frontiers | Research Topics

# The impact of lifestyle changes on non-communicable diseases

**Edited by**
Giulia Marrone, Annalisa Noce and Attilio Parisi

**Published in**
Frontiers in Nutrition

ISSN 1664-8714
ISBN 978-2-8325-5412-8
DOI 10.3389/978-2-8325-5412-8

## About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

# The impact of lifestyle changes on non-communicable diseases

**Topic editors**
Giulia Marrone — University of Rome Tor Vergata, Italy
Annalisa Noce — University of Rome Tor Vergata, Italy
Attilio Parisi — Foro Italico University of Rome, Italy

**Citation**
Marrone, G., Noce, A., Parisi, A., eds. (2024). *The impact of lifestyle changes on non-communicable diseases*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-5412-8

# Table of contents

frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 28 August 2024
DOI 10.3389/fnut.2024.1471019



OPEN ACCESS

EDITED AND REVIEWED BY
Mauro Serafini,
University of Teramo, Italy

*CORRESPONDENCE
Giulia Marrone
giulia.marrone@uniroma2.it

RECEIVED 26 July 2024
ACCEPTED 30 July 2024
PUBLISHED 28 August 2024

CITATION
Noce A, Marrone G and Parisi A (2024) Editorial: The impact of lifestyle changes on non-communicable diseases.
*Front. Nutr.* 11:1471019.
doi: 10.3389/fnut.2024.1471019



# Editorial: The impact of lifestyle changes on non-communicable diseases

Annalisa Noce[1,2], Giulia Marrone[1]* and Attilio Parisi[3]

[1]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, [2]UOSD Nephrology and Dialysis, Policlinico Tor Vergata, Rome, Italy, [3]Department of Exercise, Human and Health Sciences, Foro Italico University of Rome, Rome, Italy

KEYWORDS

**lifestyle changes, nutritional habits, physical activity, chronic non-degenerative diseases, obesity, muscle mass**

Editorial on the Research Topic
The impact of lifestyle changes on non-communicable diseases

Non-communicable diseases (NCDs) are a series of pathological conditions, mainly caused by unhealthy behaviors but they can also depend on genetic factors, sex, age and air pollution. Currently, NCDs are the leading cause of disability and death worldwide. Moreover, NCDs imply a significant expense for their treatment and care by the National Health Systems. Lifestyle is among the modifiable factors in determining the NCDs onset. Therefore, it is very important to implement campaigns aimed at raising the general population awareness about how a healthy lifestyle is essential to maintain a good state of health and to reduce NCDs incidence. Consequently, lifestyle changes represent a new challenge involving the scientific community. At this regard, an unhealthy diet and a sedentary lifestyle can be important contributors to the development of many diseases. On the contrary, a regular physical activity, a correct diet, a nutritional supplementation and a proper sleep duration and quality can act positively in preventing and/or counteracting NCDs.

Our Research Topic has collected a number of interesting publications highlighting how lifestyle modifications can be effective in counteracting the widespread of NCDs.

Several researchers have studied the association between the intake of certain foods with the occurrence of some NCDs. Due to the development of the social economy and improvement in the living standards, the eating habits of modern people have undergone tremendous changes compared to the past. Because of their job, Chinese physicians, are more prone to follow an unregulated lifestyle, including unhealthy eating habits and for this reason they have a high risk of developing these diseases. Chen et al. investigated the association between physicians' eating habits and their health and disease perception. According to the results, the prevalence of unhealthy eating habits was high, i.e., frequent eating out-of-home, irregular meals and eating too fast were significantly related to perceived sub-optimal health status and disease occurrence.

The study conducted by Wang et al. investigated the relationship between dietary differences and the risk of oral cancer. They extracted, from the UK Biobank database, 21 dietary exposures, including 10 dietary patterns, six vitamins, and five micronutrients. According to the results of the 10 analyzed dietary patterns, eight of them showed no significant association with the risk of developing oral cancer. Consumption of dark chocolate and sweet pepper exhibited an inverse relationship with oral cancer risk.

Moreover, degenerative diseases such as osteoarthritis can be influenced by the diet. Xie and Qin investigated the effects of 45 common dietary intake habits on six osteoarthritis-related phenotypes and they identified 59 potential causal associations. Results showed that muesli intake was negatively associated with knee osteoarthritis, spine osteoarthritis and total knee replacement, while, dried fruit intake reported a negative association with osteoarthritis of knee and total knee replacement.

