-regulation of system A amino acid transport. *Biochem J.* (2000) 350(Pt 2.):361–8.

32. Rennie MJ, Bohe J, Smith K, Wackerhage H, Greenhaff P. Branched-chain amino acids as fuels and anabolic signals in human muscle. *J Nutr.* (2006) 136(1 Suppl.):264S–8S. doi: 10.1093/jn/136.1.264S

33. van Zanten AR. Glutamine and antioxidants: status of their use in critical illness. *Curr Opin Clin Nutr Metab Care.* (2015) 18:179–86. doi: 10.1097/MCO.0000000000000152

34. Yang J, Chi Y, Burkhardt BR, Guan Y, Wolf BA. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. *Nutr Rev.* (2010) 68:270–9. doi: 10.1111/j.1753-4887.2010.00282.x

35. Nair KS, Short KR. Hormonal and signaling role of branched-chain amino acids. *J Nutr.* (2005) 135(6 Suppl.):1547S–52S. doi: 10.1093/jn/135.6.1547S

36. Wilkinson DJ, Hossain T, Hill DS, Phillips BE, Crossland H, Williams J, et al. Effects of leucine and its metabolite beta-hydroxy-beta-methylbutyrate on human skeletal muscle protein metabolism. *J Physiol.* (2013) 591:2911–23. doi: 10.1113/jphysiol.2013.253203

37. Biolo G, Heer M, Narici M, Strollo F. Microgravity as a model of ageing. *Curr Opin Clin Nutr Metab Care.* (2003) 6:31–40. doi: 10.1097/00075197-200301000-00006

38. Dorfman TA, Levine BD, Tillery T, Peshock RM, Hastings JL, Schneider SM, et al. Cardiac atrophy in women following bed rest. *J Appl Physiol.* (2007) 103:8–16. doi: 10.1152/japplphysiol.01162.2006

39. Jost PD. Simulating human space physiology with bed rest. *Hippokratia.* (2008) 12(Suppl 1.):37–40.

40. Hargens AR, Vico L. Long-duration bed rest as an analog to microgravity. *J Appl Physiol.* (2016) 120:891–903. doi: 10.1152/japplphysiol.00935.2015

41. Stein TP, Schluter MD, Leskiw MJ, Boden G. Attenuation of the protein wasting associated with bed rest by branched-chain amino acids. *Nutrition.* (1999) 15:656–60. doi: 10.1016/s0899-9007(99)00120-3

42. Irwin MI, Hegsted DM. A conspectus of research on protein requirements of man. *J Nutr.* (1971) 101:387–429. doi: 10.1093/jn/101.3.385

43. Biolo G, Ciocchi B, Stulle M, Bosutti A, Barazzoni R, Zanetti M, et al. Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest. *Am J Clin Nutr.* (2007) 86:366–72. doi: 10.1093/ajcn/86.2.366

44. Biolo G, Agostini F, Simunic B, Sturma M, Torelli L, Preiser JC, et al. Positive energy balance is associated with accelerated muscle atrophy and increased erythrocyte glutathione turnover during 5 wk of bed rest. *Am J Clin Nutr.* (2008) 88:950–8. doi: 10.1093/ajcn/88.4.950

45. Di Girolamo FG, Mazzucco S, Situlin R, Mohorko N, Jenko-Praznikar Z, Petelin A, et al. Roasting intensity of naturally low-caffeine Laurina coffee modulates glucose metabolism and redox balance in humans. *Nutrition.* (2016) 32:928–36. doi: 10.1016/j.nut.2016.02.001

46. Biolo G, Di Girolamo FG, McDonnell A, Fiotti N, Mearelli F, Situlin R, et al. Effects of hypoxia and bed rest on markers of cardiometabolic risk: compensatory changes in circulating trail and glutathione redox capacity. *Front Physiol.* (2018) 9:1000. doi: 10.3389/fphys.2018.01000

47. Biolo G, Massolino B, Di Girolamo FG, Fiotti N, Mearelli F, Mazzucco S, et al. Intensive insulin therapy increases glutathione synthesis rate in surgical ICU patients with stress hyperglycemia. *PLoS One.* (2018) 13:e0190291. doi: 10.1371/journal.pone.0190291

48. Biolo G, Di Girolamo FG, Heer M, Sturma M, Mazzucco S, Agostini F, et al. Alkalinization with potassium bicarbonate improves glutathione status and protein kinetics in young volunteers during 21-day bed rest. *Clin Nutr.* (2019) 38:652–9. doi: 10.1016/j.clnu.2018.04.006

49. Londhe P, Guttridge DC. Inflammation induced loss of skeletal muscle. *Bone.* (2015) 80:131–42. doi: 10.1016/j.bone.2015.03.015

50. Costamagna D, Costelli P, Sampaolesi M, Penna F. Role of inflammation in muscle homeostasis and myogenesis. *Mediators Inflamm.* (2015) 2015:805172. doi: 10.1155/2015/805172

51. Guadagni M, Biolo G. Effects of inflammation and/or inactivity on the need for dietary protein. *Curr Opin Clin Nutr Metab Care.* (2009) 12:617–22. doi: 10.1097/MCO.0b013e32833193bd

52. Phillips SM, Glover EI, Rennie MJ. Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. *J Appl Physiol.* (2009) 107:645–54. doi: 10.1152/japplphysiol.00452.2009

53. Brocca L, Cannavino J, Coletto L, Biolo G, Sandri M, Bottinelli R, et al. The time course of the adaptations of human muscle proteome to bed rest and the underlying mechanisms. *J Physiol.* (2012) 590:5211–30. doi: 10.1113/jphysiol.2012.240267

54. Kilroe SP, Fulford J, Holwerda AM, Jackman SR, Lee BP, Gijsen AP, et al. Short-term muscle disuse induces a rapid and sustained decline in daily myofibrillar protein synthesis rates. *Am J Physiol Endocrinol Metab.* (2020) 318:E117–30. doi: 10.1152/ajpendo.00360.2019

55. de Boer MD, Selby A, Atherton P, Smith K, Seynnes OR, Maganaris CN, et al. The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. *J Physiol.* (2007) 585(Pt 1.):241–51. doi: 10.1113/jphysiol.2007.142828

56. Agostini F, Heer M, Guarnieri G, Biolo G. Physical inactivity decreases whole body glutamine turnover independently from changes in proteolysis. *J Physiol.* (2008) 586:4775–81. doi: 10.1113/jphysiol.2008.153783

57. Rennie MJ, Selby A, Atherton P, Smith K, Kumar V, Glover EL, et al. Facts, noise and wishful thinking: muscle protein turnover in aging and human disuse atrophy. *Scand J Med Sci Sports.* (2010) 20:5–9. doi: 10.1111/j.1600-0838.2009.00967.x

58. Glover EI, Yasuda N, Tarnopolsky MA, Abadi A, Phillips SM. Little change in markers of protein breakdown and oxidative stress in humans in immobilization-induced skeletal muscle atrophy. *Appl Physiol Nutr Metab.* (2010) 35:125–33. doi: 10.1139/H09-137

59. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol.* (1997) 273(1 Pt 1.):E122–9. doi: 10.1152/ajpendo.1997.273.1.E122

60. Wall BT, Dirks ML, Snijders T, van Dijk JW, Fritsch M, Verdijk LB, et al. Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion. *Am J Physiol Endocrinol Metab.* (2016) 310:E137–47. doi: 10.1152/ajpendo.00227.2015

61. Wall BT, Snijders T, Senden JM, Ottenbros CL, Gijsen AP, Verdijk LB, et al. Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men. *J Clin Endocrinol Metab.* (2013) 98:4872–81. doi: 10.1210/jc.2013-2098

62. Glover EI, Phillips SM, Oates BR, Tang JE, Tarnopolsky MA, Selby A, et al. Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. *J Physiol.* (2008) 586:6049–61. doi: 10.1113/jphysiol.2008.160333

63. Dardevet D, Remond D, Peyron MA, Papet I, Savary-Auzeloux I, Mosoni L. Muscle wasting and resistance of muscle anabolism: the "anabolic threshold concept" for adapted nutritional strategies during sarcopenia. *ScientificWorldJournal.* (2012) 2012:269531. doi: 10.1100/2012/269531

64. Biolo G, Ciocchi B, Lebenstedt M, Heer M, Guarnieri G. Sensitivity of whole body protein synthesis to amino acid administration during short-term bed rest. *J Gravit Physiol.* (2002) 9:197–8.

65. Magne H, Savary-Auzeloux I, Migne C, Peyron MA, Combaret L, Remond D, et al. Unilateral hindlimb casting induced a delayed generalized muscle atrophy during rehabilitation that is prevented by a whey or a high protein diet but not a free leucine-enriched diet. *PLoS One.* (2013) 8:e70130. doi: 10.1371/journal.pone.0070130

66. Biolo G, Ciocchi B, Lebenstedt M, Barazzoni R, Zanetti M, Platen P, et al. Short-term bed rest impairs amino acid-induced protein anabolism in humans. *J Physiol.* (2004) 558(Pt 2.):381–8. doi: 10.1113/jphysiol.2004.066365

67. Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol.* (1996) 270(4 Pt 1.):E627–33. doi: 10.1152/ajpendo.1996.270.4.E627

68. Kortebein P, Ferrando A, Lombeida J, Wolfe R, Evans WJ. Effect of 10 days of bed rest on skeletal muscle in healthy older adults. *JAMA.* (2007) 297:1772–4. doi: 10.1001/jama.297.16.1772-b

69. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab.* (2006) 291:E381–7. doi: 10.1152/ajpendo.00488.2005

70. Stipanuk MH. Leucine and protein synthesis: mTOR and beyond. *Nutr Rev.* (2007) 65:122–9. doi: 10.1111/j.1753-4887.2007.tb00289.x

71. Alvestrand A, Hagenfeldt L, Merli M, Oureshi A, Eriksson LS. Influence of leucine infusion on intracellular amino acids in humans. *Eur J Clin Invest.* (1990) 20:293–8. doi: 10.1111/j.1365-2362.1990.tb01858.x

72. Louard RJ, Barrett EJ, Gelfand RA. Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man. *Clin Sci.* (1990) 79:457–66. doi: 10.1042/cs0790457

73. Nair KS, Schwartz RG, Welle S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol.* (1992) 263(5 Pt 1.):E928–34. doi: 10.1152/ajpendo.1992.263.5.E928

74. Drummond MJ, Rasmussen BB. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Curr Opin Clin Nutr Metab Care.* (2008) 11:222–6. doi: 10.1097/MCO.0b013e3282fa17fb

75. Manifava M, Smith M, Rotondo S, Walker S, Niewczas I, Zoncu R, et al. Dynamics of mTORC1 activation in response to amino acids. *Elife.* (2016) 5:e19960. doi: 10.7554/eLife.19960

76. Son SM, Park SJ, Stamatakou E, Vicinanza M, Menzies FM, Rubinsztein DC. Leucine regulates autophagy via acetylation of the mTORC1 component raptor. *Nat Commun.* (2020) 11:3148. doi: 10.1038/s41467-020-16886-2

77. Arentson-Lantz EJ, Mikovic J, Bhattarai N, Fry CS, Lamon S, Porter C, et al. Leucine augments specific skeletal muscle mitochondrial respiratory pathways during recovery following 7 days of physical inactivity in older adults. *J Appl Physiol.* (2021) 130:1522–33. doi: 10.1152/japplphysiol.00810.2020

78. English KL, Mettler JA, Ellison JB, Mamerow MM, Arentson-Lantz E, Pattarini JM, et al. Leucine partially protects muscle mass and function during bed rest in middle-aged adults. *Am J Clin Nutr.* (2016) 103:465–73. doi: 10.3945/ajcn.115.112359

79. Harper AE, Miller RH, Block KP. Branched-chain amino acid metabolism. *Annu Rev Nutr.* (1984) 4:409–54. doi: 10.1146/annurev.nu.04.070184.002205

80. Aftring RP, Block KP, Buse MG. Leucine and isoleucine activate skeletal muscle branched-chain alpha-keto acid dehydrogenase in vivo. *Am J Physiol.* (1986) 250(5 Pt 1.):E599–604. doi: 10.1152/ajpendo.1986.250.5.E599

81. May RC, Piepenbrock N, Kelly RA, Mitch WE. Leucine-induced amino acid antagonism in rats: muscle valine metabolism and growth impairment. *J Nutr.* (1991) 121:293–301. doi: 10.1093/jn/121.3.293

82. Cemin HS, Tokach MD, Woodworth JC, Dritz SS, DeRouchey JM, Goodband RD. Branched-chain amino acid interactions in growing pig diets. *Transl Anim Sci.* (2019) 3:1246–53. doi: 10.1093/tas/txz087

83. Wolfe RR. Branched-chain amino acids and muscle protein synthesis in humans: myth or reality? *J Int Soc Sports Nutr.* (2017) 14:30. doi: 10.1186/s12970-017-0184-9

84. Pinilla J, Aledo JC, Cwiklinski E, Hyde R, Taylor PM, Hundal HS. SNAT2 transceptor signalling via mTOR: a role in cell growth and proliferation? *Front Biosci.* (2011) 3:1289–99. doi: 10.2741/e332

85. Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, et al. Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell.* (2009) 136:521–34. doi: 10.1016/j.cell.2008.11.044

86. Drummond MJ, Glynn EL, Fry CS, Timmerman KL, Volpi E, Rasmussen BB. An increase in essential amino acid availability upregulates amino acid transporter expression in human skeletal muscle. *Am J Physiol Endocrinol Metab.* (2010) 298:E1011–8. doi: 10.1152/ajpendo.00690.2009

87. Drummond MJ, Fry CS, Glynn EL, Timmerman KL, Dickinson JM, Walker DK, et al. Skeletal muscle amino acid transporter expression is increased in young and older adults following resistance exercise. *J Appl Physiol.* (2011) 111:135–42. doi: 10.1152/japplphysiol.01408.2010

88. Drummond MJ, Dickinson JM, Fry CS, Walker DK, Gundermann DM, Reidy PT, et al. Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults. *Am J Physiol Endocrinol Metab.* (2012) 302:E1113–22. doi: 10.1152/ajpendo.00603.2011

89. Koyama H, Nishizawa Y, Yamashita N, Furumitsu Y, Hagiwara S, Ochi H, et al. Measurement of composition changes using dual-photon absorptiometry in obese patients undergoing semistarvation. *Metabolism.* (1990) 39:302–6. doi: 10.1016/0026-0495(90)90051-d

90. Blottner D, Salanova M, Puttmann B, Schiffl G, Felsenberg D, Buehring B, et al. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. *Eur J Appl Physiol.* (2006) 97:261–71. doi: 10.1007/s00421-006-0160-6

91. Belavy DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D. Differential atrophy of the lower-limb musculature during prolonged bed-rest. *Eur J Appl Physiol.* (2009) 107:489–99. doi: 10.1007/s00421-009-1136-0

92. Armbrecht G, Belavy DL, Gast U, Bongrazio M, Touby F, Beller G, et al. Resistive vibration exercise attenuates bone and muscle atrophy in 56 days of bed rest: biochemical markers of bone metabolism. *Osteoporos Int.* (2010) 21:597–607. doi: 10.1007/s00198-009-0985-z

93. Moriggi M, Vasso M, Fania C, Capitanio D, Bonifacio G, Salanova M, et al. Long term bed rest with and without vibration exercise countermeasures: effects on human muscle protein dysregulation. *Proteomics.* (2010) 10:3756–74. doi: 10.1002/pmic.200900817

94. Belavy DL, Armbrecht G, Richardson CA, Felsenberg D, Hides JA. Muscle atrophy and changes in spinal morphology: is the lumbar spine vulnerable after prolonged bed-rest? *Spine.* (2011) 36:137–45. doi: 10.1097/BRS.0b013e3181cc93e8

95. Miokovic T, Armbrecht G, Gast U, Rawer R, Roth HJ, Runge M, et al. Muscle atrophy, pain, and damage in bed rest reduced by resistive (vibration) exercise. *Med Sci Sports Exerc.* (2014) 46:1506–16. doi: 10.1249/MSS.0000000000000279

96. Gao R, Chilibeck PD. Nutritional interventions during bed rest and spaceflight: prevention of muscle mass and strength loss, bone resorption, glucose intolerance, and cardiovascular problems. *Nutr Res.* (2020) 82:11–24. doi: 10.1016/j.nutres.2020.07.001

97. Kilroe SP, Fulford J, Jackman S, Holwerda A, Gijsen A, van Loon L, et al. Dietary protein intake does not modulate daily myofibrillar protein synthesis rates or loss of muscle mass and function during short-term immobilization in young men: a randomized controlled trial. *Am J Clin Nutr.* (2021) 113:548–61. doi: 10.1093/ajcn/nqaa136

98. Dirks ML, Wall BT, Nilwik R, Weerts DH, Verdijk LB, van Loon LJ. Skeletal muscle disuse atrophy is not attenuated by dietary protein supplementation in healthy older men. *J Nutr.* (2014) 144:1196–203. doi: 10.3945/jn.114.194217

99. Backx EMP, Horstman AMH, Marzuca-Nassr GN, van Kranenburg J, Smeets JS, Fuchs CJ, et al. Leucine supplementation does not attenuate skeletal muscle loss during leg immobilization in healthy, young men. *Nutrients.* (2018) 10:635. doi: 10.3390/nu10050635

100. Edwards SJ, Smeuninx B, McKendry J, Nishimura Y, Luo D, Marshall RN, et al. High-dose leucine supplementation does not prevent muscle atrophy or strength loss over 7 days of immobilization in healthy young males. *Am J Clin Nutr.* (2020) 112:1368–81. doi: 10.1093/ajcn/nqaa229

101. Holloway TM, McGlory C, McKellar S, Morgan A, Hamill M, Afeyan R, et al. A novel amino acid composition ameliorates short-term muscle disuse atrophy in healthy young men. *Front Nutr.* (2019) 6:105. doi: 10.3389/fnut.2019.00105

102. Reidy PT, McKenzie AI, Brunker P, Nelson DS, Barrows KM, Supiano M, et al. Neuromuscular electrical stimulation combined with protein ingestion preserves thigh muscle mass but not muscle function in healthy older adults during 5 days of bed rest. *Rejuvenation Res.* (2017) 20:449–61. doi: 10.1089/rej.2017.1942

103. Arentson-Lantz EJ, Fiebig KN, Anderson-Catania KJ, Deer RR, Wacher A, Fry CS, et al. Countering disuse atrophy in older adults with low-volume leucine supplementation. *J Appl Physiol.* (2020) 128:967–77. doi: 10.1152/japplphysiol.00847.2019

104. Deutz NE, Pereira SL, Hays NP, Oliver JS, Edens NK, Evans CM, et al. Effect of beta-hydroxy-beta-methylbutyrate (HMB) on lean body mass during 10 days of bed rest in older adults. *Clin Nutr.* (2013) 32:704–12. doi: 10.1016/j.clnu.2013.02.011

105. Rudwill F, O'Gorman D, Lefai E, Chery I, Zahariev A, Normand S, et al. Metabolic inflexibility is an early marker of bed-rest-induced glucose intolerance even when fat mass is stable. *J Clin Endocrinol Metab.* (2018) 103:1910–20. doi: 10.1210/jc.2017-02267

106. Owen PJ, Armbrecht G, Bansmann M, Zange J, Pohle-Frohlich R, Felsenberg D, et al. Whey protein supplementation with vibration exercise ameliorates lumbar paraspinal muscle atrophy in prolonged bed rest. *J Appl Physiol.* (2020) 128:1568–78. doi: 10.1152/japplphysiol.00125.2020

107. Stein TP, Blanc S. Does protein supplementation prevent muscle disuse atrophy and loss of strength? *Crit Rev Food Sci Nutr.* (2011) 51:828–34.

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Personalized dietary advices provided by a dietitian increase calcium intake in outpatients with multiple sclerosis—Results from a randomized, controlled, single-blind trial

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Background and aims: Multiple sclerosis (MS) is associated with osteoporosis, possibly due to neurological disability and decreased calcium intake. The objective of this study was to evaluate the efficacy of a personalized nutritional advice program by a dietitian compared to the delivery of a standard advice form to optimize dietary calcium intake in outpatients with MS.

Methods: We performed a randomized, controlled, parallel trial comparing the efficacy of a personalized dietary advice (PDA) program to standard advice form (SAF) to increase daily calcium intake in MS patients. The study population was composed by patients with relapsing-remitting MS aged 18–69 years old. PDA program consisted in dietary advice delivered by a dietitian at baseline, 1 month, and 3 months. Calcium and nutrient intake in patients from both groups was evaluated at baseline and 6 months using a dietary survey.

Results: Of the 194 patients screened for inclusion, 182 patients were included (79% female, median age of 42 years, and median EDSS of 2.0), and randomized to SAF (n = 92) or PDA (n = 90). At 6 months, median calcium intake increased by 241 mg/day in the PDA group and decreased by 120 mg/day in the SAF group ($p < 0.0001$). However, the median calcium intake was 947 mg/day in the SAF group and 778 mg/day in the PDA group at baseline (p = 0.0077), potentially favoring the effect of dietary advice. Complementary analyses focusing on patients with insufficient calcium

intakes at baseline revealed comparable values in both groups (p = 0.69). Of those, patients included in the PDA group obtained significantly higher calcium intakes at 6 months than patients from the SAF group (p = 0.0086) independently of EDSS, PASAT, HADS and EQ-5D scores.

Conclusion: This work shows the efficacy of dietary management based on personalized advice program over 3 months to durably increase calcium consumption in MS patients with insufficient calcium intake.

Clinical trial registration: clinicaltrials.gov, identifier NCT02664623.

KEYWORDS

calcium intake, dietary advice, multiple sclerosis, coaching/accompaniment, personalized medicine

Introduction

Calcium is an essential nutrient playing a key role in skeletal mineralization, as well as a wide range of biologic functions (1). Prolonged insufficient calcium intake promotes the occurrence of osteoporosis or worsens ongoing osteoporosis, resulting in decreased bone mineral density (BMD) and disruption of bone micro-architecture, consequently increasing bone fragility and predisposing to a higher risk of bone fractures, most often of the wrist, vertebrae and femoral neck (2, 3). Osteoporosis can be prevented by adequate calcium and vitamin D intake (4, 5).