According to Yang Y. et al., dietary factors, including intake of alcohol, non-oily fish, beef, fresh fruit, oily fish, salad/raw vegetables, dried fruit etc., contribute to determining or reducing the possibility of developing the various forms of hernia. Results evidenced that alcoholic drinks *per* week reduce the risk of inguinal hernia, while alcohol intake frequency does not affect the risk of the inguinal hernia. Cheese and dried fruit intake decrease the risk of ventral hernia, while cooked vegetable intake increase the risk of ventral hernia. Lastly, the intake of non-oily fish increases the risk of inguinal hernia.

Diet during pregnancy can also influence the occurrence of NCDs in unborn children. The study by Miyake et al. investigated the impact of maternal fiber intake during pregnancy on fetal neurodevelopment in Japanese population, showing that pregnant Japanese women had a lower fiber intake compared to the recommended one. Therefore, these data confirm how it is essential a correct diet during pregnancy in order to reduce the onset of health problems in newborns and how it is essential a multidisciplinary approach, which also includes the professional figure of the nutritionist during pregnancy.

Furthermore, interventions through oral supplementation or through a changing in the percentage of macronutrients in the diet seem to have a positive effect on several diseases. According to the study of Massimino et al., supplementation for 3 months with a low dose of medium-chain triglycerides (MCTs) has increased muscle mass and function in frail older adults. These findings indicate the potential practical use of MCTs in daily life for the treatment of sarcopenia. In fact, dietary lipid manipulation in the nutritional management of glycogen storage disease type III (GSD III) has been shown to be effective in reducing associated muscle damages. A low-CHO (32%)/high-fat (45%)/high-protein (23%) diet was safe, sustainable and effective in reducing muscle damages without worsening cardiometabolic profile in GSD III.

Similarly, physical activity plays a pivotal role in preventing or treating several NCDs, supporting the dietary treatment. In example, a low-calorie diet, which is commonly used in the treatment of obesity, induces a lean body mass loss, when it is not combined with an adapted exercise training. As evidenced by Monsalves-Álvarez et al., a 3 months high intensity interval training (HIIT) prevents a muscle mass loss caused by a hypocaloric-Mediterranean diet in overweight and obese women.

Recent findings evidence a relationship between lifestyle and the microorganisms diversity populating the intestine. Mancini et al., investigated the role of lifestyle (active vs. sedentary) on saliva microbiota composition in Italian school children. One-hundred-fourteen children were enrolled in a Turin neighborhood school. The results showed that children with an active lifestyle compared to sedentary children had an enrichment of the saliva microbiota species and genera mainly associated with a healthier profile, while the species, developed in the sedentary group, could be linked to human diseases.

Dietary habits, as well as the sleep quality, may increase the incidence of NCDs. The study conducted by Yang W. et al. has investigated the association between the sleep duration, the regularity of breakfast and overweight in 1,178 university students. The authors find out that only 34.1% of the study population ate a breakfast every day, while students that consumed breakfast from 1 to 3 times/week showed a higher risk to develop overweight. Moreover, short sleep duration may be the main reason for irregular breakfast, leading to overweight.

In conclusion, it is very important to follow a correct lifestyle not only to counteract the NCDs onset but also to act as an adjuvant therapy in the clinical management of NCDs patients.

## Author contributions

AN: Writing – original draft, Writing – review & editing. GM: Writing – original draft, Writing – review & editing. AP: Writing – original draft, Writing – review & editing.

## Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

## Acknowledgments

We would like to thank Gabriella Venafro for the English language revision.

## 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

frontiers | Frontiers in Nutrition

TYPE Case Report
PUBLISHED 11 May 2023
DOI 10.3389/fnut.2023.1178348



OPEN ACCESS

EDITED BY
Giulia Marrone,
University of Rome Tor Vergata, Italy

REVIEWED BY
Sumei Zhao,
Yunnan Agricultural University, China
Karolina M. Stepien,
Salford Royal NHS Foundation Trust,
United Kingdom

*CORRESPONDENCE
Brunella Capaldo
✉ brunellacapaldo@gmail.com

RECEIVED 02 March 2023
ACCEPTED 13 April 2023
PUBLISHED 11 May 2023

CITATION
Massimino E, Amoroso AP, Lupoli R, Rossi A and Capaldo B (2023) Nutritional management of glycogen storage disease type III: a case report and a critical appraisal of the literature. *Front. Nutr.* 10:1178348. doi: 10.3389/fnut.2023.1178348



# Nutritional management of glycogen storage disease type III: a case report and a critical appraisal of the literature

Elena Massimino[1], Anna Paola Amoroso[1], Roberta Lupoli[2], Alessandro Rossi[3] and Brunella Capaldo[1]*

[1]Department of Clinical Medicine and Surgery, University Federico II, Naples, Italy, [2]Department of Molecular Medicine and Medical Biotechnology, University Federico II, Naples, Italy, [3]Department of Translational Medicine, Section of Pediatrics, University Federico II, Naples, Italy

Glycogen storage disease Type III (GSD III) is an autosomal recessive disease due to the deficiency of the debranching enzyme, which has two main consequences: a reduced availability of glucose due to the incomplete degradation of glycogen, and the accumulation of abnormal glycogen in liver and cardiac/skeletal muscle. The role of dietary lipid manipulations in the nutritional management of GSD III is still debated. A literature overview shows that low-carbohydrate (CHO) / high-fat diets may be beneficial in reducing muscle damage. We present a 24-year GSD IIIa patient with severe myopathy and cardiomyopathy in whom a gradual shift from a high-CHO diet (61% total energy intake), low-fat (18%), high-protein (21%) to a low-CHO (32 %) high-fat (45%) / high-protein (23%) diet was performed. CHO was mainly represented by high-fiber, low glycemic index food, and fat consisted prevalently of mono and polyunsaturated fatty acids. After a 2-year follow-up, all biomarkers of muscle and heart damage markedly decreased (by 50–75%), glucose levels remained within the normal range and lipid profile was unchanged. At echocardiography, there was an improvement in geometry and left ventricular function. A low -CHO, high-fat, high-protein diet seems to be safe, sustainable and effective in reducing muscle damage without worsening cardiometabolic profile in GSDIIIa. This dietary approach could be started as early as possible in GSD III displaying skeletal/cardiac muscle disease in order to prevent/minimize organ damage.

KEYWORDS

**glycogen storage disease, dietary intervention, high-fat diet, cardiomyopathy, myopathy, continuous glucose monitoring (CGM)**

## 1. Introduction

Glycogen storage disease Type III (GSD III) (#MIM 232400) is an autosomal recessive disease due to deficiency of the debranching enzyme (GDE) encoded by the AGL gene, located on chromosome 1p21. GDE allows the cleavage of glucose molecules from glycogen through two distinct catalytic activities: 1) the 1,4-α-D-glucan 4-α-D-glycosyl transferase, which transfers the terminal three glucose molecules to the parent chain and 2) the amylo-1,6-glucosidase component, which cleaves the alpha 1,6 bond to release free glucose. GDE deficiency has two main consequences: a reduced availability of energy /glucose due to the incomplete degradation of glycogen, and the accumulation in liver and cardiac/skeletal muscle of abnormal glycogen with short external ramifications (limit dextrin). Functionally, patients with GSD III have defective glycogenolysis while glycolysis and gluconeogenesis

are preserved. There are two major clinical subtypes of GSD III: GSD IIIa (85% of cases)– involving liver and cardiac/skeletal muscle, and GSD IIIb, where only the liver is affected (1, 2). In childhood, the disease usually presents with ketotic hypoglycemia, hepatomegaly, growth retardation and elevated transaminase levels. Progressive skeletal myopathy, and often cardiomyopathy, appear in adulthood (3).

Traditionally, the dietary management of GSD III includes a high proportion of carbohydrates distributed throughout the day, together with uncooked cornstarch supplements to maintain euglycemia. Protein intake is also increased to provide substrates for neoglucogenesis (4). However, some criticisms have been raised regarding this dietary approach. First, a high-carbohydrate diet can induce reactive hyperinsulinemia with activation of glycogen synthesis and further accumulation of abnormal glycogen in the affected tissues, with worsening of organ damage; furthermore, carbohydrate overtreatment can lead to suppression of lipolysis, ketogenesis, and gluconeogenesis with reduced availability of alternative energy substrates. Based on these considerations, diets containing high amount of fat and/or protein have recently been proposed as a valid alternative to the traditional approach (5, 6). In particular, dietary lipid manipulations, including modified ketogenic diets with or without medium-chain triglycerides (MCT), have been shown to be associated with a reduction in liver enzymes, creatinkinase, and interventricular septum thickness, although with some variability between children and adults with GSD III (5).

Here, we present a patient with GSD IIIa, severe myopathy and heart failure in whom a gradual transition from a high CHO-diet to a high-fat high-protein diet was performed under careful monitoring of glucose and biochemical parameters. The main steps of dietary management and their impact on the course of organ damage are described.