Multiple sclerosis (MS), a chronic, auto-immune, demyelinating disease of the central nervous system affecting mainly young women, is a multifactorial disease that appears to be influenced by genetic and environmental factors (6). MS is the leading non-traumatic cause of acquired severe disability in young patients (7). MS patients have a greater risk of osteoporosis than the general population as low BMD appears to occur in the early-stage of the disease (8–11), attributed to neurological disability, specific treatments, concomitant use of corticosteroids and lack of exposure to sunlight (25-hydroxyvitamin D—25OHD—deficiency). In addition, patients diagnosed with an auto-immune disease such as MS often choose to follow a gluten-free diet and reduce or avoid consuming dairy products, as these diets have been suggested to improve disease outcomes (12–15). In fact, the low-saturated fat (e.g., the so-called Swank diet) and Paleolithic diets have shown promise for MS symptoms, although inadequate calcium and vitamin D intake have been observed due to the restriction of specific foods (16, 17). Milk and dairy products on average provide 50–60% of daily calcium intake, so should be replaced by other calcium rich elements like certain mineral waters, canned fish such as sardines, and fruit and vegetables rich diet (18). With a regular dietary survey, an exogenous supplementation can compensate the calcium deficiency to reach the recommended dietary allowance (RDA) of calcium (18). MS is also associated with malnutrition in advanced disease stages because of increasing motor and cognitive disability that should be detected and treated (19, 20). Doing so, it is important to consider multiple factors like age, sex and education level that are known to influence diet quality and health behavior patterns (21–23).

We hypothesized that a dietitian, by proposing appropriate nutritional strategy, would contribute to optimize calcium intake in MS patients. We designed a randomized controlled trial in MS patients comparing the efficacy of a personalized dietary advice (PDA) program to standard advice form (SAF) to modify patient behavior and thus improve calcium intake. We also analyzed the impact of different conditions associated with MS (disability, cognitive status, anxiety, depression and quality of life) on these interventions.

Abbreviations: ANSES, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail; BMD, bone mineral density; BMI, body mass index; CIQUAL, French Information Center on Food Quality; EDSS, Expanded Disability Status Scale; FDR, false discovery rate; GRIO, Groupe de Recherche et d'Information sur les Ostéoporoses; HADS, Hospital Anxiety and Depression Scale questionnaire; HEL, high education level; IQR, interquartile range; LEL, low education level; MS, multiple sclerosis; PDA, personalized dietary advice; PASAT, Paced Auditory Serial Addition Test; PNNS, Programme National Nutrition Santé; RDA, recommended dietary allowance; RRMS, recurrent remittent multiple sclerosis; SAF, standard advice form.

Materials and methods

Study design and participants

This study was a randomized, controlled, single-blinded, parallel trial. The study population consisted of MS outpatients with Expanded Disability Status Scale (EDSS) score <6.5 followed in the departments of Neurology of Alès Hospital and University Hospital of Nîmes. Eligible patients were aged between 18 and 70 years with confirmed RRMS, without previous dietary consultation for calcium intake. Patients

were excluded if they were unable to complete the self-questionnaire, if we identified at screening that they had vitamin D deficiency related to a history of progressive gastro-intestinal or systemic disorder, moderate renal impairment (creatinine clearance <60 ml/min) or if they were vulnerable to hypercalcemia (e.g., history of cardiac arrhythmia or disease, renal lithiasis, or undergoing treatment with digitalis drug products). Patients meeting the inclusion criteria were contacted by phone within 30 days before the next follow-up consultation by the neurologist to determine if they were interested in participating in the study conducted following their next neurology consultation. Interested patients were sent the study information leaflet and a dietary diary to complete by post. The study dietitian called the patient within 10 days prior to the visit to verify that the documents had been received and explained how to complete the dietary diary. All patients assessed in the study signed the consent form.

This study was conducted in compliance with law no. 2004-806 of 9 August 2004 relating to public health policy and to its application decisions, the declaration of Helsinki and Good Clinical Practice. The study received ethics approval by the French national agency for the safety of medicines and health products (ANSM) and by National Persons Protection Committee (CPP) Sud Méditerranée III (# 2015.11.01 ter) and was prospectively registered on clinicaltrials.gov (NCT02664623).

Intervention

All patients were included by a neurologist during the neurology visit and randomized into two groups: personalized dietary advice (PDA) and standard advice form (SAF). They next attended a dietitian interview during which calcium, nutrient and energy intakes were evaluated over the last three days, coupled with an evaluation of food consumption habits (gluten free, lactose free, vegetarian, and other specific diets). These interviews were conducted according to the recommendations of the French Association of Dietitians Nutritionists (AFDN) (24). The patient's meals of the last three days were entered into a survey calculation software (DATAMEAL) allowing the calculation of calcium and others nutrients intakes based on CIQUAL database (database from the ANSES website Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES), Ciqual (Table de composition nutritionnelle des aliments) (25). In the Ciqual database, energy, macronutrients (proteins, carbohydrates, lipids) and micronutrients (vitamins and minerals), among others, are indicated per 100g of food. The average intake was calculated over these 3 days and considered for the study. Then, all patients were delivered an advice form from GRIO (Groupe de Recherche et d'Information sur les Ostéoporoses) (26). This learned society, created 30 years ago by physicians and rehabilitation specialists, aims at educating care givers, informing the public and promoting research on medical bone pathologies, particularly osteoporosis. This document provided information to patients in both groups on how to improve calcium intakes. The GRIO form is a two-page document in French, indicating the optimal target for daily calcium intake and provides information on the calcium content of different food groups such as fruits, vegetables, calcium-rich mineral waters and other protein-rich foods (**Supplementary Figure 1**).

Patients randomized to the PDA group had an additional personalized interview at baseline with the dietitian lasting at least 20 min, to propose a healthcare plan with negotiated objectives appropriate for the patient's situation, optimizing calcium intake and limiting risk of fractures. They aimed at optimizing calcium intake by increasing the consumption of calcium-rich foods compatible with the patient's dietary profile (choice of dairy products, fatty fish such as sardines, calcium-rich mineral waters, vegetables, oleaginous fruits) and at limiting the risk of osteoporotic fractures (balanced diet sufficiently rich in proteins and adapted physical activity, limitation of overweight, etc.). They also attended two other consultations with the study dietitian at 1 and 3 months to enhance the motivational levers concerning the nutritional changes recommended at baseline. At this stage of the patient's care, the qualitative evaluation of calcium intake (survey of consumption frequencies, not quantified) provided the professional with information on the implementation of the proposed advice, on the obstacles encountered and possible adjustments. Nutritional coaching were dedicated to refinement of calcium objectives, readjustment of calcium intake and reduction of osteoporosis risk factors (vitamin D deficiency, insufficient physical activity, low BMI, smoking, etc.). At the end of the M1 or M3 visits, patients from the PDA group received again the GRIO form annotated with personalized and readjusted advice. The same dietitian followed the patient in the PDA group during this study at baseline, M1 and M3, in order to reproduce the personalized educational management scheme.

Patients from both groups had a further neurology and dietitian consultation at 6 months with another dietitian blinded from randomization to evaluate calcium intake based on CIQUAL data using the same procedure that at baseline.

Objectives

The primary objective of this study was to evaluate the change in calcium intake (mg/day) at 6 months from baseline between PDA and SAF groups, evaluated by a dietary survey.

The secondary objectives of the study were to examine the effect of PDA approach on 25OHD levels and to correlate these to calcium intake. We also evaluated the impact of MS conditions (disability, cognitive status, anxiety, depression and quality of life) on the efficacy of the interventions and the impact

of diet modifications after MS diagnosis on calcium intake at baseline, as an indirect effect of the disease.

Sample size

To determine the sample size, we relied on results of previous studies (27, 28). One study described a 12% increase in calcium intake 1 month after a therapeutic education program including a dietary consultation (27). We hypothesized that the increase would be higher in the PDA arm, approximately 20% at 3 months after three consultations with a dietitian. Based on another study, we assumed a baseline calcium intake of 917 ± 271 mg/day (28). So, hypothesizing a 5% increase in the SAF group (963 mg/day after the intervention, i.e., +27 mg/day) and 20% in the PDA group (1,100 mg/day after intervention, i.e., +183 mg/day), 82 patients per group were calculated to be necessary to demonstrate this difference with a power of 90% and a type 1 error alpha of 5%. Anticipating 10% unevaluable data, 91 patients would be needed per group for a total of 182 patients (the common standard deviation of the difference has been set at 271).

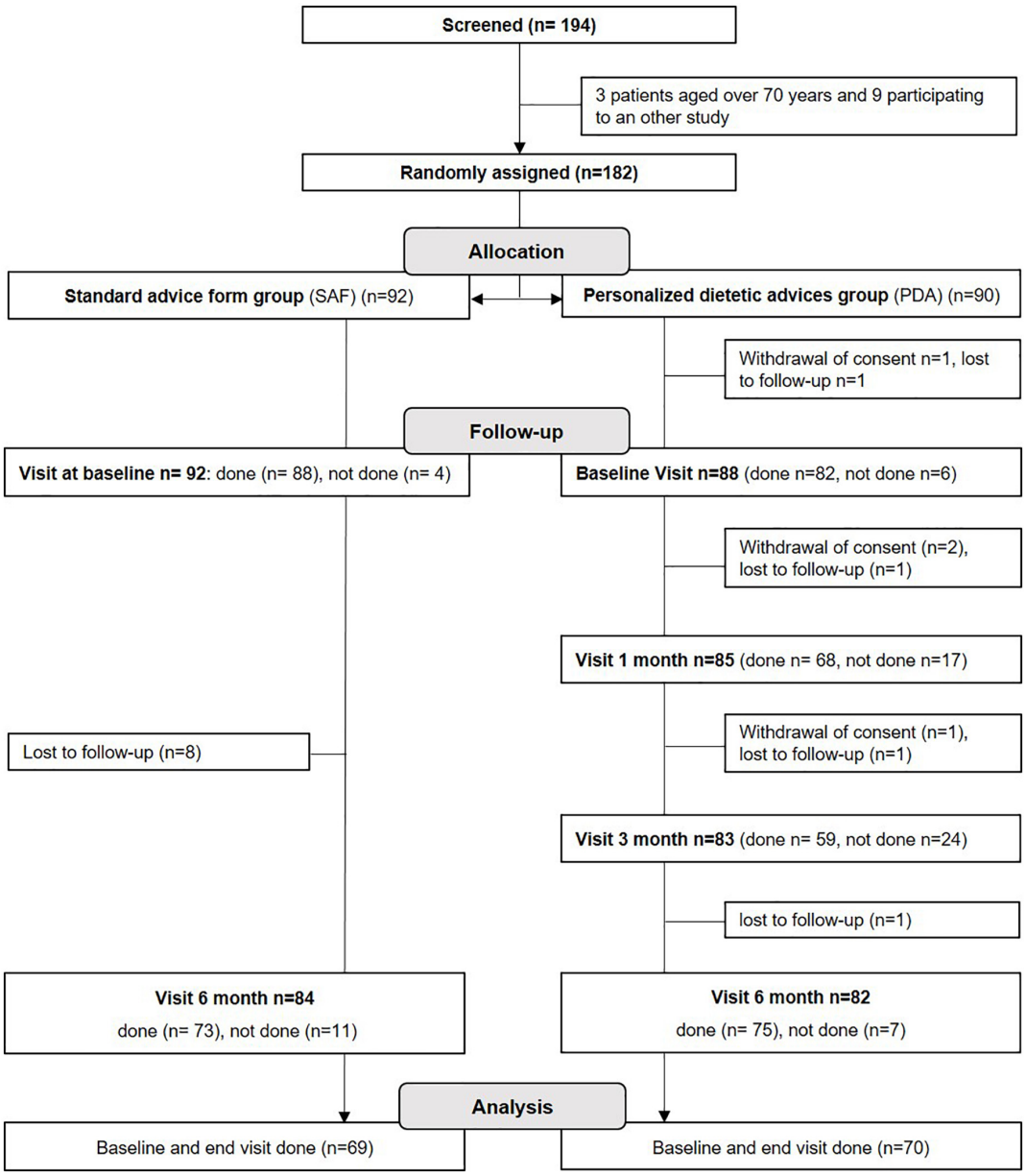

FIGURE 1
Study flow chart.

Stratification

Calcium recommended dietary allowances (RDA) by the ANSES in 2016 (ANSES 2016) were 1200 mg/day for men over 65 years old and postmenopausal women aged over 51 years old, and 900 mg/day for other adults (29). We defined three classes of patients according to these recommendations (**Supplementary Table 1**): (1) the InfraRDA for patients with insufficient daily calcium intake (<900 mg/day for men >65 years and postmenopausal women >51 years and <750 mg/day for other patients); (2) the SubOptiRDA for patients whose calcium intake is close to the recommendation (between 900 and 1,200 mg/day for men >65 years and postmenopausal women >51 years, and between 750 and 900 mg/day for other

TABLE 1 Baseline characteristics.

Variable	Total (n = 182)		SAF (n = 92)		PDA (n = 90)		P-value
Sex, male/female	182	39 (21.4%)/143 (78.6%)	92	18 (19.6%)/74 (80.4%)	90	21 (23.3%)/69 (76.7%)	0.5356[a]
Education level, (LEL/HEL)	180	69 (38.3%)/111 (61.7%)	90	36 (40%)/54 (60%)	90	33 (36.7%)/57 (63.3%)	0.6456[a]
Age, years	182	42 [34; 49]	92	39.5 [33; 46.5]	90	43 [34; 53]	**0.0351**[b]
Weight, kg	167	65 [57; 79]	86	66.5 [58; 82]	81	65 [56; 78]	0.3801[b]
Height, cm	180	166 [160.5; 173]	91	166 [162; 173]	89	167 [160; 173]	0.7203[b]
BMI, kg/m^2	167	23.2 [20.8; 28.7]	86	23.4 [21.3; 28.7]	81	22.9 [20.7; 28.5]	0.6253[b]
Calcium intake, mg	170	865.5 [612; 1095]	88	948 [764.5; 1149.5]	82	772 [579; 941]	**0.0027**[b]
Proteins, g	170	73 [58; 93]	88	76 [62.5; 100]	82	67.5 [57; 89]	**0.0231**[b]
Lipids, g	170	74.5 [57; 98]	88	78 [61; 110]	82	72.5 [55; 91]	0.1081[b]
Carbohydrates, g	170	181 [134; 240]	88	179.5 [137; 245.5]	82	182.5 [131; 222]	0.8275[b]
Energy, kCa	170	1767 [1411; 2191]	88	1827.5 [1448.5; 2255.5]	82	1744.5 [1349; 2049]	0.1764[b]
Vitamin D (μg)	170	2.0 [1.3; 3]	88	2.0 [1.5; 3.8]	82	2.0 [1.1; 2.4]	**0.0100**[b]
25OHD (nmol/L)	172	62 [45; 90]	86	60 [41; 87]	87	64 [47; 95]	0.3497[b]
PASAT score	152	39 [30.5; 49]	78	38.5 [31; 49]	74	39 [29; 46]	0.8453[b]
EDSS score	181	2.0 [1; 4]	92	1.5 [1; 4]	89	2.0 [1; 4]	0.3929[b]
MS duration (years)	182	6 [3;14]	92	5 [2; 12]	90	7 [4; 16]	**0.0176**[b]

SAF, standard advice form approach; PDA, personalized dietary advice approach; LEL, low education level; HEL, high education level; PASAT, Paced Auditory Serial Addition Test; EDSS, Expanded Disability Status Scale.
Data presented as number (%) or median [q1;q3]. P-values in bold denote significant differences.
[a]Statistical significance between groups was calculated by Chi-square test.
[b]Statistical significance between groups was calculated by Wilcoxon–Mann–Whitney test.

TABLE 2 Calcium intake of total and stratified baseline population in PDA and SAF groups and evolution of calcium intake at 6 months in each subgroup.

	Baseline					At 6 months				
	SAF		PDA		P-value	SAF		PDA		P-value
Total population	69	947 [760; 1,150]	70	778 [583; 941]	**0.0077**	69	816 [617; 1,023]	70	1,016.5 [822; 1,391]	**0.0004**
InfraRDA	17	578 [494; 668]	35	583 [497; 650]	0.6909	17	617 [525; 704]	35	939 [791; 1,067]	**0.0086**
SubOptiRDA	16	837.5 [790; 896.5]	10	812 [790; 844]	0.4363	16	817.5 [680.5; 946]	10	932.5 [787; 1,218]	0.227
SupraRDA	36	1,149.5 [1,027; 1,431.5]	25	1,169 [940; 1,340]	0.5165	36	945 [753.5; 1,068]	25	1,283 [1,059; 1,765]	**0.0007**

Daily calcium intake is presented as median [q1; q3]. P-values in bold denote significant differences.
Statistical significance between groups was calculated by Wilcoxon–Mann–Whitney test.
SAF, standard advice form approach; PDA, personalized dietary advice approach; InfraRDA, baseline patients with insufficient daily calcium intake (<900 mg/day for men 65+ years old and postmenopausal women 51+ years old and <750 mg/day for others); SubOptiRDA, baseline patients whose calcium intake is close to the recommendation (900–1,200 mg/day for men 65+ years old and postmenopausal women 51+ years old, and 750–900 mg/day for others); SupraRDA, baseline patients with a calcium intake above the recommendations (>1,200 mg/day for men 65+ years old and postmenopausal women 51+ years old with a daily calcium intake and >900 mg/day for others).

10.3389/fnut.2022.919336

patients); (3) the SupraRDA for patients with a calcium intake above the recommendations (>1,200 mg/day for men >65 years and postmenopausal women >51 years and >900 mg/day for other patients). According to this classification, we first stratified baseline population for analysis of calcium intake change over 6 months. At 6 months, patients were classified again to examine

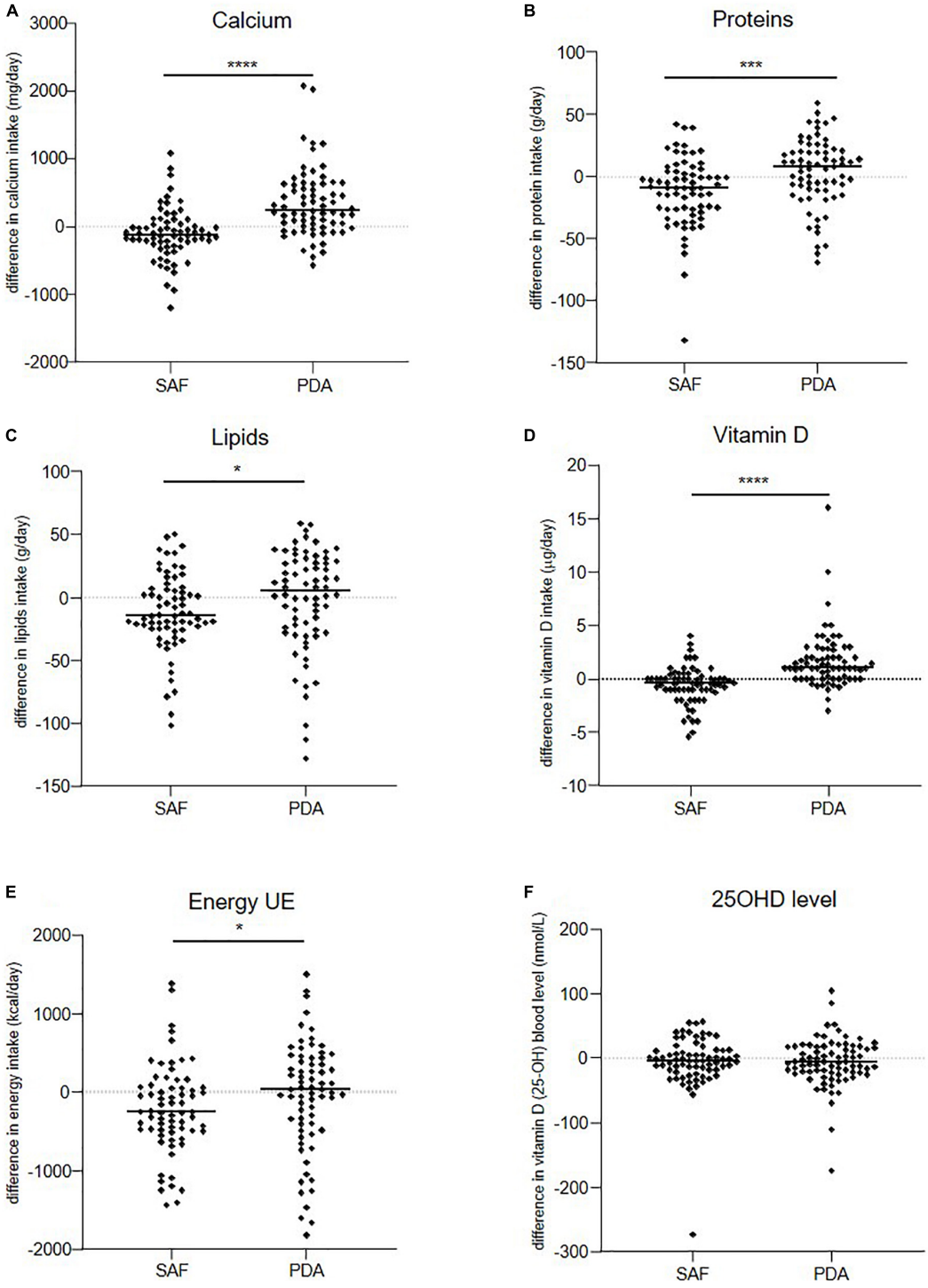

FIGURE 2
Evolution over 6 months of patients' daily intakes. **(A)** Calcium, **(B)** proteins, **(C)** lipids, **(D)** vitamin D, **(E)** energy, and **(F)** level of serum 25OHD in SAF and PDA groups. Means are shown as horizontal bars. Statistical significance was determined using the Wilcoxon–Mann–Whitney test. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

TABLE 3 Daily calcium intake evolution over 6 months in PDA and SAF groups.