## 2. Case presentation

The patient is a 24- year-old man with GSD IIIa, born to non-consanguineous parents. The diagnosis was made at age 9 months, based on 1–6 glycosidase deficiency in erythrocytes and confirmed by molecular analysis, which showed a homozygous IVS21 + 1A/G (c.2681 + 1G>A) mutation in the *AGL* gene.

Since the diagnosis, the patient was under follow-up at the Pediatric Unit of Federico II University Hospital in Naples. Over the years, the patient presented regular growth and development. Dietary regimen was based on high CHO intake, including administration of cornstarch. Starting with puberty, a progressive worsening of his muscle and cardiac biomarkers was observed. At age 16, due to the occurrence of asthenia and reduced exercise capacity, the patient underwent a cardiological assessment, which evidenced diffuse ventricular repolarization anomalies and signs of left ventricular hypertrophy. At the age of 22, the patient was referred to the outpatient clinic for rare metabolic diseases in adults of the Department of Clinical Medicine and Surgery of Federico II University in Naples for clinical management and follow-up. Upon admission, the patient underwent a careful assessment of his clinical status and complications, which was repeated during the dietary intervention according to the timeline reported in Table 1. Body weight was within the normal range, while fat-free mass (FFM) was slightly reduced. Resting energy expenditure (REE), evaluated through indirect calorimetry, was 22% higher than the predicted value calculated using the Harris Benedict equation. Glucose profile, monitored for 4 weeks by means of FreeStyle Libre 2 (Abbott), showed quite large glycemic fluctuations, ranging from 54 to 140 mg/dl (3–7.78 mmol/L), with 9% of time spent in hypoglycemia (<70 mg/dl, <3.89 mmol/L). Both plasma insulin and HOMA index were elevated, indicating a condition of insulin resistance. Lipid profile showed high levels of both cholesterol and triglycerides. Circulating markers of cardiac and skeletal muscle were markedly elevated. Echocardiography revealed increased LV mass and reduced global longitudinal strain (GLS), indicating myocardial injury. Cardiac MRI confirmed hypertrophic cardiomyopathy with preserved biventricular systolic function. AST and ALT were 5-fold higher than normal values and the liver ultrasound showed enlargement of the organ and diffuse hepatic steatosis. The patient's diet, evaluated through a 7-day food record, is reported in Table 2. Calorie intake was quite high (3132 Kcal/day) mainly due to an elevated intake of CHO (513 g/day, 61% of Total Energy Intake). The amount of cornstarch was 215 g/day (3.16 g/kg BW) divided into two administrations in daytime and one at nighttime, in addition, to 80g/day of maltodextrin – a high glycemic index (GI) polysaccharide sugar. Fiber intake was low (11 g/day), due to reduced consumption of fruit and vegetables. Protein intake was 2.4 g/kg BW (163 g/day, 21% TEI) of which 28% (45g/die) as a protein powder supplement (Protifar, Nutricia) divided into two daily doses. Total fat intake was rather low (18% TEI), with percentages of monounsaturated, polyunsaturated, and saturated fatty acids of 9, 2, and 5%, respectively.

The patient was treated with oral L-Carnitine (2gx2 times /day) and Vitamin D (10,000 U /ml, 30 drops/wk).

## 3. Dietary intervention

We decided to implement a gradual shift from a diet with a high CHO content to one with a high fat content, under careful monitoring of glucose levels. Dietary changes were performed in 2 steps, each lasting 6 months, which were interrupted for 1 year, due to the COVID-19 pandemic, during which the patient partly adhered to the 1st dietary step.

Based on the results of the calorimetric assessment showing a high resting energy expenditure (REE), we prescribed an isocaloric diet to maintain body weight stable. During the 1st step, maltodextrins were progressively eliminated, and the cornstarch supplementation was reduced by more than 50%. In the 2nd step, the CHO content halved compared to initial levels (from 61 to 32% TEI) and cornstarch supplementation was gradually reduced to 1.1 g/kg bw/day under the guide of CGM data. Much attention was paid to CHO quality, preferring—among starchy sources—foods rich in fiber with a low glycemic index (wholemeal foods, legumes, vegetables). In parallel, lipid intake was progressively increased from 18% upon admission to 24% in the 1st step and to 45% in the 2nd step. Foods rich in monounsaturated and polyunsaturated fats (olive oil, almonds, nuts) were preferred, to achieve a percentage of saturated fatty acids below 10% of TEI, as recommended by international nutritional guidelines. Regarding protein intake, according to guidelines (4), we maintained a

10.3389/fnut.2023.1178348

TABLE 1 Timeline of clinical, metabolic and organ damage biomarkers before and during the nutritional intervention.

| | | On admission (0 m) | 1st step intervention (6 m) | After 1-yr COVID lock-down (18 m) | 2nd step intervention (24 m) | Range values |
|---|---|---|---|---|---|---|
| Antropometric | Weight (Kg) | 68 | 68 | 70 | 70 | |
| | BMI (Kg/m$^2$) | 23.5 | 23.5 | 24.2 | 24.2 | 20–25 |
| | REE (Kcal/die) | 2,073 | | | 1,862 | |
| | REE (% predicted) | 122% | | | 110% | |
| | QR | 0.9 | | | 0.9 | |
| | FFM (Kg) (%) | 50.2 (76) | | | 51.4 (73) | 80–90% |
| Metabolic | Glucose (mg/dl) (mmol/L) | 96 (5.33) | 80 (4.44) | 86 (4.78) | 94 (5.22) | 70–110 (3.89–6.11) |
| | Time spent at IG 70–140 mg/dl (%) | 91 | | | 96 | |
| | Time spent at IG < 69 mg/dl (%) | 9 | | | 4 | |
| | Insulin (μU/ml) | 41 | 15.2 | 10 | 12.8 | 3–25 |
| | HOMA-index | 9.7 | 3.0 | 2.1 | 3.0 | |
| | Lactate (mol/L) | 5.7 | 2.4 | 1.8 | 0.9 | 0.5–2.2 |
| | Total Cholesterol (mg/dl) (mmol/L) | 213 (5.5) | 217 (5.61) | 228 (5.89) | 213 (5.5) | <190 (4.91) |
| | HDL-Chol (mg/dl) (mmol/L) | 35 (0.9) | 37 (0.96) | 37 (0.96) | 39 (1.01) | >40 (1.03) |
| | LDL -Chol (mg/dl) (mmol/L) | 153 (3.95) | 166 (4.29) | 176 (4.55) | 151 (3.9) | <115 (2.97) |
| | Triglyceride (mg/dl) (mmol/L) | 205 (2.31) | 206 (2.33) | 172 (1.94) | 197 (2.22) | <150 (1.69) |
| Muscle/Heart | CK (U/L) | 22,423 | 12,968 | 12,590 | 10,431 | 30–200 |
| | LDH (U/L) | 2,612 | 1,651 | 1,507 | 1,128 | 125–243 |
| | CK MB (ng/ml) | >600 | 418 | 398 | 205 | 0–7.2 |
| | Troponin I (pg/ml) | 336 | 387 | 442 | 282 | 0–34 |
| | Pro-BNP (pg/ml) | 1,175 | 787 | 980 | 362 | <125 |
| | LV Mass Index (g/m $^{2.7}$) | 65 | | | 58 | <45 (F), 49 (M) |
| | Interventricular Septum (mm) | 15 | | | 14 | <11 |
| | Global Longitudinal Strain (%) | 16,4 | | | 17,7 | >20 |
| Liver | AST (U/L) | 292 | 226 | 229 | 220 | 0–34 |
| | ALT (U/L) | 216 | 181 | 239 | 255 | 0–55 |
| | ALP (U/L) | 191 | 174 | 165 | 150 | 40–150 |
| | Liver longitudinal diameter (right/left lobe) (mm) | 191/156 | | | 187/147 | |

IG, interstitial glucose; HOMA-Index (7).

TABLE 2 Daily dietary intake (7d-diary) on admission and during dietary intervention.

| | On admission (0 m) | After 1st step intervention (6m) | After 1-yr Covid lock-down (18m) | After 2nd step intervention (24 m) |
|---|---|---|---|---|
| Energy (kcal) (KJ) | 3,132 (13,114) | 3,105 (13000) | 2,870 (12016) | 3,046 (12,752) |
| Carbohydrates (g) (%TEI) | 513 (61) | 422 (51) | 396 (52) | 260 (32) |
| Protein (g) (%TEI) | 163 (21) | 192 (25) | 194 (27) | 177 (23) |
| Lipid (g) (%TEI) | 62 (18) | 84 (24) | 68 (21) | 152 (45) |
| Monounsaturated (%TEI) | 9 | 14 | 9 | 27 |
| Polyunsaturated (%TEI) | 2 | 4 | 4 | 6 |
| Saturated (%TEI) | 5 | 5 | 6 | 10 |
| Corn starch (g) | 215 | 100 | 100 | 80 |
| Maltodextrin (g) | 80 | 0 | 0 | 0 |
| Fiber (g) | 11 | 28 | 28 | 26 |

percentage of ≈25% TEI including a protein powder supplement. To reinforce the patient's adherence to dietary intervention, daily menus were provided (Appendix A).