	Calcium intake evolution at 6 months				
	SAF		PDA		
Group	*N*	**Median IQR[q1;q3]**	*N*	**Median IQR[q1;q3]**	***P*-value**
Total population	**69**	**−120 [−257; 42]**	**70**	**241.5 [7; 629]**	**<0.0001**
InfraRDA	**17**	**66 [−45; 251]**	**35**	**358 [172; 631]**	**0.0082**
SubOptiRDA	**16**	**−78 [−144.5; 108.5]**	**10**	**120.5 [−84; 436]**	0.1389
SupraRDA	**36**	**−232 [−513.5; −140]**	**25**	**124 [−92; 540]**	**0.0004**

Data presented as median [q1; q3]. *P*-values in bold denote significant differences. Statistical significance between groups was calculated by Wilcoxon–Mann–Whitney test. SAF, standard advice form approach; PDA, personalized dietary advice approach.

potential class change during follow-up in each intervention group. As ANSES recommendations (ANSES 2019) changed during the study (**Supplementary Table 1**), we also performed an ancillary analysis using these new recommendations (30).

Randomization and blinding

After checking the eligibility criteria, patients were randomly assigned (1:1) to PDA group or SAF group according to a randomization list stratified on the center. This list was established by an independent statistician of CHU de Nîmes using specifically designed SAS (Cary, NC, USA) program. Only this statistician knew the number of subjects by block size of 4 or 6. Random allocation sequence was centralized to an online application to which recruiting investigators had access via connection with personal login and password. An independent dietitian evaluating calcium intake at 6 months was blinded to group assignment. Blinding was not possible for patient and study dietitian.

Data collection

During the neurology visit at baseline and 6 months, all patients underwent neurological disability evaluation according to the EDSS (31), scoring disability according to eight systems (bowel and bladder, vision, mental and other) or functional parameters (pyramidal function, cerebellar function, sensory function, and brain stem function). Patients' age, sex and education level [low education level (LEL) < school diploma ≤ high Education level (HEL)] were collected at baseline. Calcium and nutrient intake were evaluated at baseline and at 6 months by a dietary survey calculated using the nutritional composition of foods described in the CIQUAL database (Consulted between 13 July 2016 to 1 January 2020) (25). The Paced Auditory Serial Addition Test (PASAT) (32) was used to evaluate the ability to process information, assess cognitive status and measure sustained and divided attention. The Hospital Anxiety and Depression Scale questionnaire (HADS) (33) validated in French (34) was used to assess anxiety and depressive disorders. It contains seven questions each on anxiety and depression. The final score classifies anxiety and depression symptoms as follows, 0–7: normal; 8–10: average; 11–14: moderate; 15–21: severe; and we considered patients with a score <11 as having no or low disorder and those with a score ≥11 as having a serious disorder (moderate or severe). Quality of life was evaluated by the EQ-5D (35), the total score of the questionnaire is a utility value calculated in relation to the "France" reference with the tool available on the EuroQOL website (36). Five dimensions are measured (mobility, self-sufficiency, routine activities, pain/discomfort, anxiety/depression), on a 5-point Likert scale ("no problem" to "extreme problems"). The responses can be combined in a 5-digit number describing

TABLE 4 Evolution of baseline population distribution in the classes after 6 months (number of patients).

			6 months			
			InfraRDA	SubOptiRDA	SupraRDA	Total
Baseline	SAF	InfraRDA	13	0	4	**17**
		SubOptiRDA	7	6	3	**16**
		SupraRDA	9	8	19	**36**
		Total	**29**	**14**	**26**	**69**
	PDA	InfraRDA	12	8	15	**35**
		SubOptiRDA	2	3	5	**10**
		SupraRDA	1	3	21	**25**
		Total	**15**	**14**	**41**	**70**

SAF, standard advice form approach; PDA, personalized dietary advice approach.

the patient's health status. We chose a cut off at 0.7 (median score of the study population) and we evaluated patient's quality of life as follows: bad $< 0.7 \leq$ good. Blood samples from participants were collected at baseline and 6 months. Circulating levels of 25OHD (concentration in nmol/L) were determined from EDTA serum samples with the Elecsys vitamin D total II kit using the cobas e 801 analytical unit (Roche Diagnostics, USA). This assay reflects vitamin D2 and vitamin D3 sources, although the majority is vitamin D3 given the very limited sources of vitamin D2 unless a participant is taking a vitamin D2 supplement. The laboratory is certified for French quality standards NF ISO15189 and ISO22870. The intra-assay precision was 5.8% for low-level (27.28 nmol/L) and 3.1% for medium level (78.95 nmol/L) measures. The inter-assay precision was 6.56% for low-level measure (27.28 nmol/L) and 5.36% for medium level (78.95 nmol/L). For 2020, the bias (long term) was 0.2%.

Data analysis

Categorical variables are expressed as counts, percentages, and continuous variables as medians and interquartile ranges (IQR) because of their distribution. Baseline characteristic

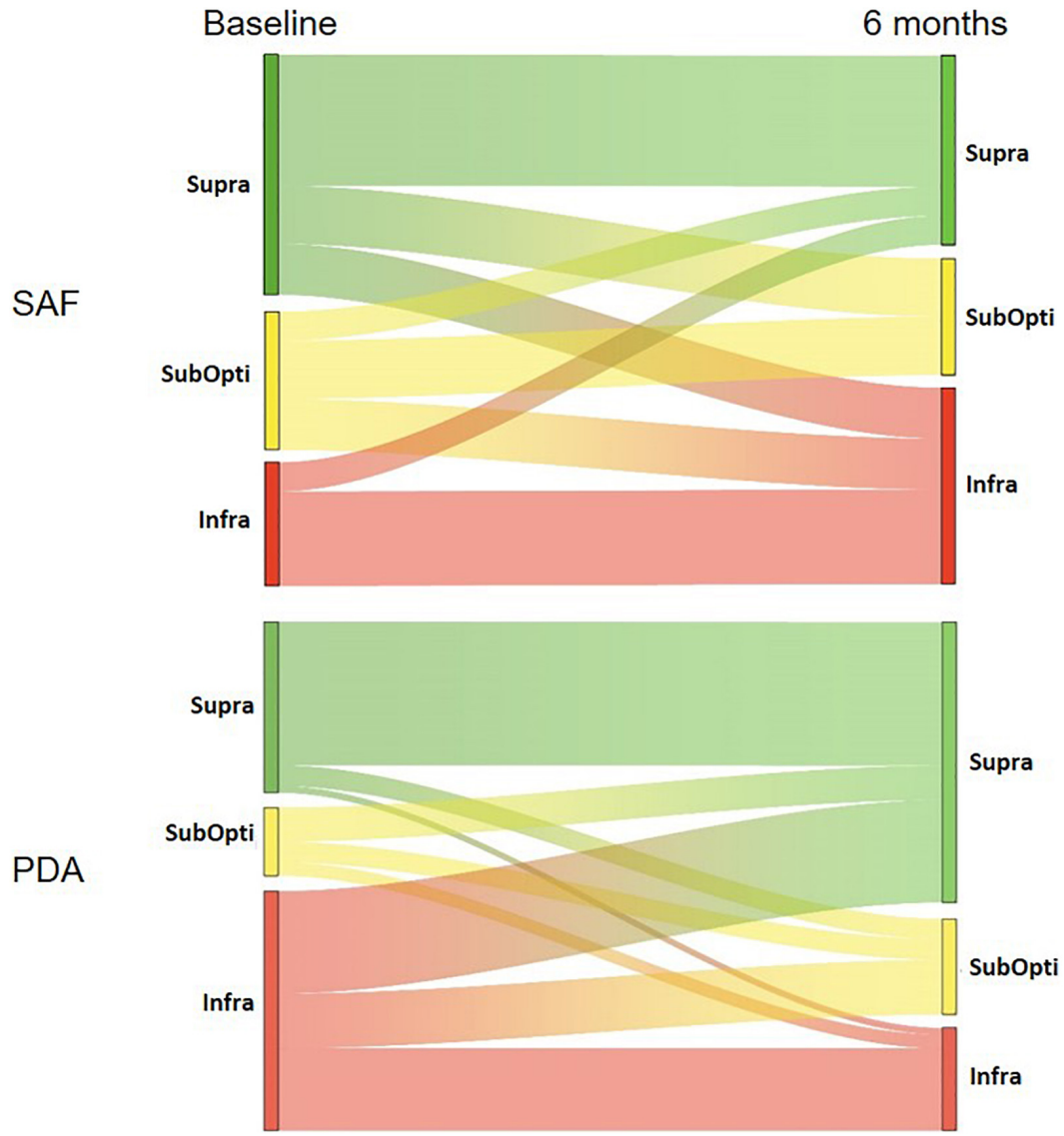

FIGURE 3
Sankey diagrams showing the interclass evolution of patient according to the ANSES 2016 recommendations. Patients were classified into InfraRDA, SubOptiRDA or SupraRDA at baseline and 6 months. Interclass evolution during follow-up is depicted in SAF and PDA groups. For each class, the height of bars corresponds to the number of patients (see Table 4).

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TABLE 5 Daily nutrients intake evolution over 6 months.

	Nutrient evolution at 6 months				
	SAF		PDA		
Nutrient	N	Median IQR [q1;q3]	N	Median IQR [q1;q3]	*P*-value
Protein, g/day	69	−9 [−26; 4]	70	8 [−11; 20]	**0.0007**
Lipids,g/day	69	−15 [−25; 6]	70	5 [−26; 29]	**0.0321**
Carbohydrates, g/day	69	−13 [−53; 15]	70	−4 [−64; 39]	0.5261
Energy, Kcal/day	69	−258 [−495; 55]	70	30.5 [−413; 427]	**0.0277**
Vitamin D, µg/day	69	−0.4 [−1; 0]	70	1 [0; 2.7]	**<0.0001**
Serum 25OHD, nmol/L	64	−5 [−18; 12]	69	−8 [−21; 15]	0.6076

Data presented as median change [q1; q3]. *P*-values in bold denote significant differences. Statistical significance between groups was calculated by Wilcoxon–Mann–Whitney test. SAF, standard advice form approach; PDA, personalized dietary advice approach.

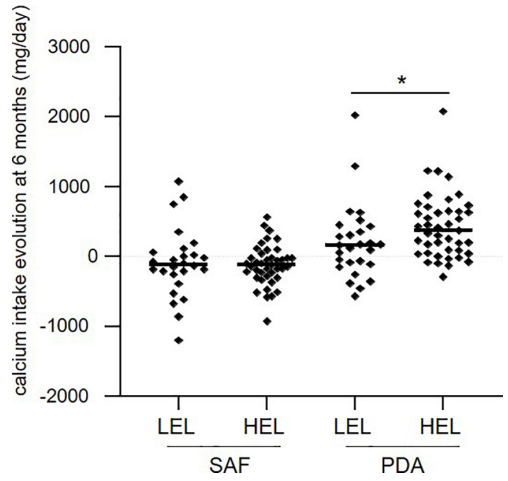

FIGURE 4
Evolution of calcium intake at 6 months in each group, according to the level of education. LEL, low education level; HEL, high education level. Means are shown as horizontal bars. Statistical significance was determined using the Wilcoxon–Mann–Whitney test. *$p < 0.05$.

population and calcium intakes between SAF vs. PDA groups were compared using the chi-square or Fisher's exact test for qualitative variables and the Wilcoxon-Mann-Whitney test for quantitative variables. Spearman r was used for correlation between calcium intakes and levels of 25OHD. Wilcoxon-Mann–Whitney test have been used to compare calcium intake between SAF and PDA subgroups (InfraRDA, subOptiRDA, and SupraRDA) after stratification. Holm and false discovery rate (FDR) correction were used for multiple comparisons. Group effect on 6 months calcium intake was analyzed with adjustment factors using linear model. Analyses were performed with a bilateral alpha level of 0.05 using SAS software, version 9.4 (SAS Institute, Cary, NC, USA).

Results

The flowchart of the study is shown in Figure 1. Out of the 194 patients screened for the study, 182 were recruited between July 2016 and April 2019. Final follow-up visits were completed by October 2019. Ninety-two patients were assigned to the SAF group and 90 to the PDA group. Three patients withdrew consent, 12 patients were lost to follow-up and 28 patients did not attend the baseline or the 6 month visit. Altogether, data from 139 patients who completed the study were analyzed (n = 69 from the SAF group and n = 70 from the PDA group). Analysis of serious adverse events during the study showed that the safety was good as only 10 serious adverse events were reported and none of them were related to the interventions. Serious adverse events occurring are related to an independent medical examination (n = 1, lymph-node biopsy), to surgical and medical procedures (n = 6, 2 induced abortions, 1 cesarean procedure for fetal cardiac rhythm disorder at 30 weeks of amenorrhea, 1 cholecystectomy, 1 sinus surgery by endoscopy, 1 case of botulinum toxin injection for the treatment of overactive bladder syndrome), to gastro-intestinal disorders (n = 2, 1 subileus and 1 intestinal perforation), or to MS (1 case of vertigo and nausea attributed to MS) (data not shown).

Baseline characteristics

Baseline demographic and clinical parameters of the entire population (n = 182) are shown in Table 1. Median age was 42.0, 79% were females, median disease duration was 6 years and median BMI was 23.2. Evaluation of neurological disability and cognitive impairment revealed a median EDSS of 2.0 and a median PASAT of 39.0. Median daily lipid, carbohydrate and protein intake was 74.5, 181, and 73 g, respectively. Median daily calcium intake was 865.5 mg, vitamin D consumption 2.0 µg (Table 1), and median serum level of 25OHD was 62 nmol/L. Calcium intake at inclusion was not correlated with age, disease duration, EDSS, PASAT, and serum levels of 25OHD (Spearman r = 0.02, −0.04, −0.11, 0.18, and 0.011, respectively). Five patients (including 4 PDA and 1 SAF patients) reported calcium supplementation and 33 patients (including 20 PDA and 13 SAF patients) reported Vitamin D supplementation at baseline. Despite randomization, baseline calcium intake in the SAF group was higher (947 mg/day) than in the PDA group (778 mg/day, p = 0.0077).

Evolution of calcium intake at 6 months

Over the 6-month follow-up period, 139 participants were analyzed, 9 patients reported calcium supplementation (including 6 PDA and 3 SAF patients) and 51 patients (29 PDA and 22 SAF patients) reported Vitamin D supplementation at baseline. The median calcium intake for the SAF group was 816 mg vs. 1016.5 mg for the PDA group ($p = 0.0004$) showing at 6 months, a significantly greater increase in median calcium intake in the PDA group (+241 mg/day) than in the SAF group (−120 mg/day) ($p < 0.0001$) (**Table 2** and **Figure 2A**). This significant group effect was confirmed by an analysis with adjusted linear model on baseline calcium intake ($p < 0.0001$). To complete our analysis, we stratified, in each group, the baseline population according to the recommendations of ANSES 2016 and focused on InfraRDA patients with insufficient calcium intake (**Supplementary Tables 1**, **2**). Among the 139 patients who completed the study, 52 patients were InfraRDA (17 in the SAF group and 35 in the PDA group), 26 patients were SubOptiRDA (16 in the SAF group and 10 in the PDA group) and 61 patients were SupraRDA (36 in the SAF group and 25 in the PDA group) at baseline (**Table 3**).

Considering only the InfraRDA patients at baseline, there was no significant difference in the median calcium intake between the two groups (578 mg/day for SAF and 583 mg/day for PDA, $p = 0.69$, Wilcoxon–Mann–Whitney) (**Table 2**). At 6 months, we observed a median increase of 66 mg of calcium intake per day in the SAF group and of 358 mg in the PDA group ($p = 0.0082$; Wilcoxon Mann–Whitney) (**Table 3**). To examine interclass evolution of patients during follow-up, we reclassified the entire population according to ANSES 2016 recommendations at 6 months. Among the 35 InfraRDA patients at baseline, only 12 patients (34%) remained in the InfraRDA class after 6 months of follow-up in the PDA group, compared to 13 patients of 17 (76%) in SAF group (**Table 4** and **Figure 3**). Moreover, the number of InfraRDA patients increased in the SAF group (29 vs. 17 patients at baseline) while the number of SupraRDA patients decreased (26 vs. 36 patients at baseline). Indeed, 17 out of 36 SupraRDA patients in the SAF group reduced their calcium intake [9 patients (25%) moved to the InfraRDA class and 8 (22%) moved to the SubOptiRDA class] (**Table 4** and **Figure 3**). In the same group, 7 patients in the SubOptiRDA class (43%) moved to the InfraRDA class at the end of the follow-up period (**Table 4** and **Figure 3**).

Analysis of calcium intake using the 2019 ANSES guidelines

As the ANSES guidelines changed over the course of the study (30), data were analyzed considering the new recommendations (950 mg/day for adults and 1,000 mg/day for adults under the age of 24 years). We stratified the study population according to these recommendations (**Supplementary Table 1**). The effect of the new classification produced only minor changes in the distribution of patients between groups. The results of the analyses of the primary objective reported similar results, confirming the efficacy of personalized advice by a dietitian (**Supplementary Table 3** and **Supplementary Figure 2**).

Evolution of other nutrient intakes and disease outcomes at 6 months

We also evaluated patient intake of other nutrients between groups after 6 months (**Table 5** and **Figures 2B–D**). In the PDA group, proteins, lipids, and vitamin D intakes increased with a median increase of 8 g/day, 5 g/day, and 1 μg/day, respectively. In contrast, the median intake of these nutrients decreased in the SAF group by 9 g/day, 15 g/day, and 0.4 μg/day, respectively ($p = 0.0007$, $p = 0.032$, and $p < 0.001$, respectively). The nutrient increase in the PDA group was associated with global increase in energy intake (+30.5 Kcal/day), and nutrient intake decrease in the SAF group was associated with a strong decrease in energy intake (−258 Kcal/day) ($p = 0.0277$) (**Table 5** and **Figure 2E**). No difference was observed for 25OHD levels between groups at 6 months (**Table 5** and **Figure 2F**). No influence of the intervention was observed on MS outcomes (PASAT, EDSS, HADS, EQ-5D) (data not shown).

Disease effects on calcium intake

Because MS is associated with cognitive disorders, neurological disability, altered quality of life and mood disorders that may affect the understanding and practical implementation of dietary recommendations, we analyzed the effect of these conditions on calcium intake evolution between groups (**Supplementary Table 2**). We compared neurological disability (EDSS score), cognitive status (PASAT), anxiety (HADS A), depression (HADS D), and quality of life (EQ-5D) in both groups at baseline and at 6 months. Analysis of evolution of calcium intake in each group according to these conditions showed that the efficacy of PDA was still significant in the population with no or low disability (EDSS ≤ 4) (q-value = 0.0002) and population with low or high cognitive status (low < 39 answers < high) (q-value = 0.0002 and 0.0009, respectively) (**Supplementary Table 2**). We observed similar results for anxiety and depression independently of the level of symptoms (low or serious) and for the quality of life (good or bad) (**Supplementary Table 2**).

We also analyzed how changes in diet after MS diagnosis, as an indirect effect of MS, could affect calcium intake, as some patients reduce the consumption of dairy products and

gluten after diagnosis. We observed that 24% of the patients included in this study changed their eating habits because of MS diagnosis. Of note, the majority of participant declared not following any restrictive or specific diet [89.6% (SAF) and 87.8% (PDA)], while very few patients (<3%) declared following a vegetarian or a gluten free diet in the SAF and in the PDA groups. However, no difference was observed between the median calcium intake at inclusion of patients who had not changed their diet (871 mg/day) and those who had changed their diet (840 mg/day) (p = 0.8385, **Supplementary Table 4**). Interestingly, 34 patients (19%) of patients avoided lactose at baseline while only one patient avoided lactose at 6 months.

Influence of education level on calcium intake

We examined the influence of education level on the evolution of calcium intake after 6 months. Patients in the PDA group with high education level (HEL) had a greater median increase of calcium intake after 6 months (373 mg/day) compared to patients with a low education level (LEL) (159 mg/day) (p = 0.023, **Figure 4**), while in the SAF group, the education level did not affect the evolution of calcium intake over 6 months (−115.5 mg/day for LEL and −120 mg/day for HEL, p = 0.87, **Figure 4**). These results strongly suggest that education level affects the effectiveness of the PDA approach.

Discussion

In this study, we show that personalized advice by a dietitian appears to be more effective than the delivery of written recommendations to increase daily calcium intake in MS patients. Indeed, MS patients with insufficient calcium intake (InfraRDA) in the PDA group showed a significant increase in their calcium intake as compared to those from the SAF group. This positive effect of personalized dietary management is confirmed in the SubOptiRDA class in which 50% of patients moved to the SupraRDA in the PDA group and only 19% in the SAF group.

In addition, given that low BMI is a risk factor of osteoporosis, the increase of other nutrients intake and energy intake in the PDA group could constitute a complementary beneficial effect of the PDA approach (37). Moreover, efficient increase in calcium intake based on diet improvement could minimize the need for calcium supplementation potentially at risk of lithiasis (38). Here, very few patients used calcium supplementation and no SAE was observed, especially in the SupraRDA class in the PDA group, suggesting that increasing calcium intake by dietary means might be preferable to calcium supplementation. Interestingly, despite a significant increase of dietary vitamin D intakes between baseline and 6 months in the PDA group, our study population had 25OHD levels below the recommended concentration range of 75–150 nmol/L, suggesting that vitamin D supplementation might still be necessary in MS patients to sustain or improve BMD as these patients show low bone mass, even in early-stage disease (9–11, 39). In this case, vitamin D supplementation needs to be controlled as severe calcium deficiency and excessive vitamin D supplementation may lead to bone density loss and osteoporosis (39).

Looking at factors influencing the PDA efficacy, we showed that efficacy was not affected by age, level of disability or cognitive status, suggesting that this approach would not be altered by the severity of the disease and may be suitable for all MS patients. These results may also suggest that PDA could constitute a more effective approach than SAF in the population at high risk of osteoporosis.

In our study the median calcium intake of the SAF group decreased significantly at 6 months compared to inclusion (p = 0.025), suggesting that the written recommendations may be misinterpreted by MS patients, leading to a paradoxical negative effect. This effect could be the consequence of the disease and/or the GRIO form. MS is associated with cognitive disorders, neurological disability, altered quality of life and mood disorders that may affect the understanding and practical implementation of written dietary recommendations. The GRIO form only provides information on daily-recommended calcium intake and calcium content from enriched food and water. This information seems to be insufficient for MS patients to change their eating habits and might also explain why in the SAF group we did not observe an increase of other nutrients (proteins, lipids, and vitamin D) after 6 months. Long-term follow-up would be necessary to confirm this effect.

Our results highlight the importance of a personalized nutrition advice program as compared to general written recommendations. Several studies have tested the value of nutritional programs in increasing calcium intake to prevent osteoporosis. A study from Beaudoin et al. on 1175 women compared two nutritional education programs (40). One program provided written recommendations (WM) and the other written recommendations and video support explaining the recommendations in detail (VC). Results showed that in women over the age of 50 years, the video program was not more effective than written recommendations alone. Median calcium intake increased by 91 mg/day in the WM group and 93mg/day in the VC group, showing the positive effect of written recommendations in increasing calcium intake in osteoporotic patients. However, another study (41) involving 80 younger participants (19–29 years) that used a 45-min slide show at inclusion and then 8 weeks later, showed that the mean calcium intake of these patients decreased slightly from 961.3 to 905.0 mg/day, demonstrating the lack of effectiveness of this approach. In a study conducted by

Chan et al., 20 women followed a nutritional educational program consisting of a nurse delivering personalized advice accompanied by phone call for follow-up (42). This program changed the eating habits of participants and significantly increased their consumption of dairy products, contrary to the situation for participants receiving no advice. However, the authors did not analyze the calcium intake of participants. Finally, a study on Chinese female immigrants in the United States suffering from severe calcium deficiencies showed that personalized and interactive nutritional workshops resulted in a significant increase in calcium intake as compared to the control group with no dietary intervention (43). The increase achieved in this study was comparable to the results obtained in our study (−26.3 mg/day in the control group vs. +213.2 mg/day in the intervention group), although these patients remained with insufficient intake despite changing their diet.

All these studies suggest the effectiveness of personalized nutritional programs, although less efficiently than in our study. It is worth noting that the above studies were carried out on female populations and, although the majority of participants in our study were women, it also included male individuals (143 women and 39 men).

Other studies have also investigated the impact of dietary advice in other diseases such as cancer. One study investigated the effect of individual dietary advice versus protein-enriched nutritional supplements on nutrition, morbidity and quality of life in colorectal cancer patients three months after undergoing radiotherapy, showing that both interventions had beneficial effects on health during radiotherapy (44). However, only dietary advice was able to maintain these positive effects three months after radiotherapy. Similarly, Insering et al. reported that in cancer patients undergoing radiotherapy, personalized dietary advice resulted in better nutritional intake than written and general recommendations (45). These studies therefore suggest that even in a context of severe disease, the use of dietary advice is probably more effective by promoting better collaboration with the patient and appropriate support.

Inadequate diets play a major role in the increasing prevalence of malnutrition in all its forms and can even become a risk factor for osteoporosis or other diseases (46, 47). In our study, 24% of patients reported changing their eating habits due to MS, but we did not observe any significant difference in their median calcium intake at baseline as compared to the other patients of the study, suggesting that diet changes instigated by these patients did not impact calcium intake.

Education level is a socio-economic factor that influences dietary pattern. Several studies have shown that unhealthy diets are observed more often in people of lower socioeconomic status, based on education level, income level, and type of occupation (21–23, 48). In our study, the PDA approach was less effective in patients with low education level than for those with high education level. This effect could be explained by the fact that this population is less compliant with nutritional recommendations or dietary guidelines (22, 48). Longer follow-up may be necessary to markedly change eating habits in this population.

Our study has some limitations. Despite randomization, the difference in basal calcium intake between the study groups was significant, probably contributing to the negative effect of SAF on calcium intake evolution in this group. Although we perform an analysis with adjusted linear model on baseline calcium intake and a *post-hoc* analysis based on the analysis of patients with insufficient or suboptimal calcium intake at baseline, this cannot replace a stratified random sampling on baseline calcium intake that would have been much preferable. In our study, almost one quarter of patients did not attend all the visits, resulting in a limited power of statistical analyses. The impact of this factor was probably limited since these patients were spread equally between both groups.

Conclusion

This work shows for the first time the importance of the effect of a three-month dietary management program based on personalized interviews in improving calcium intakes in MS patients. Although, long term follow-up coupled to BMD assessment would be necessary to demonstrate a positive effect on osteoporosis prevention in MS patient. Early systematic screening for calcium deficiencies in MS patients seems to be an interesting and promising approach. The safety of dietary management appears to be excellent in view of the total absence of serious adverse events linked to the PDA approach. In addition, it minimizes drug consumption and the risks of iatrogenesis. More generally, due to the potential benefits of nutritional education on the overall health of patients, dietary management might be proposed to integrate the general therapeutic education program in these patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Persons Protection Committee (CPP) Sud Méditerranée III (# 2015.11.01 ter). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ET and SF: conceptualization. TC: methodology. SF, ET, GC, PP, ES, VV, and CB: investigation. SL-C and SA: statistical analysis supervision-validation. ET, HA, SL-C, and SF: data analysis and interpretation. ET and HA: writing—original draft preparation. ET, HA, EE, SL-C, and DR: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.919336/full#supplementary-material

References

1. Peacock M. Calcium metabolism in health and disease. *Clin J Am Soc Nephrol.* (2010) 5:S23–30. doi: 10.2215/CJN.05910809

2. The American Journal of Medicine. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med.* (1993) 94:646–50. doi: 10.1016/0002-9343(93)90218-E

3. Holroyd C, Cooper C, Dennison E. Epidemiology of osteoporosis. *Best Pract Res Clin Endocrinol Metab.* (2008) 22:671–85. doi: 10.1016/j.beem.2008.06.001

4. Tang B, Eslick G, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet.* (2007) 370:657–66. doi: 10.1016/S0140-6736(07)61342-7

5. Jackson R, LaCroix A, Gass M, Wallace R, Robbins J, Lewis C, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* (2006) 354:669–83.

6. Compston A, Coles A. Multiple sclerosis. *Lancet.* (2008) 372:1502–17. doi: 10.1016/S0140-6736(08)61620-7

7. French Federation of Neurology. *National Agency for Accreditation and Evaluation in Health. Conférence de Consensus: La Sclérose en Plaques.* (2001). Available online at: https://www.has-sante.fr/upload/docs/application/pdf/sclerose3.pdf (accessed April 10, 2022).

8. Gupta S, Ahsan I, Mahfooz N, Abdelhamid N, Ramanathan M, Weinstock-Guttman B. Osteoporosis and multiple sclerosis: risk factors, pathophysiology, and therapeutic interventions. *CNS Drugs.* (2014) 28:731–42. doi: 10.1007/s40263-014-0173-3

9. Moen S, Celius E, Sandvik L, Nordsletten L, Eriksen E, Holmøy T. Low bone mass in newly diagnosed multiple sclerosis and clinically isolated syndrome. *Neurology.* (2011) 77:151–7. doi: 10.1212/WNL.0b013e3182242d34

10. Dobson R, Ramagopalan S, Giovannoni G. Bone health and multiple sclerosis. *Mult Scler J.* (2012) 18:1522–8. doi: 10.1177/1352458512453362

11. Bisson E, Finlayson M, Ekuma O, Leslie W, Marrie R. Multiple sclerosis is associated with low bone mineral density and osteoporosis. *Neurol Clin Pract.* (2019) 9:391–9. doi: 10.1212/CPJ.0000000000000669

12. von Geldern G, Mowry E. The influence of nutritional factors on the prognosis of multiple sclerosis. *Nat Rev Neurol.* (2012) 8:678–89. doi: 10.1038/nrneurol.2012.194

13. Calder P, Albers R, Antoine J, Blum S, Bourdet-Sicard R, Ferns G, et al. Inflammatory disease processes and interactions with nutrition. *Br J Nutr.* (2009) 101:1–45. doi: 10.1017/S0007114509377867

14. Yadav V, Marracci G, Kim E, Spain R, Cameron M, Overs S, et al. Low-fat, plant-based diet in multiple sclerosis: a randomized controlled trial. *Mult Scler Relat Disord.* (2016) 9:80–90. doi: 10.1016/j.msard.2016.07.001

15. Farinotti M, Vacchi L, Simi S, Pietrantonj C, Brait L, Filippini G. Dietary interventions for multiple sclerosis. *Cochrane Database Syst Rev.* (2012) 12:CD004192. doi: 10.1002/14651858.CD004192.pub3

16. Titcomb T, Bisht B, Moore D, Chhonker Y, Murry D, Snetselaar L, et al. Eating pattern and nutritional risks among people with multiple sclerosis following a modified paleolithic diet. *Nutrients.* (2020) 12:1844. doi: 10.3390/nu12061844

17. Titcomb T, Brooks L, Smith K, Ten Eyck P, Rubenstein L, Wahls T, et al. Change in micronutrient intake among people with relapsing-remitting multiple sclerosis adapting the Swank and Wahls diets: an analysis of weighed food records. *Nutrients.* (2021) 13:3507. doi: 10.3390/nu13103507

18. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin D, Ross A, Taylor C, Yaktine A, Del Valle H editors. *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: National Academies Press (2011).

19. Schwarz S, Leweling H. Multiple sclerosis and nutrition. *Mult Scler Houndmills Basingstoke Engl.* (2005) 11:24–32. doi: 10.1191/1352458505ms1119oa

20. Sorgun M, Yucesan C, Tegin C. Is malnutrition a problem for multiple sclerosis patients? *J Clin Neurosci.* (2014) 21:1603–5. doi: 10.1016/j.jocn.2013.12.024

21. McKay D, Houser R, Blumberg J, Goldberg J. Nutrition information sources vary with education level in a population of older adults. *J Am Diet Assoc.* (2006) 106:1108–11. doi: 10.1016/j.jada.2006.04.021

22. Thorpe M, Milte C, Crawford D, McNaughton S. Education and lifestyle predict change in dietary patterns and diet quality of adults 55 years and over. *Nutr J.* (2019) 18:67. doi: 10.1186/s12937-019-0495-6

23. Woo J, Leung S, Ho S, Sham A, Lam T, Janus E. Influence of educational level and marital status on dietary intake, obesity and other cardiovascular risk factors in a Hong Kong Chinese population. *Eur J Clin Nutr.* (1999) 53:461–7. doi: 10.1038/sj.ejcn.1600777

24. Association Française des Diététiciens Nutritionnistes. *Le Soin Diététique Réalisé Par Un Diététicien En Établissement De Santé Recommandations*. (2011). Available online at: https://www.afdn.org/

25. ANSES. *Table de Composition Nutritionnelle Des Aliments Ciqual*. (n.d.). Available online at: https://ciqual.anses.fr/

26. GRIO. *Une Alimentation Équilibrée Et Riche En Calcium Pour des os Plus Solides*. (n.d.). Available online at: http://www.grio.org/documents/page85/alimentationassurant-apport-calcique-1.pdf

27. Eric L, Jaffre C, Loiseau P, Villequenault N, Blot B, Benhamou C. *Est-ce Qu'un Programme D'éducation Augmente Les Apports Calciques?*. (2009). Available from: https://www.sciencedirect.com/journal/revue-du-rhumatisme/vol/76/issue/10

28. Ramsaransing G, Mellema S, De Keyser J. Dietary patterns in clinical subtypes of multiple sclerosis: an exploratory study. *Nutr J.* (2009) 8:36. doi: 10.1186/1475-2891-8-36

29. ANSES. *Actualisation des Repères du PNNS: Révision des Repères de Consommations Alimentaires*. (2016). Available online at: https://www.anses.fr/fr/system/files/NUT2012SA0103Ra-1.pdf

30. ANSES. *Avis de l'Agence Nationale de Sécurité Sanitaire de L'alimentation, de L'environnement et du Travail relatif à l'actualisation des Repères Alimentaires du PNNS pour les Femmes dès la Ménopause et les Hommes de plus de 65 ans*. (2019). Available online at: https://www.anses.fr/fr/system/files/NUT2017SA0143.pdf (accessed December 2019).

31. Kurtzke J. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* (1983) 33:1444–52. doi: 10.1212/WNL.33.11.1444

32. Gronwall D, Sampson H. *The Psychological Effects of Concussion*. Oxford: Auckland University Press (1974). p. 118.

33. Zigmond A, Snaith R. The hospital anxiety and depression scale. *Acta Psychiatr Scand.* (1983) 67:361–70. doi: 10.1111/j.1600-0447.1983.tb09716.x

34. Untas A, Aguirrezabal M, Chauveau P, Leguen E, Combe C, Rascle N. Anxiété et dépression en hémodialyse : validation de l'hospital anxiety and depression scale (HADS). *Néphrol Thér.* (2009) 5:193–200. doi: 10.1016/j.nephro.2009.01.007

35. Essink-Bot M, Stouthard M, Bonsel G. Generalizability of valuations on health states collected with the EuroQolc-questionnaire. *Health Econ.* (1993) 2:237–46. doi: 10.1002/hec.4730020307

36. EuroQol Organization. *EQ5D-5L | Valuation | Crosswalk Index Value Calculator*. (n.d.). Available online at: https://euroqol.org/eq-5d-instruments/valuation-of-eq-5d/

37. World Health Organization Technical Report Series. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser.* (2003) 916:i–viii,1–149.

38. Curhan G, Willett W, Speizer F, Spiegelman D, Stampfer M. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Ann Intern Med.* (1997) 126:497–504. doi: 10.7326/0003-4819-126-7-199704010-00001

39. Veldurthy V, Wei R, Oz L, Dhawan P, Jeon Y, Christakos S. Vitamin D, calcium homeostasis and aging. *Bone Res.* (2016) 4:16041. doi: 10.1038/boneres.2016.41

40. Beaudoin C, Bessette L, Jean S, Ste-Marie L, Brown J. The impact of educational interventions on modifiable risk factors for osteoporosis after a fragility fracture. *Osteoporos Int.* (2014) 25:1821–30. doi: 10.1007/s00198-014-2618-4

41. Bohaty K, Rocole H, Wehling K, Waltman N. Testing the effectiveness of an educational intervention to increase dietary intake of calcium and vitamin D in young adult women. *J Am Acad Nurse Pract.* (2008) 20:93–9. doi: 10.1111/j.1745-7599.2007.00281.x

42. Chan M, Ko C, Day M. The effectiveness of an osteoporosis prevention education programme for women in Hong Kong: a randomized controlled trial. *J Clin Nurs.* (2005) 14:1112–23. doi: 10.1111/j.1365-2702.2005.01224.x

43. Lv N, Brown J. Impact of a nutrition education program to increase intake of calcium-rich foods by Chinese-American women. *J Am Diet Assoc.* (2011) 111:143–9. doi: 10.1016/j.jada.2010.10.005

44. Ravasco P, Monteiro-Grillo I, Vidal P, Camilo M. Dietary counseling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. *J Clin Oncol.* (2005) 23:1431–8. doi: 10.1200/JCO.2005.02.054

45. Isenring E, Bauer J, Capra S. Nutrition support using the American dietetic association medical nutrition therapy protocol for radiation oncology patients improves dietary intake compared with standard practice. *J Am Diet Assoc.* (2007) 107:404–12. doi: 10.1016/j.jada.2006.12.007

46. Rizzoli R. Nutritional aspects of bone health. *Best Pract Res Clin Endocrinol Metab.* (2014) 28:795–808. doi: 10.1016/j.beem.2014.08.003

47. Micha R, Peñalvo J, Cudhea F, Imamura F, Rehm C, Mozaffarian D. Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. *JAMA.* (2017) 317:912–24. doi: 10.1001/jama.2017.0947

48. Finger J, Tylleskär T, Lampert T, Mensink G. Dietary behaviour and socioeconomic position: the role of physical activity patterns. *PLoS One.* (2013) 8:e78390. doi: 10.1371/journal.pone.0078390

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Targeted nutritional intervention with enhanced recovery after surgery for carotid endarterectomy: A prospective clinical trial

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Ischemic stroke is the most common cerebrovascular disease, and vascular obstruction is an important cause of this disease. As the main method for the management of carotid artery stenosis, carotid endarterectomy (CEA) is an effective and preventive treatment measure in ischemic cerebrovascular disease. This study aims to propose the application of a new enhanced recovery after surgery (ERAS) nutritional support regimen in CEA, which can significantly improve the perioperative nutritional status of patients. A total of 74 patients who underwent CEA were included and randomly divided into two groups: 39 patients received nutritional therapy with the ERAS protocol (ERAS group) and 35 patients received routine perioperative nutritional support (control group). Our results showed that the levels of major clinical and biochemical parameters (albumin, hemoglobin, creatinine, calcium and magnesium levels, etc.) in the ERAS group were significantly higher than those in the control group after surgery ($p<0.05$). Additionally, patients in the ERAS group had dramatically shorter postoperative length of stay and reflected higher mean satisfaction at discharge ($p<0.001$). Moreover, no statistically significant differences were observed in postoperative complication rates and Mini-mental State Examination scores at discharge. The emergence of this neurosurgical ERAS nutritional support program can effectively intervene in perioperative nutritional status, and notably reduce postoperative hospital stays.

KEYWORDS

carotid endarterectomy, enhanced recovery after surgery, nutrition, rehabilitation, ischemic stroke

Introduction

The causes of ischemic stroke are extremely complex and diverse, and mainly result from thromboembolic occlusion of the major cerebral artery or its branches and severe narrowing (stenosis) of the carotid artery (1). The two major approaches, neuroprotection and improvement of cerebral blood circulation, are used to treat ischemic stroke, the latter of which uses thrombolytic drugs or mechanical devices to recanalize occluded vessels (2, 3). Carotid endarterectomy (CEA) is a surgical procedure for patients with vascular stenosis or obstruction that can remove the carotid intima thickened atherosclerotic plaque to prevent stroke caused by

plaque peeling off (4–6). Carotid surgery treatment may reduce the risk of stroke and the incidence of cerebral infarction, and effectively control postoperative and long-term stroke recurrence and mortality (7). However, patients undergoing CEA surgery are often at an advanced age and have comorbid organ diseases, along with degeneration of body functions and other factors. Therefore, postoperative weakness, metabolic disturbance and malnutrition are prone to occur after invasive surgery under general anesthesia due to the stress response of surgical trauma and postoperative fasting. Based on this situation, our research group put forward the application of a nutritional protocol for enhanced recovery after surgery (ERAS) in CEA.

ERAS refers to the implementation of various effective methods in the perioperative period to reduce the complications of patients undergoing surgery and speed up the recovery of patients (8, 9). ERAS pays attention to reducing the perioperative stress response of surgical patients, including physical and psychological stress. Although there is no report on the application of ERAS in CEA for nutritional support, referring to the clinical application of other disciplines, it is expected to potentially improve the nutritional status of patients during the perioperative period, enhance the immune function of patients, and promote postoperative recovery (10–13).

To the best of our knowledge, the ERAS protocol for CEA, especially with regard to its nutrition domain, has not been established. Recently, our research group developed a multidisciplinary ERAS protocol for CEA based on the best available evidence. The aim of the present study was to prospectively propose a novel, multidisciplinary, evidence-based, neurosurgical ERAS nutritional protocol for CEA. We wished to evaluate the safety and effectiveness of the ERAS nutritional regimen, and to expectantly evaluate there was a significant postoperative improvement in physical condition and recovery in patients compared with those receiving standard care in our institution.

Materials and methods

Patient recruitment

This study was carried on between February 2020 and July 2021 at the Department of Neurosurgery, Tangdu Hospital, Air Force Medical University (Xi'an, China) and was registered with the Chinese Clinical Trial Registry (ChiCTR2000029570). The randomized control trial was approved by the Institutional Human Research and Ethics Committee of Tangdu Hospital. All patients were provided with all the information concerning the study, including detailed explanations and written notifications. Informed and signed consent was obtained from all patients to participate and all patients with CEA were screened for eligibility. Patients that required emergency surgery, had serious consciousness and movement disorders before surgery, required emergency surgery pathologically, or who had other confounding factors that may affect postoperative recovery (such as paralysis, spinal deformity, autoimmune diseases, myocardial infarction, serious infection, liver or kidney dysfunction or serious psychological or mental diseases) were excluded.

A total of 74 patients who met the selection criteria were randomized into either an ERAS group or control group. The selection sequence was computer generated and the results were reviewed by a statistician, which ensured the objectivity and randomness of the experiment. First, 35 patients were placed in the control group and received routine perioperative care according to the practice mode of the institution. The remaining 39 patients were assigned to the ERAS group and received treatment according to new ERAS nutritional protocol described in this study. The researchers responsible for the follow-up visit and the surgeons were all masked to treatment assignment during the study phase of CEA. Through these measures, the study was not affected by subjective human factors.

Nutritional risk screening

The screening tool we used was nutritional risk screening 2002 (NRS 2002), which was proposed by European Society for Parenteral and Enteral Nutrition (ESPEN) guidelines on the basis of analysis of controlled clinical trials (14–16). A total score greater than 3 indicated that the patient was malnourished or at risk of nutrition and should avail of nutritional support. If the score was 0–3, patients only had a slight risk were excluded from the study. Their status was reviewed weekly and these patients were received no nutritional support.