At the end of each step, clinical, biochemical and imaging organ exams were performed to evaluate the efficacy of the dietary intervention.

## 4. Results

The patient well tolerated the dietary changes and showed a good adherence to prescriptions. He reported an improved feeling of wellbeing and greater exercise capacity (also evidenced by the SF36 questionnaire). The changes in clinical, metabolic and organ damage biomarkers during the nutritional intervention are presented in Table 1. Body weight remained substantially stable during the nutritional intervention (except for a 2-kg increase due to a more sedentary lifestyle during the lockdown due to COVID restrictions). Although REE decreased by ≈200 Kcal, it was still 10% higher than the predicted value). No significant changes were found in body composition. Since the 1st step of dietary intervention, there was a remarkable reduction in insulin levels (from 41 to 15 μU/ml) and in insulin resistance index (from 9.7 to 3.0) resulting from the reduced CHO intake. It is noteworthy that despite the substantial reduction in CHO intake, no severe hypoglycemia occurred; instead, during the nutritional intervention both the number and the average duration of hypoglycemia (<70 mg/dl, <3.89 mmol/L) decreased (from 41 episodes/4 week on admission to 24 episodes/4 week after the 2nd step, and from 99 to 56 min, respectively). Notably, lipid profile remained unchanged despite the consistent increase in fat intake. Since the 1st step intervention, both muscle and cardiac enzymes markedly decreased, with a 50% reduction in CK (from 22,423 to 10,431 U/L) and LDH (from 2,612 to 1,128 U/L) and a 70% reduction in Pro-BNP (from 1,175 to 362 pg/ml). Cardiac ultrasound evidenced an improvement in geometry and LV function as evidenced by a significant reduction in LV mass and an increase in global longitudinal strain (GLS). Liver enzymes remained substantially stable and a trend toward a reduction in liver size was found at ultrasound examination.

## 5. Discussion

By chronologically reviewing the literature on the dietary management of patients with GSD III (Table 3), it is evident that over the years several attempts have been made to define the best nutritional approach capable of maintaining euglycemia and, in the meantime, preventing/limiting the long-term complications of the disease involving heart, muscle and liver. Unfortunately, the analysis of the available literature does not lead to firm conclusions due to a large variation in patients' ages (from 2 months to 47 years) (8, 9), duration of follow-up (from 4 months to 5 years), types of nutritional intervention—which are often not sufficiently detailed in terms of bromatological composition and, finally, the clinical outcomes (heart, muscle, liver).

The hallmark of all these dietary programs is a reduction in CHO intake associated with an increase in protein and, more recently, in fat intake. The rationale being to avoid excessive glycemic elevation with consequent reactive hyperinsulinemia and tissue deposition of abnormal glycogen and, in the meanwhile, to provide alternative fuel for body needs.

The clinical benefits of a high protein diet were first demonstrated in a case report showing an improvement in strength and muscle mass in a 7-year-old patient receiving a protein intake of 25% of TEI (10). Another clinical case reported a partial remission of subacute respiratory failure in a 47-year-old GSD III patient receiving a 30–35% protein diet (9). Subsequently, the benefits of a high protein diet (30–37% TEI) were described in a patient with hypertrophic cardiomyopathy at pre-transplant stage (11, 12). More recently, a high fat diet has gained much attention following the evidence from cardiovascular research that ketone bodies are an efficient metabolic substrate for the failing heart since they require less oxygen per molecule of ATP generated (13). In addition, the administration of D,L-3-hydroxybutyrate was found to be beneficial in patients with cardiomyopathy secondary to defects of fatty acid oxidation (14). Finally, Valayannopoulus et al. published the successful treatment of a 2-month-old infant with severe cardiomyopathy, by means of ketone bodies supplementation (D, L-3-hydroxybutyrate), ketogenic and high protein diet (8). Overall, these data lent support to the hypothesis

TABLE 3 Chronological overview of clinical case reports of patients with GSD III treated with dietary manipulation.