Nutritional protocol for ERAS and conventional care

The nutrition plan for this ERAS was designed for patients undergoing CEA, based on concepts from other established plans and drawing on extensive and current evidence-based support for perioperative nutritional interventions. We have studied traditional nutritional support programs and improved them according to the specific conditions of individual patient, and creatively proposed a new set of ERAS nutritional programs. Briefly, our protocol includes the following aspects: (1) preoperative management and assessment, which aimed at correcting malnutrition, improving nutritional status and optimizing body composition. A nutritional support working group was established to cooperate closely with clinicians and support staff from the ultrasound, anesthesiology, inpatient and surgical care, and nutritional services departments. Patients in the ERAS group abstained from solid food for 6 h before surgery, took no more than 400 ml of carbohydrates orally 2 h before surgery and had their fasting blood glucose and preoperative blood glucose monitored. Meanwhile, for malnourished patients, preoperative nutritional support was required. The preferred method was enteral nutrition (EN) support 7–10 days before surgery, usually with oral nutrition supplementation. However, if the patient had an intake disorder, tube feeding was required. (2) Intraoperative surgery and anesthesiology management, although not the key to the ERAS nutritional program, was a standard intraoperative measure and an important prerequisite for ERAS. Firstly, selected the appropriate surgical position and approach, and designed a reasonable surgical incision. Secondly, ropivacaine was given before surgery for subcutaneous local anesthesia (when the operation time > 3 h, anesthesia was given again), concomitantly, general anesthesia combined with regional nerve block anesthesia was selected, and the systemic application of opioids was reduced. Next, it was necessary to strictly control arterial blood pressure and maintain the stability of cerebral flow during the operation, and monitor end-tidal carbon dioxide to prevent

hyperventilation. Finally, optimized the suture of the incision and avoided routine drain placement. (3) Postoperative nutritional support: patients in the ERAS group were given parenteral nutrition (PN) and EN support according to their own gastrointestinal conditions. The timing and dose of early postoperative eating or EN were determined according to the patient's gastrointestinal function and tolerance. Liquid food was taken 6 h after the operation, after which the patients could be changed to semi-liquid food after the intestinal ventilation was restored, and the intake could then be gradually increased according to the tolerance of the gastrointestinal tract. During this period, the oral administration or tube feeding of EN solution, as the core of ERAS nutritional care, played an irreplaceable role in postoperative nutritional support. In particular, when the patient was not suitable to receive EN, nutrients were provided by continuous infusion through the peripheral intravenous route of PN. In cases where EN could not be initiated, PN was supplemented as soon as possible if the patient was at high nutritional risk (NRS score $\geq$ 5). Supplemental PN was administered if EN intake and protein levels remained below 60% of target after 7–10 days of EN treatment. In addition, blood glucose continued to be strictly monitored postoperatively to keep it within the ideal range of 7.77–9.99 mmol/L.

Correspondingly, the control group adopted the traditional regimen, fasting for 8 h before surgery, with no water intake for 4 h before surgery. A liquid diet was given 1 day after surgery, which gradually returned to normal. It should be noted that the measures presented above are only one part of the ERAS scheme and must be highly compatible with other steps of ERAS in order to achieve better clinical treatment effect (Figure 1).

Patients received conventional perioperative care in our unit. Preoperative care mainly included psychological care of patients, ward environmental protection, advice on smoking and alcohol cessation, application of preventive antibiotics and antithrombotic therapy, etc. Meanwhile, postoperative care, including monitoring of vital signs every 1–2 h, nebulization of the airway, sleep management and setting discharge criteria was also implemented. Patients were also advised to perform appropriate rehabilitation exercise, which was conducive to the patient's positive mood and a more ideal prognosis.

Health guidance after discharge

After discharge, it was recommended that patients limit heavy physical activity and avoid strenuous exercise for 3 to 4 weeks. Also, patients were instructed to maintain emotional stability and avoid excessive tension, excitement, or mood swings. In addition, it was also essential to develop good living habits, such as quitting smoking and drinking, eating a reasonable diet, and resting frequently. In terms of nutrition, for most surgical patients, low-salt, low-fat and easily digestible food was recommended to maintain a balanced diet and satisfy the body's needs for various nutrients. If the patient lose weight significantly after surgery, it was recommended to increase the intake of calories and protein to meet the needs of rehabilitation. In particular, oral nutritional supplements were an important component of post-discharge dietary plans for surgical patients. For severely malnourished patients and patients with long postoperative hospital stay or intensive care units stay, oral nutritional supplements were instructed to be used for 3 to 6 months after surgery. Nursing personnel instructed the patient to self-observe bleeding tendency and take medication as prescribed. Finally, the patients needed to actively cooperate with telephone follow-up and outpatient follow-up.

Data collection and observation indicators

Preoperatively, demographic variables including age, sex, height, weight, body mass index, education level, occupational status, marital status, American Society of Anesthesiologists grade, and patient comorbidities (smoking, diabetes, hypertension, hypercholesterolemia, etc.) were recorded clinically. Biochemical and clinical parameters, including albumin, hemoglobin, liver and kidney function, and electrolytes, were also measured by preoperative venous blood collection. During the operation, blood glucose, blood pressure, pulse, oxygen saturation, central venous pressure, body temperature, end-tidal carbon dioxide, and respiratory rate were monitored. Postoperatively, peripheral fasting venous blood was extracted on the first and third postoperative days to determine biochemical and clinical parameters. Furthermore, the patients' bowel movements were observed and recorded, as were other conditions during the nutritional support treatment. Clinical outcome variables comprised readmission, reoperation, postoperative surgical and non-surgical complications, as well as functional recovery [Karnofsky performance status (KPS)] at discharge and at 30-days follow-up. The primary endpoint was the postoperative length of stay (LOS), and the secondary endpoints included postoperative complications, postoperative quality-of-life (QoL), medical cost, readmission, and evaluation of patient satisfaction. At the same time, the symptoms of each group were observed during the treatment, and the prognosis was determined at the time of discharge (17, 18).

Statistical analysis

The data analysis was performed using the SPSS (Ver. 19, IBM Corp., Armonk, NY, United States). Descriptive statistics were used to define baseline characteristics. The Kolmogorov–Smirnov test was used to identify the normal distribution of the variables. Group differences with continuous data with normal distribution were statistically examined using the Student's *t*-test, while data without normal distribution were analyzed using the Mann–Whitney *U*-test. Readmission, complications, and mortality were analyzed using the chi-square test (with/without Yates correction) or Fisher's accurate test. The sample size was powered to be 58 patients in each group based on the hypothesis that the primary outcome (postoperative LOS) would be reduced by 25% with a power of 80% and significance of 5%. Assuming a maximal dropout rate of 20%, the final sample size was determined as 74 patients per arm. In turn, analysis was planned when the minimal number of the predefined sample size was met. The data were considered to be statistically significant if $p < 0.05$.

Results

Baseline characteristics

Between February 2020 and July 2021, a total of 112 patients from our hospital were enrolled in the present study. After

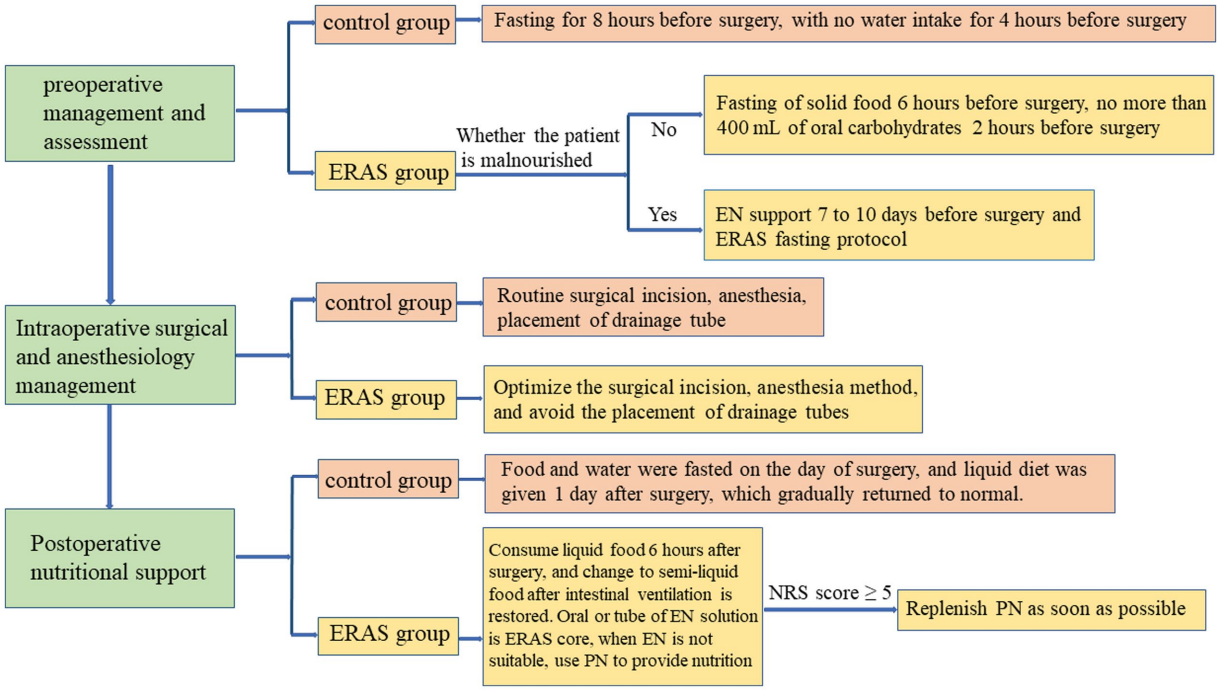

FIGURE 1
Summary of nutritional interventions in the two groups.

exclusion, a total of 74 patients (35 in the control group and 39 in the ERAS group) were included in the analysis (Figure 2). The demographic and clinical characteristics (including sex, mean age, mean BMI, ASA grade, and marital status, etc.) of the two groups of patients were not significantly different ($p > 0.05$). Concomitant diseases such as cerebral infarction, sequelae of cerebral infarction, cardiac/hypertension, diabetes mellitus, hypercholesterolemia, liver/gallbladder, lung, and miscellaneous were equally distributed between the two groups. In terms of nutrition, most patients both groups had normal nutritional status. Both groups of patients underwent CEA by the same experienced surgical team, and all patients received the assigned intervention. Characteristics of patients are shown in Table 1.

Surgery characteristics

The main surgical results were shown in Table 2. The differences in mean duration of surgery, cross-clamping time, carotid plaque size, blood transfusion, lateral location, and blood loss >300 ml between the two groups were not remarkable. The mean duration of surgery of the ERAS group was 150.08 ± 19.89 min and that of the control group was 149.11 ± 21.71 min ($p = 0.843$). For intraoperative monitoring of CEA patients, blood loss is an important indicator. There were 9 and 11 patients whose blood loss volume was over 300 ml in the ERAS group and control group, respectively ($p = 0.419$). Additionally, two patients in the ERAS group required blood transfusion (i.e., red blood cell and blood plasma transfusions) during the operation, while none of the patients in the control group required a transfusion.

Clinical and biochemical parameters

Table 3 mainly described the changes in the content of various substances in the blood before and after surgery. There was no significant difference in the levels of various nutrients between the two groups before surgery. However, at postoperative day (POD) 1, there was a significant trend toward an increase of albumin, hemoglobin and calcium in the ERAS group compared with the control group ($p < 0.001$), which continued at POD 3 ($p < 0.05$). The overall pattern of creatinine and magnesium levels was similar in ERAS group, which increased at POD 1 and then decreased over the next couple of days. In the control group, creatine and magnesium was increased at POD 1 and decreased to the preoperative level at POD 3, while the contents of other substances were decreased at POD 1 and increased at POD 3.

Postoperative hospital stays and hospitalization expenses

Evaluation of LOS, cost, and postoperative recovery were shown in Table 4. The data showed that LOS was significantly lower in the ERAS group than in the control group (4.31 ± 0.98 days vs. 6.71 ± 2.09 days, $p < 0.001$). Additionally, there was also a significant difference in overall cost between the two groups (2.43 ± 0.18 10,000 yuan in the ERAS group vs. 2.57 ± 0.26 10,000 yuan in the control group, $p < 0.05$).

Assessment of patient satisfaction

All patients completed the discharge satisfaction survey questionnaire. The figure below showed the changes and differences

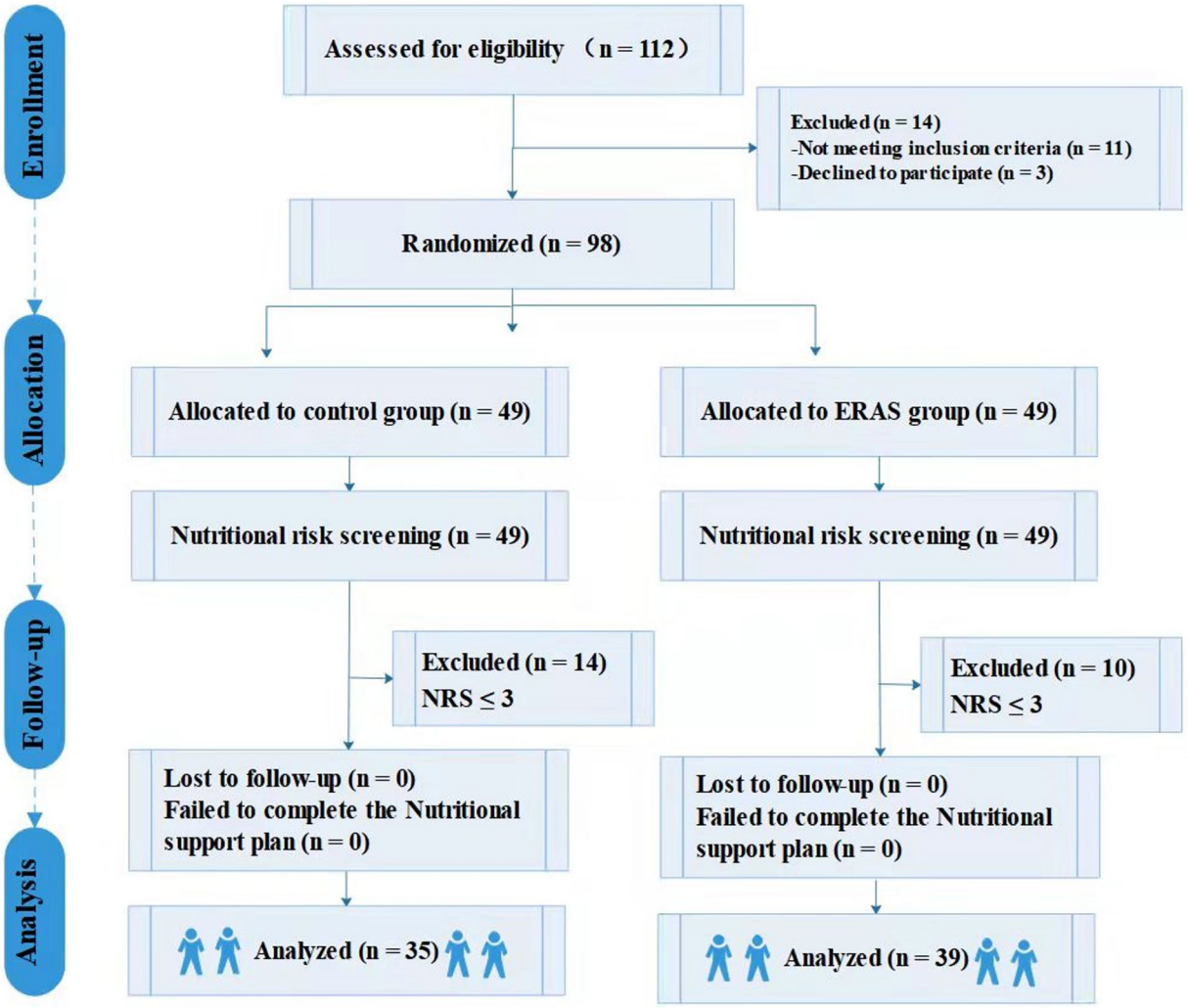

FIGURE 2
Flow diagram for study participants.

in QoL and Mini-mental State Examination (MMSE) scores between the two groups before surgery, at discharge, and at postoperative month (POM) 3 (Figure 3). The mean overall satisfaction of patients in the ERAS group at discharge was significantly higher than that of the control group (89.82 ± 3.52 score vs. 80.31 ± 4.04 score). Similarly, there were differences in satisfaction between the two groups, with obvious differences in medical care (18.46 ± 1.23 score in the ERAS group vs. 17.23 ± 1.82 score in the control group), nursing care (18.95 ± 1.07 score in the ERAS group vs. 17.49 ± 1.80 score in the control group), and enhanced recovery (18.26 ± 1.48 score in the ERAS group vs. 13.49 ± 2.85 score in the control group; $p < 0.001$).

There was little difference in QoL and MMSE scores between the two groups in preoperative period ($p > 0.05$), but differences were evident after discharge. At discharge, the QoL score of the ERAS group was 73.00 ± 3.93 score and that of the control group was 67.71 ± 5.20 score ($p < 0.001$). However, at POM 3, the QoL and MMSE scores of two groups showed no statistical difference ($p > 0.05$; Figure 3).

Follow-up results showed that survey satisfaction in the ERAS group was significantly higher than that in the control group ($p < 0.001$). There were significant differences in grip strength improvement at POM3 between the two groups (5.95 ± 2.08 score in the ERAS group vs. 4.54 ± 2.31 score in the control group, $p = 0.007$). However, no remarkable difference between the two groups was noted in the other outcomes relating to the KPS score (90.00 ± 3.83 score in the ERAS group vs. 89.95 ± 3.41 score in the control group, $p = 0.952$). Detailed patient satisfaction scores according to each module are shown in Table 4.

Postoperative complications after discharge

Table 5 showed the postoperative complications. Stroke, as an important complication after CEA, occurred in one patient (2.56%) in the ERAS group and two patients (5.71%) in the control group ($p = 0.493$). Two patients in the ERAS group and none in the control group had deep vein thrombosis ($p = 0.130$). None of the patients experienced symptoms of dyspnea or surgical site infection/subcutaneous effusion in the ERAS group. Additionally, postoperative nausea and vomiting intensity scale and nausea visual analog scale were performed postoperatively in both groups, but the proportions of patients with mild, moderate, and severe disease varied.

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TABLE 1 Sociodemographic and clinical features [Mean+SD, *n*(%)].

Variable	Control group (*n*=35)	ERAS group (*n*=39)	*p*-value
Mean age (year)	72.09 ± 10.05	70.41 ± 8.42	0.438
Gender (n)			0.116
Male	21 (60.00%)	30 (76.92%)	
Female	14 (40.00%)	9 (23.08%)	
Mean BMI(kg/m^2)	20.90 ± 1.02	21.36 ± 1.94	0.163
Education (*n*)			0.417
No education/primary school	8 (22.86%)	11 (28.21%)	
Secondary school/high school	13 (37.14%)	9 (23.08%)	
College/more than college	14 (40.00%)	19 (48.72%)	
Occupation (*n*)			0.84
Employed	9 (25.71%)	10 (25.64%)	
Unemployed	11 (31.43%)	9 (23.08%)	
Retired	9 (25.71%)	11 (28.21%)	
Home maker	6 (17.14%)	9 (23.08%)	
Marital status (*n*)			0.805
Unmarried (single/divorced)	3 (8.57%)	4 (10.26%)	
Married	32 (91.43%)	35 (89.74%)	
ASA grade (*n*)			0.81
ASA I	9 (25.71%)	11 (28.21%)	
ASA II	26 (74.29%)	28 (71.79%)	
Concomitant diseases (*n*)			
Cerebral infarction	8 (22.86%)	9 (23.08%)	0.982
Sequelae of cerebral infarction	2 (5.71%)	3 (7.69%)	0.735
Cardiac/hypertension	25 (71.43%)	30 (76.92%)	0.589
Diabetes mellitus	23 (65.71%)	27 (69.23%)	0.747
Smoker	4 (11.43%)	9 (23.08%)	0.189
Hypercholesterolemia	27 (77.14%)	32 (82.05%)	0.6
Liver/gallbladder	3 (8.57%)	5 (12.82%)	0.714
Lung	3 (8.57%)	2 (5.13%)	0.662
Miscellaneous	4 (11.43%)	3 (7.69%)	0.701
Nutrition (*n*)			0.874
Normal	31 (88.57%)	35 (89.74%)	
Mild malnutrition	1 (2.86%)	2 (5.13%)	
Moderate malnutrition	2 (5.71%)	1 (2.56%)	
Severe malnutrition	1 (2.86%)	1 (2.56%)	

ASA, American Society of Anesthesiologists grade.

TABLE 2 Surgery characteristics [Mean+SD, *n*(%)].

Variable	Control group (*n*=35)	ERAS group (*n*=39)	*P*-value
Lateral location (*n*)			0.451
Right	21 (60.00%)	20 (51.28%)	
Left	14 (40.00%)	19 (48.72%)	
Mean duration of surgery (min)	149.11 ± 21.71	150.08 ± 19.89	0.843
Cross-clamping time (min)	21.29 ± 3.97	19.67 ± 5.63	0.162
Carotid plaque size (cm)	2.67 ± 0.57	2.79 ± 0.52	0.328
Blood loss > 300 mL (*n*)	11 (31.43%)	9 (23.08%)	0.419
Blood transfusion (*n*)	0 (0.00%)	2 (5.13%)	0.174

Discussion

The traditional nutritional regimen includes fasting before surgery, a liquid diet on the first postoperative day, after which the patient gradually transition to a normal diet. Preoperative fasting depletes the body's carbohydrate reserves, resulting in reduced preoperative comfort. In addition, fasting may change the body's endocrine and metabolic response and reduce the body's ability to resist stress after surgery, thereby increasing postoperative complications. Thus, it can be seen that conventional nutritional support for the patient remains problematic, unsystematic and not fully aligned with clinical care and other perioperative steps.

Due to the importance of the perioperative nutritional status of CEA patients, nutritional improvement measures for patients have become diverse and complex (19, 20). It is of great significance to propose a new and more reasonable perioperative nutritional therapy based on existing mature experiences of medical staff, research literature, and research progress. Briefly, the nutritional measures combined both EN and PN, with EN as the key factor to improve the measures and the main nutritional mode. Compared with the control group, which adopted a conventional nutritional regimen, the ERAS group had better preoperative mental states, visual field vision, language, body activity, and limb muscle strength. It is beneficial to the success of the operation, and significantly promotes the postoperative intervention and nursing, as well as the rehabilitation of patients.

The results of this study showed that serum albumin decreased in patients undergoing CEA surgery within a short period of admission. After 3 days of nutritional support treatment, serum albumin and hemoglobin increased more significantly in the ERAS group than in the control group, indicating that the ERAS group was more conducive to protein synthesis. In terms of specific postoperative physiological parameters, such as blood albumin, hemoglobin, cholesterol, and creatinine, among others, the ERAS group had better results than those of the control group, which may reflect the result's wider significance that patients in the ERAS group had increased immunity, better body function, reduced complications, and shorter length of hospital stay. According to the personalized evaluation of patients, the satisfaction of the ERAS group was also much higher than that of the control group. Indeed, the patients in the ERAS group had better clinical compliance of postoperative follow-up. Thus, the nutritional measures had a good effect on the patients' physical condition and significantly improved postoperative recovery. Furthermore, it had a positive effect on patients' subjective feelings and inner emotions.

In recent years, it has been recognized that the gastrointestinal tract is not only an organ of digestion and absorption, but an important immune organ (21, 22). Based on this, the advantages of EN are not only reflected in the direct absorption and utilization of nutrients through the intestine, more physiological, convenient administration and low cost, but helped to maintain the integrity of intestinal mucosal structure and barrier function (23, 24). The ESPEN guidelines propose that normal food intake or EN should start early

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TABLE 3 Laboratory characteristics [Mean+SD].

Variable	Control group (n=35)	ERAS group (n=39)	*P* value
Albumin (mg)			
Pre-operation	37.78 ± 2.63	37.10 ± 2.76	0.286
POD 1	37.23 ± 2.81	39.73 ± 3.00***	<0.001
POD 3	40.09 ± 2.46	41.93 ± 2.44*	0.002
Hemoglobin (g/L)			
Pre-operation	122.60 ± 8.01	124.29 ± 7.44	0.351
POD 1	121.71 ± 7.38	130.83 ± 4.79***	<0.001
POD 3	132.20 ± 6.28	135.59 ± 6.93*	0.032
Cholesterol (mmol/L)			
Pre-operation	6.30 ± 1.85	6.32 ± 1.64	0.959
POD 1	6.14 ± 1.81	6.54 ± 1.58	0.322
POD 3	6.54 ± 1.74	6.45 ± 1.49	0.809
Blood urea nitrogen (mmol/L)			
Pre-operation	5.77 ± 1.31	5.40 ± 1.23	0.207
POD 1	5.55 ± 1.33	5.75 ± 1.44	0.543
POD 3	6.05 ± 1.43	5.97 ± 1.27	0.794
Creatinine (umol/L)			
Pre-operation	74.91 ± 12.89	77.60 ± 11.76	0.35
POD 1	78.47 ± 12.62	79.71 ± 11.48	0.66
POD 3	75.51 ± 13.44	78.91 ± 12.10	0.256
Calcium (mmol/L)			
Pre-operation	2.37 ± 0.05	2.35 ± 0.08	0.185
POD 1	2.31 ± 0.05	2.45 ± 0.09***	<0.001
POD 3	2.45 ± 0.13	2.53 ± 0.08*	0.003
Magnesium (mmol/L)			
Pre-operation	0.89 ± 0.10	0.88 ± 0.09	0.517
POD 1	0.91 ± 0.08	0.92 ± 0.10	0.818
POD 3	0.88 ± 0.09	0.88 ± 0.10	0.82
Phosphorus (mmol/L)			
Pre-operation	1.16 ± 0.10	1.16 ± 0.10	0.747
POD 1	1.15 ± 0.12	1.19 ± 0.10	0.211
POD 3	1.16 ± 0.10	1.16 ± 0.09	0.99

POD, Postoperative day.
*$P < 0.05$; ***$P < 0.001$.

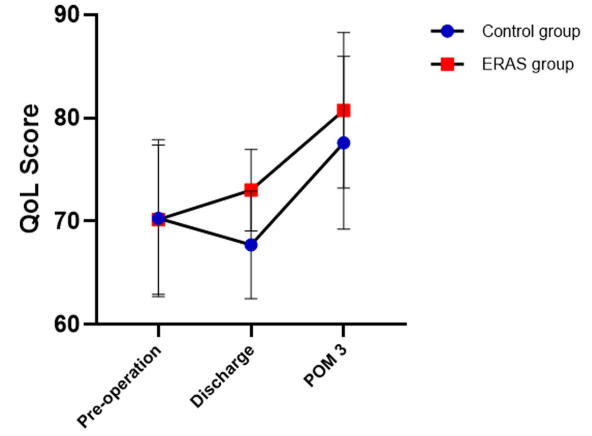
TABLE 4 Postoperative recovery [Mean+SD].

Variable	Control group (*n*=35)	ERAS group (*n*=39)	*P*-value
Postoperative LOS(day)	6.71 ± 2.09	4.31 ± 0.98***	<0.001
Overall cost, 10,000 (Yuan; Chinese Yuan Renminbi)	2.57 ± 0.26	2.43 ± 0.18*	0.017
Overall satisfaction(score)	80.31 ± 4.04	89.82 ± 3.52***	<0.001
Information	15.80 ± 2.61	17.44 ± 1.68***	0.002
Medical care	17.23 ± 1.82	18.46 ± 1.23***	<0.001
Nursing care	17.49 ± 1.80	18.95 ± 1.07***	<0.001
Enhanced recovery	13.49 ± 2.85	18.26 ± 1.48***	<0.001
Comfort and others	16.31 ± 2.41	16.72 ± 2.14	0.448
QoL (score)			
Pre-operation	70.29 ± 7.59	70.15 ± 7.21	0.939
Discharge	67.71 ± 5.20	73.00 ± 3.93***	<0.001
POM 3	77.60 ± 8.37	80.74 ± 7.52	0.093
MMSE (score)			
Pre-operation	18.60 ± 4.95	19.38 ± 5.28	0.513
Discharge	20.54 ± 4.53	22.97 ± 4.40*	0.022
POM 3	24.83 ± 3.61	25.44 ± 3.80	0.484
GSI POM 3(score)	4.54 ± 2.31	5.95 ± 2.08*	0.007
KPS(score)	90.00 ± 3.83	89.95 ± 3.41	0.952

KPS, Postoperative Karnofsky performance status; Postoperative LOS, length of stay, days; QoL, quality of life; POM, Postoperative month; MMSE, Mini-mental State Examination; GSI POM 3, grip strength improvement in 3 months after surgery. *$P < 0.05$; ***$P < 0.001$.

after surgery (25). An analysis was conducted to investigate the relationship between perioperative nutritional intervention, especially preoperative intervention and surgical effect in the ERAS group. Patients receiving the perioperative nutrition regimen had a shorter hospital stay, faster recovery of intestinal function, and greater patient satisfaction compared with patients in the control group. Furthermore, immunity was enhanced and there were less postoperative complications compared with the control group. Early preoperative nutrition status was associated with a significant reduction in postoperative overall complications. According to the experimental results, the extremely low incidence of postoperative complications may be related to long-term preoperative training. This effect was more pronounced in patients who received longer periods of preoperative nutrition. In addition, their physical condition and mental outlook were better in the early postoperative period than those in the control group. This was mainly reflected in their significantly better physical condition and earlier participation in postoperative exercise recovery, and their compliance and overall satisfaction were better than those in the control group. In conclusion, we have shown that preoperative nutritional intervention played a key role in the prognosis of patients undergoing surgery.

Satisfaction evaluation was a balance between the patients' expectations of care and the actual care provided, reflecting the changes in health status caused by the effectiveness of hospital care (26, 27). The analysis of the satisfaction test results showed that patients found value in using personalized clinical nursing measures. Furthermore, important results were through data collation: clinical compliance of the patients (such as quitting smoking and drinking, taking medication regularly, and exercising regularly) was significantly associated with patient satisfaction. Compared with the control group, no significant difference in the ERAS group was found in regard to satisfaction with clinical nursing. However, in terms of self-subjective feelings, the survey results showed that the ERAS group had more positive emotions and better expectations for both the near and distant future. This is obviously of great value to the clinical rehabilitation and follow-up treatment of patients. These predictors could be interpreted as the determinants of patient satisfaction in each group when other factors do not change greatly within the group.



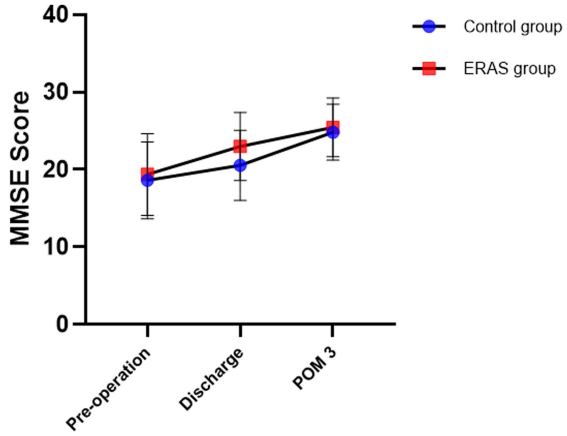


FIGURE 3
Comparison of MMSE and QoL scores between the two groups.

TABLE 5 Postoperative complications *n* (%).

Variable	Control group (*n*=35)	ERAS group (*n* =39)	*P*-value
Mortality (*n*)	0	0	—
PONV VAS (*n*)			0.101
Mild (0–4)	22 (62.86%)	33 (84.62%)	
Moderate (5–6)	9 (25.71%)	4 (10.26%)	
Severe (7–10)	4 (11.43%)	2 (5.13%)	
Dyspnea (*n*)	0(0.00%)	0 (0.00%)	—
Surgical site infection/ subcutaneous effusion (*n*)	1 (2.86%)	0 (0.00%)	0.288
Stroke (*n*)	2 (5.71%)	1 (2.56%)	0.493
Cardiovascular (*n*)	0 (0.00%)	1 (2.56%)	0.340
Gastrointestinal (*n*)	1 (2.86%)	1 (2.56%)	0.938
Urinary tract (*n*)	2 (5.71%)	1 (2.56%)	0.493
DVT (*n*)	0 (0.00%)	2 (5.71%)	0.130

PONV, postoperative nausea and vomiting; VAS, Visual Analog Scale; DVT, deep vein thrombosis.

The current study had some limitations. The main weakness of this study was the absence of important nutritional indices, such as calorie needs, energetic needs, protein needs etc., which we tried to compensate for with albumin and other biochemical markers. Advantages were that we used NRS score and preoperative EN before surgery, and we followed the patients nutritional support suggestions after discharge. Furthermore, while our data supported the efficacy and safety of our perioperative nutrition support program, larger multicenter studies are needed to assess its applicability in patients undergoing CEA surgery.

Conclusion

According to our study, perioperative nutrition in ERAS program had a positive effect on postoperative rehabilitation and improved postoperative complications in CEA patients. The LOS and the cost of hospitalization were, in turn, significantly reduced. Finally, under dedicated nursing care, the mental state and subjective feelings of patients were greatly improved. Further research is needed to demonstrate the effect of clinical nutrition support in a pragmatic manner.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Human Research and Ethics Committee of Tangdu Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

BL and YQ conducted the study design. Y-QL, X-PQ, and L-WP completed the writing of the manuscript. Later revisions were done by BL, YQ, J-YA, X-WL, and YZ. Y-QL, X-PQ, L-WP, J-YA, X-WL, YZ, CW, XJ, LG, GL, D-LW, and D-CZ participated in the data collection, while data analysis is done by CW, XJ, LG, GL, D-LW, and D-CZ. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Tissue plasminogen activator for acute ischemic stroke. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. (1995) 333:1581–8. doi: 10.1056/NEJM199512143332401

2. Smith LN, James R, Barber M, Ramsay S, Gillespie D, Chung C. Rehabilitation of patients with stroke: summary of SIGN guidance. *BMJ*. (2010) 340:c2845. doi: 10.1136/bmj.c2845

3. Uk NCCF. *Stroke: National Clinical Guideline for diagnosis and initial Management of Acute Stroke and Transient Ischaemic Attack (TIA)*. London, UK: Royal College of Physicians (2008).

4. Brott TG, Hobson RN, Howard G, Roubin GS, Clark WM, Brooks W, et al. Stenting versus endarterectomy for treatment of carotid-artery stenosis. *N Engl J Med*. (2010) 363:11–23. doi: 10.1056/NEJMoa0912321

5. Moore WS. Commentary. Prevention of disabling and fatal strokes by successful carotid endarterectomy in patients without recent neurological symptoms: randomized controlled trial. *Perspect Vasc Surg Endovasc Ther*. (2005) 17:62-1–3. doi: 10.1177/153100350501700112

6. Rosenfield K, Matsumura JS, Chaturvedi S, Riles T, Ansel GM, Metzger DC, et al. Randomized trial of stent versus surgery for asymptomatic carotid stenosis. *N Engl J Med*. (2016) 374:1011–20. doi: 10.1056/NEJMoa1515706

7. Grant EG, Benson CB, Moneta GL, Alexandrov AV, Baker JD, Bluth EI, et al. Carotid artery stenosis: gray-scale and Doppler US diagnosis--Society of Radiologists in ultrasound consensus conference. *Radiology*. (2003) 229:340–6. doi: 10.1148/radiol.2292030516

8. Kehlet H, Wilmore DW. Evidence-based surgical care and the evolution of fast-track surgery. *Ann Surg*. (2008) 248:189–98. doi: 10.1097/SLA.0b013e31817f2c1a

9. Ljungqvist O, Scott M, Fearon KC. Enhanced recovery after surgery: a review. *JAMA Surg*. (2017) 152:292–8. doi: 10.1001/jamasurg.2016.4952

10. Jones EL, Wainwright TW, Foster JD, Smith JR, Middleton RG, Francis NK. A systematic review of patient reported outcomes and patient experience in enhanced recovery after orthopaedic surgery. *Ann R Coll Surg Engl*. (2014) 96:89–94. doi: 10.1308/003588414X13824511649571

11. Smith MD, Mccall J, Plank L, Herbison GP, Soop M, Nygren J. Preoperative carbohydrate treatment for enhancing recovery after elective surgery. *Cochrane Database Syst Rev*. (2014):CD009161. doi: 10.1002/14651858.CD009161.pub2

12. Pedziwiatr M, Kisialeuski M, Wierdak M, Stanek M, Natkaniec M, Matlok M, et al. Early implementation of enhanced recovery after surgery (ERAS(R)) protocol—compliance improves outcomes: a prospective cohort study. *Int J Surg*. (2015) 21:75–81. doi: 10.1016/j.ijsu.2015.06.087

13. Gustafsson UO, Scott MJ, Hubner M, Nygren J, Demartines N, Francis N, et al. Guidelines for perioperative Care in Elective Colorectal Surgery: enhanced recovery after surgery (ERAS((R))) society recommendations: 2018. *World J Surg*. (2019) 43:659–95. doi: 10.1007/s00268-018-4844-y

14. Kondrup J, Rasmussen HH, Hamberg O, Stanga Z. Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. *Clin Nutr*. (2003) 22:321–36. doi: 10.1016/S0261-5614(02)00214-5

15. Hersberger L, Bargetzi L, Bargetzi A, Tribolet P, Fehr R, Baechli V, et al. Nutritional risk screening (NRS 2002) is a strong and modifiable predictor risk score for short-term and long-term clinical outcomes: secondary analysis of a prospective randomised trial. *Clin Nutr*. (2020) 39:2720–9. doi: 10.1016/j.clnu.2019.11.041

16. Mercadal-Orfila G, Lluch-Taltavull J, Campillo-Artero C, Torrent-Quetglas M. Association between nutritional risk based on the NRS-2002 test and hospital morbidity and mortality. *Nutr Hosp*. (2012) 27:1248–54. doi: 10.3305/nh.2012.27.4.5791

17. Nagata S, Fukuzawa K, Iwashita Y, Kabashima A, Kinoshita T, Wakasugi K, et al. Comparison of enteral nutrition with combined enteral and parenteral nutrition in post-pancreaticoduodenectomy patients: a pilot study. *Nutr J*. (2009) 8:24. doi: 10.1186/1475-2891-8-24

18. Chen CC, Li HC, Liang JT, Lai IR, Purnomo J, Yang YT, et al. Effect of a modified hospital elder life program on delirium and length of hospital stay in patients undergoing abdominal surgery: a cluster randomized clinical trial. *JAMA Surg*. (2017) 152:827–34. doi: 10.1001/jamasurg.2017.1083

19. Vacas S, Van de Wiele B. Designing a pain management protocol for craniotomy: a narrative review and consideration of promising practices. *Surg Neurol Int*. (2017) 8:291. doi: 10.4103/sni.sni_301_17

20. Nechay T, Sazhin A, Titkova S, Tyagunov A, Anurov M, Melnikov-Makarchuk K, et al. Evaluation of enhanced recovery after surgery program components implemented in laparoscopic appendectomy: prospective randomized clinical study. *Sci Rep*. (2020) 10:10749. doi: 10.1038/s41598-020-67591-5

21. Galli G, Purchiaroni F, Lahner E, Sacchi MC, Pilozzi E, Corleto VD, et al. Time trend occurrence of duodenal intraepithelial lymphocytosis and celiac disease in an open access endoscopic population. *United European Gastroenterol J*. (2017) 5:811–8. doi: 10.1177/2050640616680971

22. Buchan AM. An immunocytochemical study of endocrine pancreas of snakes. *Cell Tissue Res*. (1984) 235:657–61. doi: 10.1007/BF00226965

23. Saito H, Trocki O, Alexander JW, Kopcha R, Heyd T, Joffe SN. The effect of route of nutrient administration on the nutritional state, catabolic hormone secretion, and gut mucosal integrity after burn injury. *JPEN J Parenter Enteral Nutr*. (1987) 11:1–7. doi: 10.1177/014860718701100101

24. Hosoda N, Nishi M, Nakagawa M, Hiramatsu Y, Hioki K, Yamamoto M. Structural and functional alterations in the gut of parenterally or enterally fed rats. *J Surg Res*. (1989) 47:129–33. doi: 10.1016/0022-4804(89)90076-0

25. Plauth M, Cabre E, Campillo B, Kondrup J, Marchesini G, Schutz T, et al. ESPEN guidelines on parenteral nutrition: hepatology. *Clin Nutr*. (2009) 28:436–44. doi: 10.1016/j.clnu.2009.04.019

26. Pascoe GC. Patient satisfaction in primary health care: a literature review and analysis. *Eval Program Plann*. (1983) 6:185–210. doi: 10.1016/0149-7189(83)90002-2

27. Johansson P, Oleni M, Fridlund B. Patient satisfaction with nursing care in the context of health care: a literature study. *Scand J Caring Sci*. (2002) 16:337–44. doi: 10.1046/j.1471-6712.2002.00094.x

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Efficacy of short-chain polypeptide-based EEN formulas in alleviating intestinal injury in children with Crohn's disease: a single-center study in China

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Short-chain polypeptides are composed of three to nine amino acids, which can be absorbed by the intestinal tract without digestive enzymes and ATP energy. Crohn's disease (CD) is a chronic non-specific disease derived from inflammation and damage of the gastrointestinal tract. In this study, we aim to investigate the effect of short-chain polypeptide-based exclusive enteral nutrition (EEN) formulas on intestinal injury in Chinese children with active CD. From January 2013 to January 2019, a total of 84 consecutive children with a diagnosis of Crohn's disease (CD) in the Department of Pediatric Gastroenterology, Children's Hospital of Nanjing Medical University, were divided into mild and moderate-to-severe active CD groups. Each group was further divided into two subgroups: drug group and short-chain polypeptide plus drug group. Tests were carried out on the levels of intestinal fatty acid binding protein (I-FABP) in the blood, fecal calprotectin (FC), and occludin protein in the intestinal mucosa 1day before treatment and 8weeks after treatment. Endoscopic and histopathological observations were detected to compare the changes in intestinal injury in children with active CD. After 8weeks of treatment, the SES-CD scores and Chiu scores of the ileocecal area and terminal ileum of children with mild active CD and the ileocecal area of children with moderate-to-severe active CD in short-chain polypeptide plus drug group were significantly lower than those in the drug group. The OD value of occludin in the terminal ileum and ileocecal area of children with mild active CD and the ileocecal area of children with moderate-to-severe active CD after short-chain polypeptide-based EEN formulas and drug treatment was significantly higher than those in the drug group (p <0.05). Meanwhile, the levels of FC and I-FABP were significantly decreased (p <0.05). The results showed that short-chain polypeptide-based EEN formulas effectively alleviate intestinal injury in children with active CD.

KEYWORDS

short-chain polypeptide, exclusive enteral nutrition, Crohn's disease, intestinal injury, children

Introduction

Crohn's disease (CD) involves chronic relapsing inflammation and damage in the gastrointestinal tract (1). Up to 15% of patients are diagnosed with CD before the age of 20 years (2). Worldwide, the incidence rate of CD in children is between 2.5/100000 and 11.4/100000 (3).

During the occurrence and development of CD, the intestinal epithelial tight junction (TJ) barrier plays an important role (4). A defective intestinal TJ barrier has been implicated as an important pathogenic factor in inflammatory diseases of the gut including CD (4). Occludin protein, as an important part of TJ, is expressed at the top junction between intestinal epithelial cells and regulates the permeability of cell membranes through interaction with the TJ complex (5). The loss or decrease of occludin levels can cause the rupture of TJ, leading to intestinal epithelial TJ barrier dysfunction (6). Intestinal fatty acid binding protein (I-FABP) is a water-soluble protein secreted by monolayer columnar epithelial cells and only exists in gastrointestinal mucosa. After the intestinal mucosa is damaged, I-FABP quickly enters the blood circulation through the cell membrane, capillaries, lymphatic capillaries, and portal vein (7). Therefore, the levels of I-FABP in the blood reflect the degree of intestinal mucosal damage (8).

Severe gastrointestinal injury in pediatric CD causes nutrition and growth retardation, which influences the therapeutic effect and prognosis of children (9). Therefore, early nutrition intervention can promote the improvement of gastrointestinal injury growth and development (10). Although exclusive enteral nutrition (EEN) is one of the effective treatments for pediatric CD and has been recommended as the first-line treatment (11, 12), the influence of different types of nutritional formulas, especially different protein compositions, on the effectiveness of EEN for treatment of active CD is still unclear (13). Short-chain polypeptides are composed of three to nine amino acids, which can be absorbed by the intestinal tract without digestive enzymes and ATP energy. Peptide-based formulas have been shown to improve nitrogen balance and visceral protein synthesis, reduce bacterial translocation and diarrhea, and restore gut integrity (14). Studies have shown that short-chain polypeptides can promote the development of the structure and function of intestinal epithelial cells and contribute to the recovery of the damaged intestinal mucosa (15, 16). However, no clear recommendations are available on the use of short-chain polypeptide-based nutritional formulas in ill children with active CD.