| Study | Patients | Diagnosis | Diet description | | | | | Follow-up (months) | Main clinical benefits |
|---|---|---|---|---|---|---|---|---|---|
| | | | Kcal | CHO | Prot | Fat | Supplements | | |
| Slonim (10) | ♂ 7-yr | Debrancher deficiency (Liver and Muscle biopsy) | 1,600 | 50% | 25% | 25% | Sustacal* | 20 | - Improved muscle strength and growth. |
| Kiechl (9) | ♀ 47-yr | Debrancher deficiency (Muscle biopsy) | nr | nr | 30–35% | nr | | 4 | - CK ↓<br>- normalization of muscle strength and spirometry |
| Dagli (11) | ♂ 22-yr | Retention of limit dextrin in cultured fibroblasts | nr | nr | 30% | nr | Cornstarch: 1,36g/Kg | 12 | - CK ↓<br>- Decreased Left ventricular mass index |
| Valayannopoulos (8) | ♂ 2 months | Homozygosity for the mutation: c.2157+IG>T | nr | 20% | 15% | 65% | 3OHB at the dose of 400 to 800 mg/Kg/d | 24 | - Decreased Interventricular wall thickness |
| Sentner (12) | ♀ 32-yr | Homozygosity for the mutation: c.753_756delCAGA | 900§ | 61% | 37% | 2% | | 4 | - CK ↓<br>- Decreased Interventricular Septum (IVS) |
| | | | 1,370 | nr | 4,3% | nr | Cornstarch: 2 doses | 32 | |
| Mayorandan (15) | 1) ♂ 9-yr | Homozygosity for the mutation: c.4256dupC | nr | 0,4g/Kg | 7g/Kg | 8g/Kg | | 32 | - CK ↓<br>- ProBNP ↓<br>- Decreased Ventricular Septum Thickness |
| | 2) ♂ 11-yr | Homozygosity for the mutation: c.753_756del | nr | 0,5g/Kg | 6g/Kg | 5g/Kg | | 26 | Dietary noncompliance. |

*(Continued)*





10.3389/fnut.2023.1178348

TABLE 3 (Continued)

| Study | Patients | Diagnosis | Diet description | | | | | Follow-up (months) | Main clinical benefits |
|---|---|---|---|---|---|---|---|---|---|
| | | | Kcal | CHO | Prot | Fat | Supplements | | |
| Brambilla (19) | 1) ♀ 7-yr | Homozygosity for the mutation: p.G1087R | 1,120 | 15% | 26% | 5,9% | Protein powders | 12 | - CK ↓<br>- Decreased IVS |
| | 2) ♂ 5-yr | | 1,050 | 15% | 25% | 6,0% | | | - CK ↓<br>- Decreased IVS |
| Francini-Pesenti (16) | ♂ 34-yr | nr | nr | 20g /day | nr | nr | Cornstarch in case of hypoglycemia | 12 | - CK ↓<br>- Increased Steps/24h<br>- Improved ejection fraction |
| Fisher (18) | 1) ♂ 37-yr | Homozygosity for the mutation: (c.753_756delCAGA; p.ASP251Glufs*23) | nr | 5-20% | 9-23% | 70-78% | MCT: 11-23% of total daily fat | 60 | - CK ↓<br>- Stabilization of blood glucose |
| | 2) ♂ 40-yr | | | | | | | | |
| Marusic (21) | ♀ 15-yr | Homozygosity for the mutation: c.3980G>A | nr | 2% | 11% | 87% | | 48 | - Improved Hepatic, Muscle and Cardiac markers<br>- Decreased Left Ventricular Mass Index and septal Wall |
| Olgac (17) | 1) ♂4,5-yr<br>2) ♂11-yr<br>3) ♂11-yr<br>4) ♂9-yr<br>5) ♀ 31-yr<br>6) ♀ 3-yr | Nr | nr | 10% | 20% | 70% | Vitamins and minerals | Range: 3–7 | - CK ↓ |
| Kumru Akin (20) | ♂ 9-yr | Homozygosity for the mutation: c.1783C > T | 1,400 | 30% | 2,0% | 50% | Protifar** (1g/Kg) and Glycosade®ᶜ (2g/Kg). | 18 | - CK ↓<br>- Improved left ventricular outflow tract<br>- Decreased interventricular septum |

*Sustacal contains 24% protein, 55% carb. and 21% fat.

**Protifar contains 20% casein and 80% whey.

ᶜCornstarch (Vitaflo International Ltd. Liverpool, UK).