This study aimed to evaluate the effects of drug regimens with or without short-chain polypeptide-based EEN formulas on intestinal injury in pediatric CD patients.

Materials and methods

Patients

A total of 114 children with active CD who were hospitalized in the Department of Pediatric Gastroenterology at the Children's Hospital of Nanjing Medical University, from January 2013 to January 2019, with the diagnostic criteria according to the ESPGHAN revised porto criteria were selected as the research subjects (17). Failure to receive short-chain polypeptide-based EEN feeding according to the protocol resulted in the exclusion of eight patients. In addition, 22 patients were excluded as they did not receive endoscopy after treatment. Finally, 84 consecutive patients, including 41 with mild active CD and 43 with moderate-to-severe active CD were studied. Patients treated with short-chain polypeptide-based EEN formulas ($n = 42$) and without short-chain polypeptide-based EEN formulas ($n = 42$) were considered as the study and control groups. This study was approved by the ethics committee of the Children's Hospital of Nanjing Medical University, and its clinical trial registration number is Chir 180018278 in Chinese Clinical Trial Registry.

Inclusion and exclusion criteria

The following inclusion criteria were used to screen children with CD for inclusion in this clinical study: (1) the age of children included was between 1 and 10 years; (2) children who were newly diagnosed as CD; and (3) children with active CD.

The exclusion criteria for children with CD included: (1) the age of children with CD was more than 10 years or less than 1 year; (2) children with CD in remission; (3) children with recurrent CD; (4) children with other chronic intestinal infectious diseases, such as Behcet's disease, intestinal malignant lymphoma, tuberculosis infection, and hematological diseases; and (5) children with isolated oral ulcers or perianal lesions.

According to the pediatric CD activity index (PCDAI), children with CD were divided into mild active CD (10.0–27.5 points) and moderate-to-severe active CD (≥30.0 points).

Clinical data

The general information (name, gender, and age), clinical manifestations, and personal and family history were obtained by asking about the patient's medical history. Clinical and laboratory indicators were collected 1 day before treatment and 8 weeks after treatment and were communicated with the parents. After obtaining the consent of the parents and signing the informed consent form, a colonoscopy (Olympus Lucera cv-260, Japan) was performed under general anesthesia. HE staining and immunohistochemical staining were performed to observe the degree of intestinal mucosal damage and occludin protein expression.

Short-chain polypeptide-based EEN and drug therapy

Short-chain polypeptide-based EEN formula (Petamen Junior, Nestle Company, Switzerland) was given by oral or nasogastric tube or nasojejunal tube (18). The total amount of short-chain polypeptide-based EEN solution was fed per day according to the children's weight. The daily amount of nutrient solution for children weighing 1–10 kg, 11–20 kg, and over 20 kg was 100 ml/kg, 1,000 ml + (children weight - 10 kg) × 50 ml/kg, and 1,500 ml + (children weight - 20 kg) × 20 ml/kg, respectively. The energy density was 100 kcal/100 ml. The drug regimens of newly diagnosed mild active CD patients included (19): 2–4 g/day of oral mesalazine for 8 weeks. The drug regimens of newly diagnosed

moderate-to-severe active CD patients included (20): corticosteroids were initiated as either 1.5 mg/(kg·day) up to a maximum of 32 mg/day of intravenous methylprednisolone for 1–2 weeks or 1 mg/(kg·day) up to a maximum of 40 mg/day of oral prednisone every morning for 4 weeks, a taper of 40 mg/day of oral prednisone every morning for 2 weeks. An endoscopic reexamination was performed after 8 weeks of treatment.

Evaluation of intestinal injury under endoscopy

According to the simple endoscopic score for Crohn's disease (SES-CD), a score of ≤2 was considered as CD endoscopic remission, 3–6 as mild inflammation, 7–15 as moderate inflammation, and ≥16 as severe inflammation (21, 22).

Assessments of intestinal histopathological changes

Colonoscopy was performed before treatment and reexamined after treatment. The cecum and terminal ileum mucosa were stained with HE. Histopathological changes were observed under a light microscope (Olympus Company, Japan). Double-blind scoring was performed by two pathologists according to the modified Chiu's score standard of small intestinal histopathology (23). The highest score observed in the sample was used to determine the degree of intestinal injury.

He staining of intestinal tissues

The specimens of ileocecal and ileal tissues were used to prepare the paraffin section. Paraffin section dewaxing to water. The sections were sequentially placed in xylene, anhydrous ethanol, and gradient concentration ethanol for dewaxing. After neutral resin-sealed slides, these samples were observed under a microscope.

Immunohistochemical staining of intestinal tissues

We took samples of ileocecal colon and terminal ileal mucosa from children with CD for paraffin embedding, sectioning, and immunohistochemical staining for occludin protein. The method was as follows: paraffin sections were dewaxed, hydrated, antigen repaired, and then diluted with occludin working solution (Rabbit anti-human occludin polyclonal antibody, Abcam company, United States). Sheep anti-rabbit IgG (Shanghai Biyuntian Biotechnology Co., Ltd., China) was incubated in a 37°C incubator for 30 min. Streptomyces anti-human occludin peroxidase solution was used to stain, dehydrate, and seal with neutral glue. Finally, 10 randomly chosen visual fields were observed on each section under a microscope for photography. We then used the Image-Pro Plus 6.0 software to determine the average optical density (OD) values of occludin-positive tissues on the staining sections, which is a quantitative occludin protein method and one of the indicators to evaluate intestinal TJ barrier function. In this study, OD values can be used as one of the quantitative values to evaluate the tight junction function of intestinal epithelium before and after CD treatment.

Determination of I-FABP and FC levels

One day before the endoscopic examination, blood and stool samples were collected from children with active CD, and I-FABP and FC were detected, respectively. After 8 weeks of treatment, we conducted an endoscopic reexamination and collected blood and stool samples again 1 day before the endoscopic examination for I-FABP and FC detection, which were compared and analyzed with those before the examination. The methods of the I-FABP and FC detection were as follows: 2 ml of blood was collected from the elbow vein and placed in a centrifuge for further testing (Beijing Baiyang Medical Equipment Co. Ltd., China). The supernatant was taken, and the I-FABP was determined using the ELISA Kit (Shanghai Xitang Technology Co. Ltd., China). Fecal samples were added to normal saline according to the ratio of 1:50 (mass/volume) and shaken for 20 min. A total of 1 ml of homogenate was placed in the centrifuge, and 0.5 ml of supernatant was taken for detection by the ELISA kit (Wuhan Xinqidi Biotechnology Co. Ltd., China).

Statistical analysis

SPSS 21 software was used for statistical analysis. The measurement data were expressed by mean ± standard deviation. The comparison between both groups was conducted by *t*-test. The chi-square test was used to compare the count data between groups, $p < 0.05$ indicated that the difference was statistically significant.

Results

The effect of short-chain polypeptide-based EEN formulas on intestinal injury of active CD

General evaluation of clinical data

There were 41 children with mild active CD, including 21 cases in the drug group and 20 cases in the short-chain polypeptide plus drug group, and 43 children with moderate-to-severe active CD, including 21 cases in the drug group and 22 cases in the short-chain polypeptide plus drug group. There were no significant differences in age, clinical manifestations, and intestinal involvement sites at the same active CD severity between both groups (Table 1).

The effect of short-chain polypeptide-based EEN formulas on PCDAI score

PCDAI is one of the important indicators to evaluate the severity of disease activity and evaluate the efficacy of pediatric CD. In this study, the PCDAI score of children with mild active CD was 25.50 ± 1.80 in the short-chain polypeptide plus drug group and

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TABLE 1 Clinical data of children with active CD.

	Mild active CD		*p* value	Moderate to severe active CD		*P* value
	Drug treatment group	Short peptide plus drug treatment group		Drug treatment group	Short peptide plus drug treatment group	
Sample size (*n*)	21	20		21	22	
Male/female	9/12	9/11	0.747	9/12	7/15	0.311
Age (year)	4.62 ± 2.54	3.58 ± 1.00	0.700	5.13 ± 2.60	4.59 ± 2.01	0.400
Positive family history	0 (0%)	1 (5%)	0.311	1 (5%)	1 (5%)	1.000
Clinical manifestation						
Abdominal pain	18 (85%)	16 (80%)	0.677	19 (90%)	19 (85%)	0.633
Diarrhea	10 (45%)	10 (50%)	0.757	11 (50%)	13 (55%)	0.752
Hematochezia	5 (25%)	4 (20%)	0.705	5 (20%)	4 (10%)	0.376
Fever	3 (10%)	1 (5%)	0.548	4 (15%)	4 (10%)	0.633
Arthralgia	1 (5%)	0 (0%)	0.311	2(5%)	5 (20%)	0.151
Montreal classification						
Age of diagnosis						
A1	21 (100%)	20 (100%)		21 (100%)	22 (100%)	
Site of intestinal involvement						
L1	6 (29%)	7 (35%)	0.658	5 (24%)	6 (27%)	0.795
L2	3 (14%)	2 (10%)	0.675	2 (10%)	2 (9%)	0.961
L3	14 (67%)	11 (55%)	0.444	14 (66%)	14 (64%)	0.835
Disease behavior						
B1	20 (95%)	19 (95%)	0.972	17 (81%)	17 (77%)	0.767
B2	1 (5%)	1 (5%)	0.972	4 (19%)	5 (23%)	0.767
p	1 (5%)	1 (5%)	0.972	4 (15%)	4 (15%)	0.942

Short peptide: short-chain polypeptide-based EEN formulas. Drug regimen in mild active CD (oral mesalazine) and moderate-to-severe active CD (intravenous methylprednisolone or oral prednisone).

25.64 ± 2.03 in the drug group before treatment. There was no significant difference between the two groups ($p > 0.05$). After treatment, the PCDAI score of children in the short-chain polypeptide plus drug group (9.43 ± 2.22) was lower than that in the drug group (11.26 ± 2.64; $p < 0.05$).

For children with moderate-to-severe active CD, the PCDAI score was 56.34 ± 7.06 in the short-chain polypeptide plus drug group and 56.79 ± 9.92 in the drug group before treatment. There was no significant difference between the two groups ($p > 0.05$). After treatment, the PCDAI score of children in the short-chain polypeptide plus drug group (10.91 ± 2.92) was significantly lower than that in the drug group (13.57 ± 2.95) ($p < 0.05$), which suggests that short-chain polypeptide-based EEN feeding reduces disease activity.

The effect of short-chain polypeptide-based EEN formulas on SES-CD score in intestinal tissues

Adequate endoscopic scoring in pediatric CD is crucial as an outcome parameter in clinical trials (21). In this study, the SES-CD score of the terminal ileum and ileocecal mucosa was 5.67 ± 0.47 and 4.67 ± 0.47 in the drug group and 5.31 ± 0.41 and 4.33 ± 1.25 in the short-chain polypeptide plus drug group before treatment for children with mild active CD. There was no significant difference at the same site between the two groups. The SES-CD score of terminal ileum mucosa in the drug group after treatment was 2.00 ± 0.41, which was significantly higher than that in the short-chain polypeptide plus drug group (1.00 ± 0.32, $p < 0.05$; Figure 1G). However, there was no significant difference in the SES-CD score of ileocecal mucosa between the drug group (2.33 ± 0.47) and the short-chain polypeptide plus drug group (2.66 ± 0.47) after treatment (Figure 1g).

For children with moderate-to-severe active CD, the SES-CD score of terminal ileum mucosa was 9.67 ± 0.47 vs. 9.00 ± 0.15 in the drug group and 6.67 ± 2.05 vs. 2.67 ± 2.05 in the short-chain polypeptide plus drug group before and after treatment, respectively. There was no significant difference between the two groups (Figure 2G). In addition, the SES-CD score of ileocecal mucosa was 9.67 ± 0.47 in the drug group and 7.66 ± 0.47 in the short-chain polypeptide plus drug group before treatment. There was no significant difference between the two groups. After treatment, the SES-CD score of ileocecal mucosa in the drug group (9.33 ± 0.47) was significantly higher than that in the short-chain polypeptide plus drug group (2.0 ± 2.16) ($p < 0.05$; Figure 2g). These results suggest that short-chain polypeptide-based EEN feeding alleviates intestinal mucosal injury of active CD under endoscopic observation.

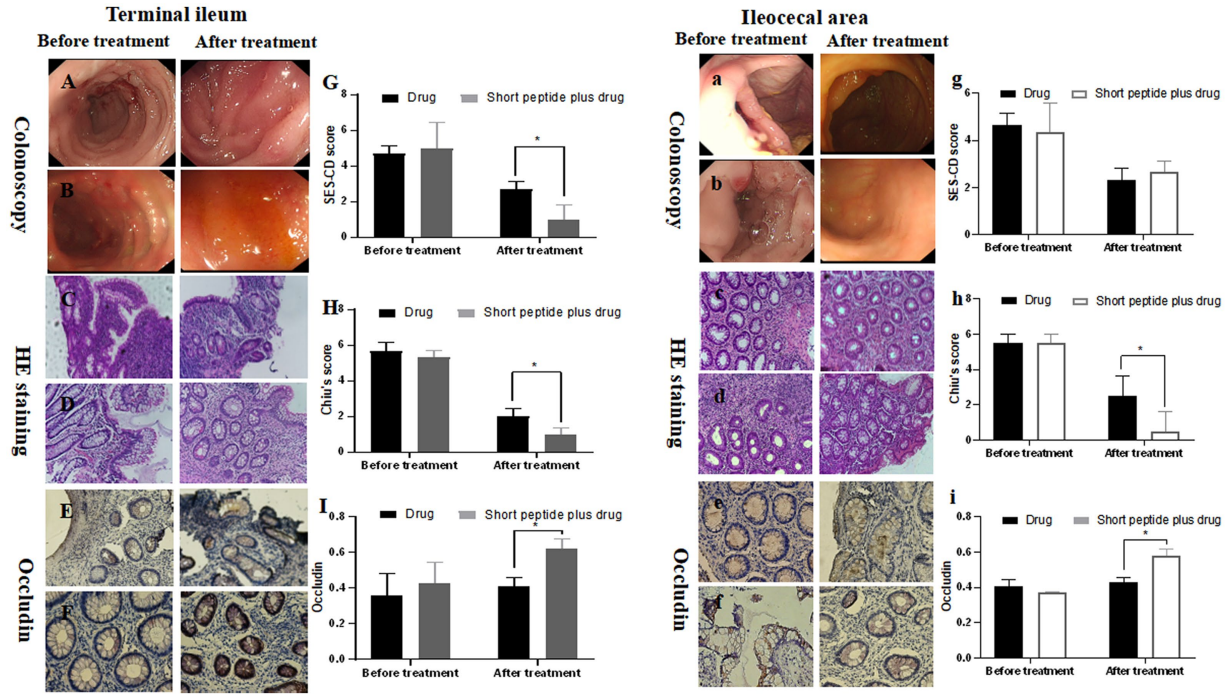

FIGURE 1
Comparison of SES-CD score, Chiu's score, and the expression of occludin in ileum mucosa between the drug group and short-chain polypeptide-based EEN plus drug group before and after treatment in children with mild active CD. **(A–I)** Ileum mucosa; **(a–i)** ileocecal mucosa. **(Aa, Cc, Ee)** Drug groups; **(Bb, Dd, Ff)** short peptide plus drug groups. Short peptide: short-chain polypeptide-based EEN formulas. Drug regimen in mild active CD: oral mesalazine. *Compared with the drug group, p <0.05.

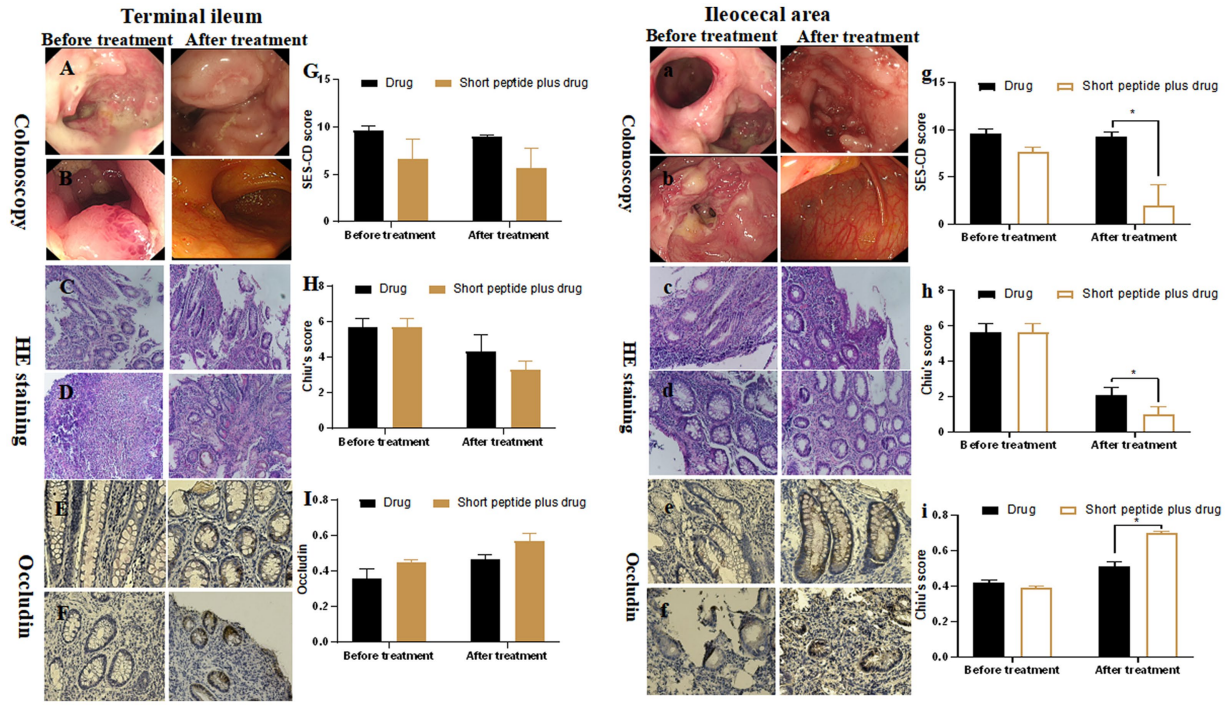

FIGURE 2
Comparison of SES-CD score, Chiu's score, and the expression of occludin between the drug group and short-chain polypeptide-based EEN plus drug group before and after treatment in children with moderate-to-severe active CD. **(A–I)** Ileum mucosa; **(a–i)** ileocecal mucosa. **(Aa, Cc, Ee)** Drug groups; **(Bb, Dd, Ff)** short-chain polypeptide-based EEN peptide plus drug groups. Short peptide: short-chain polypeptide-based EEN formulas. Drug regimen in moderate-to-severe active CD: intravenous methylprednisolone or oral prednisone. *Compared with the drug group, p <0.05.

The effect of short-chain polypeptide-based EEN formulas on Chiu's score in intestinal tissues

The results of the pathological analysis of intestinal tissue slices are shown in Figures 1, 2. No significant differences in the degree of histological damage were observed before treatment between the intestinal specimens of the drug and short-chain polypeptide plus drug groups. In addition to the terminal ileum of children in the moderate-to-severe active CD group, intestinal injury in other groups was significantly reduced after treatment compared to before treatment ($p < 0.05$).

For children with mild active CD, the Chiu's score of the terminal ileum and ileocecal mucosa was 4.67 ± 0.47 and 5.50 ± 0.50 in the drug group and 5.00 ± 1.41 and 5.50 ± 0.50 in the short-chain polypeptide plus drug group before treatment. There was no significant difference at the same site between the two groups. The Chiu's score of the terminal ileum and ileocecal mucosa in the drug group after treatment was 2.67 ± 0.47 and 2.50 ± 1.11, respectively, which was significantly higher than that in the drug group (1.00 ± 0.82 and 0.50 ± 1.11, respectively, $p < 0.05$; Figures 1H,h).

For children with moderate-to-severe active CD, the Chiu's score of terminal ileum mucosa was 5.67 ± 0.47 vs. 4.33 ± 0.94 in the drug group and 5.67 ± 0.47 vs. 3.30 ± 0.47 in the short-chain polypeptide plus drug group before and after treatment, respectively. There is no significant difference between the two groups (Figure 2H). The Chiu's score of ileocecal mucosa was 5.67 ± 0.47 in the drug group and 5.67 ± 0.47 in the short-chain polypeptide plus drug group before treatment. There was no significant difference between the two groups. After treatment, the Chiu's score of ileocecal mucosa in the drug group (2.10 ± 0.41) was significantly higher than that in the short-chain polypeptide plus drug group (1.00 ± 0.41; $p < 0.05$; Figure 2h). The results suggest that short-chain polypeptide-based EEN feeding alleviates histological damage caused by active CD.

The effect of short-chain polypeptide-based EEN formulas on the OD values of occludin in intestinal tissues

Furthermore, we tested the expression of the occludin and calculated the OD value in the intestinal tissues (Figures 1, 2). For children with mild active CD, the OD value of occludin in the terminal ileum and ileocecal mucosa was 0.34 ± 0.10 and 0.41 ± 0.04 in the drug group, 0.46 ± 0.02 and 0.37 ± 0.01 in the short-chain polypeptide plus drug group before treatment. There were no significant differences at the same site between the two groups. The OD value of occludin in the terminal ileum and ileocecal mucosa was 0.41 ± 0.05 and 0.43 ± 0.02, respectively, in the drug group after treatment, which was significantly lower than that in the short-chain polypeptide plus drug group (0.62 ± 0.05 and 0.58 ± 0.05, respectively, $p < 0.05$) (Figures 1I,i).

For children with moderate-to-severe active CD, the OD value of occludin in the terminal ileum mucosa was 0.36 ± 0.05 vs. 0.45 ± 0.01 in the drug group and 0.47 ± 0.02 vs. 0.57 ± 0.05 in the short-chain polypeptide plus drug group before and after treatment, respectively. There was no significant difference between the two groups (Figure 2I). The OD value of occludin in the ileocecal mucosa was 0.42 ± 0.01 in the drug group and 0.39 ± 0.01 in the short-chain polypeptide plus drug group before treatment. There was no significant difference between the two groups. After treatment, the OD value of occludin in the ileocecal mucosa (0.51 ± 0.03) in the drug group was significantly lower than that in the short-chain polypeptide plus drug group (0.70 ± 0.01; $p < 0.05$; Figure 2i). In all, the OD value of occludin protein in the intestines after the short-chain polypeptide-based EEN combined with drug therapy was higher than that in the drug group.

The effect of short-chain polypeptide-based EEN formulas on the levels of I-FABP in the blood and FC

I-FABP and FC are often used to evaluate inflammatory levels in intestinal injury models. Therefore, we tested I-FABP in the blood and FC in the fecal samples (Figure 3). For children with mild active CD, the concentration of I-FABP and FC was (81.13 ± 11.12) ng/ml and (1389.8 ± 229.97) ug/g in the drug group vs. (91.28 ± 24.15) ng/ml and (1256.82 ± 304.48) ug/g in the short-chain polypeptide plus drug group before treatment. There was no significant difference between the two groups. After treatment, the concentration of I-FABP and FC in the short-chain polypeptide plus drug group [(33.73 ± 7.19) ng/ml and (432.35 ± 218.46) ug/g] was significantly lower than that in the drug group [(68.39 ± 8.07) ng/ml and (956.13 ± 69.68) ug/g, $p < 0.05$; Figures 3A,C]. For children with moderate-to-severe active CD, the concentration of I-FABP and FC was (1349.8 ± 213.95) ng/ml and (1985.26 ± 277.67) ug/g in the drug group vs. (1137.17 ± 38.35) ng/ml and (2017.00 ± 484.23) ug/g in the short-chain polypeptide plus drug group before treatment. There was no significant difference between the two groups. After treatment, the concentration of I-FABP and FC in the short-chain polypeptide plus drug group [(542.21 ± 97.51) ng/ml and (1038.28 ± 130.3) ug/g] was significantly lower than that in the drug group [(854.69 ± 189.72) ng/ml and (1616.41 ± 133.54) ug/g, $p < 0.05$; Figures 3B,D]. The findings show that lower levels of inflammation occurred in the short-chain polypeptide plus drug group.

Discussion

The main therapeutic goal of pediatric CD is to induce and maintain clinical remission and mucosal healing, promote growth and development, and improve quality of life in children (24). It is increasingly important to accurately quantify the measurable concepts, including children-reported symptoms, intestinal damage and transmural inflammation, histologic appearance, as well as quality of life, disability, and other patient-centered attributes (25). Standardized indices, which show sufficient validity, reliability, and responsiveness to change are not only mandatory for implementing the treat-to-target approach but are also critical for assessing the effectiveness of emerging medications (25). Certain indicators can be accurately assessed through the use of existing measurement methods including PCDAI, SES-CD, Chiu's score, and FC and I-FABP.

PCDAI, as one of the pediatric indices and scales for assessing disease activity, is well established. In this study, the score of PCDAI in both the mild and moderate-to-severe CD groups decreased significantly after short-chain polypeptide-based EEN was combined with drugs. It suggests that the short-chain polypeptide-based EEN

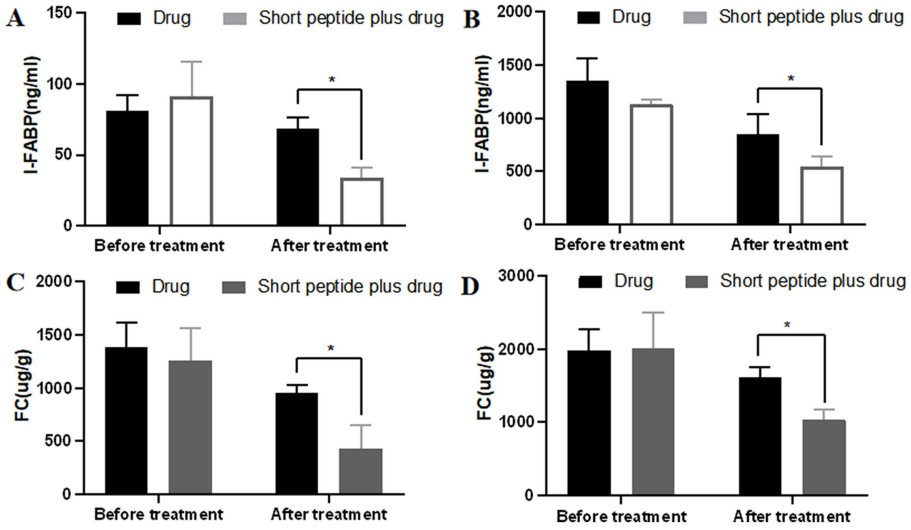

FIGURE 3
Comparison of the levels of I-FABP and FC between the drug group and short-chain polypeptide-based EEN plus drug group before and after treatment. **(A,C)** Levels of I-FABP in children with mild active CD. **(B,D)** Levels of FC in children with moderate-to-severe active CD. Short peptide: short-chain polypeptide-based EEN formulas. Drug regimen in mild active CD (oral mesalazine) and drug regimen in moderate-to-severe active CD (intravenous methylprednisolone or oral prednisone). *Compared with the drug group, p <0.05.

combined with drug regimens has more efficacy in reducing the disease activity of pediatric active CD than that of drug regimens alone.

Healing of mucosal lesions is an important endpoint in clinical trials of treatments for pediatric CD. Mucosal healing has been proposed as a new target of pediatric CD therapy. Nowadays, the CD endoscopic index of severity is the only validated endoscopic activity score. However, this index has the disadvantage of being complex and time-consuming, which limits its application in clinical practice (26). Thus, the simple endoscopic score for SES-CD as a widely used index is suitable for evaluating clinical and endoscopic activity (21). Furthermore, intestinal histopathological changes detected by HE staining and Chiu's scores are used to make more accurate assessments of the severity of the intestinal injury, which is currently the most commonly used method (23). This study found that, regardless of whether terminal ileum or ileocecal area, after treatment of short-chain polypeptide-based EEN combined with drugs, the endoscopic score for SES-CD and histopathological Chiu's scores decreased significantly in children with mild CD. While the same changes occurred in the ileocecal region of the lesion in children with moderate-to-severe active CD. It was shown that short-chain polypeptide-based EEN feeding can promote mucosal healing and induce and maintain CD remission, which is obvious in children with mild CD. For children with moderate-to-severe CD, the degree of recovery of the terminal ileum and ileocecal area is significantly different. It is speculated that the recovery speed of intestinal barrier function varies with the location where CD involves the intestines. Additionally, we found that the improvement of SES-CD endoscopic score and histopathological Chiu score in the ileocecal area of children with moderate-to-severe active CD was significantly better than that of children with mild active CD, which suggests that the degree of recovery of intestinal mucosal injury in the ileocecal area of children with moderate-to-severe CD was significantly higher than that of children with mild CD. It is speculated that the possible reason for this profound difference is that methylprednisolone or prednisone is the combined drug for the treatment of children with moderate-to-severe active CD, which is more effective than mesalazine for mild active CD treatment in inducing remission and promoting mucosal healing.

The intestinal epithelial cell apoptosis and the dysfunction of the intestinal epithelial barrier are the principal reasons for the increased intestinal permeability in CD (27). The main function of the intestinal epithelial barrier is to maintain intestinal permeability, which is formed by the dynamic changes of TJ and responds to different extracellular stimuli. Occludin can regulate the formation of TJ through protein–protein interaction (28). Previous studies have shown that injury to intestinal epithelial TJ is the leading cause of CD onset with underexpression of TJ-related proteins including occludin (29). In this study, the OD values of occludin in the terminal ileum and ileocecal area of the mild active CD and ileocecal area of moderate-to-severe active CD after short-chain polypeptide-based EEN feeding combined with drug treatment were significantly higher than those in the drug group, which suggests that the short-chain polypeptide-based EEN combined with drug regimens have more efficacy in alleviating gut damage and promoting mucosal healing of active CD than that of drug regimens alone.

FC is released by innate immune cells activated during cellular stress and injury (30). A prospective cohort study showed that the sensitivity and specificity of FC in CD were 100 and 97%, respectively (31). The study also found that the FC level in colonic active CD was significantly higher than that in ileal active CD (32). Sipponen et al. (33) showed that infliximab after 12 weeks of treatment had a significant decrease in FC, which is related to the endoscopic severity index ($\gamma = 0.561$, $p = 0.03$), indicating that FC is related to mucosal healing under endoscopy. In this study, there were no significant differences in the FC level between the two groups before treatment. However, the FC level was sharply lower in the short-chain polypeptide-based EEN plus drug group than that in the drug group, suggesting that short-chain polypeptide-based EEN feeding can promote the intestinal mucosal healing of children with active CD.

I-FABP is a type of low molecular weight (15 kDa) intracellular protein that plays a role in fatty acid transport and metabolism (34). I-FABP presents a gradient distribution in the mature small intestinal mucosa, the content of villi is higher than that of lacuna and the content of the proximal and middle 1/3 of the jejunum is higher than that of the distal 1/3 of the jejunum (35). Murat et al. showed that I-FABP is a useful systemic marker for CD activity (36). In this study, there were no differences between the two groups before treatment despite whether the children had mild or moderate-to-severe active CD. However, I-FABP levels in the blood significantly decreased after short-chain polypeptide-based EEN formulas combined with drug treatment compared with that in the drug group. The reason may be that short-chain polypeptide-based EEN feeding promotes the recovery of the surface of the intestinal mucosal villi, which is easily affected by active CD and reduces the release of I-FABP into the peripheral blood.

Conclusion

The study found that short-chain polypeptide-based EEN formulas effectively alleviate gut damage in children with active CD. When they are combined with drug regimens, they show more efficacy than drug regimens alone. Short-chain polypeptides can be absorbed by the intestinal tract without digestive enzymes and ATP energy, contributing, in particular, to the recovery of damaged intestinal mucosa of pediatric CD. Therefore, short-chain polypeptide-based EEN formulas should be recommended for the induction of remission in children with newly diagnosed active CD. Due to the limitations of a single center, a small sample size, and the lack of long-term follow-up data, it is necessary to conduct a multicenter prospective randomized trial with a larger sample size and extend the follow-up observation time to provide definite evidence to establish the extent of the benefits.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

This study was approved by the ethics committee of children's Hospital of Nanjing Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

HY and YJ conceived of the idea and were responsible for the planning, content, and structure of the article. HY wrote the initial manuscript draft. RW, JY and JC performed clinical case observation, data collection and analysis. PW, CW, WC, YW and XZ participated in the collection of intestinal tissue samples and immunohistochemical experiments, data collection and analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Marques JG, Schwerd T, Bufler P, Koletzko S, Koletzko B. Metabolic changes during exclusive enteral nutrition in pediatric Crohn's disease patients. *Metabolomics*. (2022) 18:96. doi: 10.1007/s11306-022-01953-0

2. Rosen MJ, Dhawan A, Saeed SA. Inflammatory bowel disease in children and adolescents. *JAMA Pediatr*. (2015) 169:1053–60. doi: 10.1001/jamapediatrics.2015.1982

3. Kuenzig ME, Fung SG, Marderfeld L, Mak JWY, Kaplan GG, Ng SC, et al. Twenty-first century trends in the global epidemiology of pediatric-onset inflammatory bowel disease: systematic review. *Gastroenterology*. (2022) 162:1147–1159.e4. doi: 10.1053/j.gastro.2021.12.282

4. Kaminsky LW, Al-Sadi R, Ma TY. IL-1β and the intestinal epithelial tight junction barrier. *Front Immunol*. (2021) 12:767456. doi: 10.3389/fimmu.2021.767456

5. Rawat M, Nighot M, al-Sadi R, Gupta Y, Viszwapriya D, Yochum G, et al. IL1B increases intestinal tight junction permeability by up-regulation of MIR200C-3p, which degrades Occludin mRNA. *Gastroenterology*. (2020) 159:1375–89. doi: 10.1053/j.gastro.2020.06.038

6. Chou HC, Cheng CM, Yang CH, Lin TY, Liu YW, Tan TH, et al. DUSP3 regulates phosphorylation-mediated degradation of occludin and is required for maintaining epithelial tight junction. *J Biomed Sci*. (2022) 29:40. doi: 10.1186/s12929-022-00826-x

7. Nuzzo A, Guedj K, Curac S, Hercend C, Bendavid C, Gault N, et al. Accuracy of citrulline, I-FABP and D-lactate in the diagnosis of acute mesenteric ischemia. *Sci Rep*. (2021) 11:18929. doi: 10.1038/s41598-021-98012-w

8. Logan M, MacKinder M, Clark CM, Kountouri A, Jere M, Ijaz UZ, et al. Intestinal fatty acid binding protein is a disease biomarker in paediatric coeliac disease and Crohn's disease. *BMC Gastroenterol*. (2022) 22:260. doi: 10.1186/s12876-022-02334-6

9. al-Saffar AK, Meijer CH, Gannavarapu VR, Hall G, Li Y, Diaz Tartera HO, et al. Parallel changes in Harvey-Bradshaw index, TNFα, and intestinal fatty acid binding protein in response to infliximab in Crohn's disease. *Gastroenterol Res Pract*. (2017) 2017:1–8. doi: 10.1155/2017/1745918

10. Ashton JJ, Gavin J, Beattie RM. Exclusive enteral nutrition in Crohn's disease: evidence and practicalities. *Clin Nutr*. (2019) 38:80–9. doi: 10.1016/j.clnu.2018.01.020

11. Ruemmele FM, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis*. (2014) 8:1179–207. doi: 10.1016/j.crohns.2014.04.005

12. Miele E, Shamir R, Aloi M, Assa A, Braegger C, Bronsky J, et al. Nutrition in pediatric inflammatory bowel disease: a position paper on behalf of the Porto inflammatory bowel disease group of the European society of pediatric gastroenterology, hepatology and nutrition. *J Pediatr Gastroenterol Nutr*. (2018) 66:687–708. doi: 10.1097/MPG.0000000000001896

13. Narula N, Dhillon A, Zhang D, Sherlock ME, Tondeur M, Zachos M. Enteral nutritional therapy for induction of remission in Crohn's disease. *Cochrane Database Syst Rev*. (2018) 2018:CD000542. doi: 10.1002/14651858.CD000542.pub3

14. Ibrahim H, Mansour M, El Gendy YG. Peptide-based formula versus standard-based polymeric formula for critically ill children: is it superior for patients' tolerance? *Arch Med Sci*. (2020) 16:592–6. doi: 10.5114/aoms.2020.94157

15. Yılmaz D, Sönmez F, Karakaş S, Yavaşcan Ö, Aksu N, Ömürlü İK, et al. Evaluation of nutritional status in children during predialysis, or treated by peritoneal dialysis or hemodialysis. *J Trop Pediatr*. (2016) 62:178–84. doi: 10.1093/tropej/fmv094

16. Zhang J, Yu WQ, Wei T, Zhang C, Wen L, Chen Q, et al. Effects of short-peptide-based enteral nutrition on the intestinal microcirculation and mucosal barrier in mice with severe acute pancreatitis. *Mol Nutr Food Res*. (2020) 64:e1901191. doi: 10.1002/mnfr.201901191

17. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, et al. ESPGHAN revised Porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr*. (2014) 58:795–806. doi: 10.1097/MPG.0000000000000239

18. Lu B, Xu A, Li J, Xu Z, Li H, Zhao Z. Nursing effect of nasoscopically assisted nasogastri tube and nasojejunal tube placement. *Am J Transl Res*. (2021) 13:10758–64.

19. Lim WC, Wang Y, MacDonald JK, Hanauer S. Aminosalicylates for induction of remission or response in Crohn's disease. *Cochrane Database Syst Rev*. (2016) 2016:CD008870. doi: 10.1002/ 14651858.CD008870.pub2

20. Markowitz J, Grancher K, Kohn N, Lesser M, Daum F. A multicenter trial of 6-mercaptopurine and prednisone in children with newly diagnosed Crohn's disease. *Gastroenterology*. (2000) 119:895–902. doi: 10.1053/gast.2000.18144

21. van der Does de Willebois EML, Duijvestein M, Wasmann KA, D'Haens GRAM, van der Bilt JDW, Mundt MW, et al. Endoscopic recurrence or anastomotic wound healing phenomenon after ileocolic resection for Crohn's disease: the challenges of accurate endoscopic scoring. *J Crohns Colitis*. (2022):jjac175. doi: 10.1093/ecco-jcc/jjac175

22. Kammermeier J, Dziubak R, Pescarin M, Drury S, Godwin H, Reeve K, et al. Phenotypic and genotypic characterisation of inflammatory bowel disease presenting before the age of 2 years. *J Crohns Colitis*. (2017) 11:60–9. doi: 10.1093/ecco-jcc/jjw118

23. Eszter Müller K, Laszlo Lakatos P, Papp M, Veres G. Incidence and Paris classification of pediatric inflammatory bowel disease. *Gastroenterol Res Pract*. (2014) 2014:904307. doi: 10.1155/2014/904307

24. Melek J, Štanclová M, Dědek P, Malý J, Bayer M, Pozler O, et al. Infliximab plus azathioprine is more effective than azathioprine alone in achieving mucosal healing in pediatric patients with Crohn's disease. *J Dig Dis*. (2020) 21:705–10. doi: 10.1111/1751-2980.12927

25. Ledder O, Turner D. Multi-item measures for pediatric inflammatory bowel diseases: the ABCs of all those acronyms. *J Crohns Colitis*. (2023):jjad019. doi: 10.1093/ecco-jcc/jjad019

26. Moriichi K, Fujiya M, Okumura T. The endoscopic diagnosis of mucosal healing and deep remission in inflammatory bowel disease. *Dig Endosc*. (2021) 33:1008–23. doi: 10.1111/den.13863

27. Bao CH, Wu LY, Shi Y, Wu HG, Liu HR, Zhang R, et al. Moxibustion down-regulates colonic epithelial cell apoptosis and repairs tight junctions in rats with Crohn's disease. *World J Gastroenterol*. (2011) 17:4960–70. doi: 10.3748/wjg.v17.i45.4960

28. Shi Y, Bao CH, Wu HG, Ma XP, Yu LQ, Zhang R, et al. Effect of moxibustion on colonic TNF-alpha content and influence of colonic supernatant of crohn's disease rats undergoing moxibustion on expression of occludin, claudin-1 and zonula occludens-1 proteins and genes in cultured colonic epithelial cells. *Zhen Ci Yan Jiu*. (2011) 36:235–41.

29. Ji R, Wang A, Shang H, Chen L, Bao C, Wu L, et al. Herb-partitioned moxibustion upregulated the expression of colonic epithelial tight junction-related proteins in Crohn's disease model rats. *Chin Med*. (2016) 11:20. doi: 10.1186/s13020-016-0090-0

30. Szymanska E, Bierla J, Dadalski M, Wierzbicka A, Konopka E, Cukrowska B, et al. New non-invasive biomarkers of intestinal inflammation and increased intestinal permeability in pediatric inflammatory bowel diseases and their correlation with fecal calprotectin: a pilot study. *Minerva Gastroenterol*. (2022). doi: 10.23736/S2724-5985.22.03156-4

31. von Roon AC, Karamountzos L, Purkayastha S, Reese GE, Darzi AW, Teare JP, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol*. (2007) 102:803–13. doi: 10.1111/j.1572-0241.2007.01126.x

32. Sipponen T, Björkesten CG, Färkkilä M, Nuutinen H, Savilahti E, Kolho KL. Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment. *Scand J Gastroenterol*. (2010) 45:325–31. doi: 10.3109/00365520903483650

33. Sipponen T, Savilahti E, Kärkkäinen P, Kolho KL, Nuutinen H, Turunen U, et al. Fecal calprotectin, lactoferrin, and endoscopic disease activity in monitoring anti-TNF-alpha therapy for Crohn's disease. *Inflamm Bowel Dis*. (2008) 14:1392–8. doi: 10.1002/ibd.20490

34. Funaoka H, Kanda T, Fujii H. Intestinal fatty acid-binding protein (I-FABP) as a new biomarker for intestinal diseases. *Rinsho Byori*. (2010) 58:162–8. Japanese.

35. Derikx JP, Vreugdenhil AC, Van den Neucker AM, Grootjans J, van Bijnen AA, Damoiseaux JG, et al. A pilot study on the noninvasive evaluation of intestinal damage in celiac disease using I-FABP and L-FABP. *J Clin Gastroenterol*. (2009) 43:727–33. doi: 10.1097/MCG.0b013e 31819194b0

36. Sarikaya M, Ergül B, Doğan Z, Filik L, Can M, Arslan L. Intestinal fatty acid binding protein (I-FABP) as a promising test for Crohn's disease: a preliminary study. *Clin Lab*. (2015) 61:87–91. doi: 10.7754/clin.lab.2014.140518

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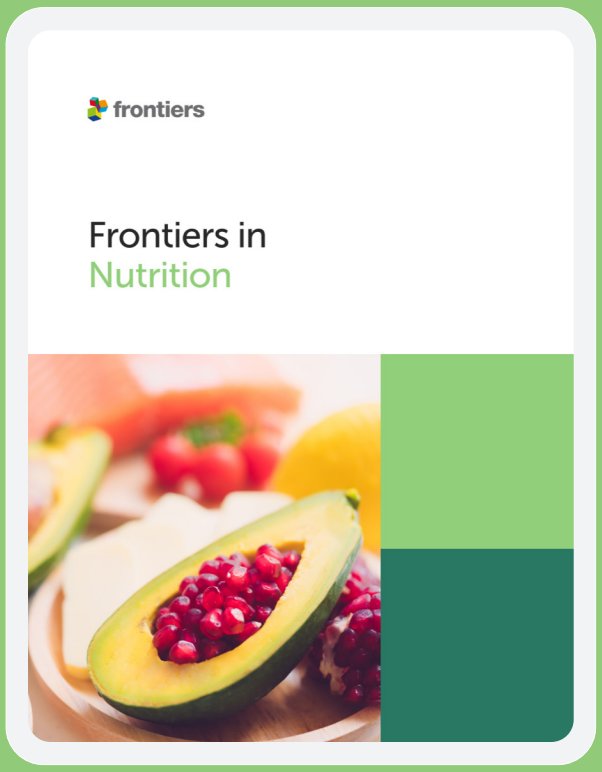

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