10.3389/fnut.2023.1178348

that a high-fat diet may help control myocardiopathy in patients with GSD III. Moving from this belief, different approaches based on variable fat intakes have been tested in patients with GSD III: the Modified Atkins Diet (MAD) (15–17), a MAD with MCT oil supplements (18), and high fat / high-protein diet (19, 20). The results show the efficacy of these approaches in reducing muscle enzymes and improving cardiomyopathy, although some concern related to patient compliance and long- term effects of these diets on bone health and lipid profile remain to be addressed. Indeed, the effect of nutritional modifications on traditional outcome parameters need to be carefully balanced against psychosocial wellbeing and quality of life in patients with hepatic GSDs (22).

The need for new therapies that can improve the quality of life of patients with GSD has prompted research toward innovative therapies such as the use of gene therapy or therapy using messenger RNA (mRNA). In principle, GSD patients would be no longer dependent on a strict dietary regimen. Early phase clinical studies are already available for GSD Ia and II and the approach appears promising also for other types of GSDs (23).

Our case report describes a patient with GSD IIIa with myopathy and severe hypertrophic cardiomyopathy in whom a gradual shift from a CHO-rich diet (61% TEI) to a high- fat (45% TEI) / high-protein (23% TEI) diet—performed under careful monitoring of glycemic and biochemical parameters—produced relevant clinical benefits and reduced organ damage. Indeed, both muscle and cardiac enzymes decreased by 50–70% and an improvement in geometry and LV function was found at echocardiography.

It is interesting to note that even a small reduction in CHO intake (from 61 to 51% TEI) together with the use of wholemeal products with a low glycemic index, were able to reduce the biochemical markers of muscle and heart damage. The unusually high CK values of our patient, compared to other cases reported, dropped from 22,423 to 12,968 U/L after only 6 months of dietary intervention. This improvement persisted throughout the COVID-19 pandemic, during which the patient kept his diet constant for a year. When CHO intake was reduced further (from 51 to 32% TEI) the biochemical parameters continued to improve, providing additional benefits. It is also interesting to note that, although lipid intake was increased from 18 to 45% of TEI, the lipid profile did not worsen, probably due to consumption of foods rich in monounsaturated and polyunsaturated fatty acids. These findings highlight the concept that beyond restricting CHO and increasing fat intake, the quality of foods is of paramount importance. In fact, low glycemic index foods and whole grains help keep glucose levels more stable, thus limiting the risk of hypoglycemia, while increasing mono- and polyunsaturated fat consumption prevents atherogenic modifications of the lipid profile– a very important finding considering that the nutritional therapy is life-long. Noteworthy, the number and duration of hypoglycemic episodes decreased during the dietary intervention despite the reduction in CHO intake. Particularly, an increase in Time In Range (TIR: 70–140 mg/dl, 3.89–7.78 mmol/L) was observed. Although optimal TIR for patients with GSD III has not been defined yet, a glycemic target for TIR has been recently proposed for adult patients with GSD Ia. Notably, the TIR observed in the present case was higher than that reported in GSD Ia patients (i.e., 96 vs. 83%), highlighting the lower contribution of liver involvement to disease phenotype in adult GSD III as compared to GSD I. CGM data from the present study support the opportunity to decrease CHO intake in older GSD III with no risk of precipitating hypoglycemia (24).

The finding of an REE value 22% higher than the predicted one observed in our patient deserves a comment. We first ruled out possible thyroid dysfunction and/or subclinical inflammatory diseases, which are known to increase energy expenditure. A possible explanation for the high REE value is that it may be related to an increased cell mass and, thus, to be an expression of organomegaly. Elevated REE values have been documented also in patients with GSD Ia (25, 26) and in patients with lysosomal storage disorders (27, 28), which are characterized by intra-organ accumulation of anomalous molecules. The slight reduction in REE (−200 kcal) during the nutritional intervention in conjunction with an albeit modest reduction in organ volumes supports this interpretation.

In conclusion, low-CHO high fat / high- protein diet showed a good efficacy in reducing organ damage and improving quality of life in our patient with GSD IIIa and severe cardiomyopathy. In addition to the low CHO intake, it is important to focus on macronutrient quality, favoring low-glycemic index food and unsaturated fats in order to preserve cardiometabolic health in the long-term. As a low CHO diet does not appear to increase the risk of hypoglycemia and is safe, this dietary approach could be started as early as possible in GSD III displaying skeletal/cardiac muscle disease in order to prevent/minimize organ damage.

## 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.

## Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

## Author contributions

EM and APA collected the clinical data, carried out the literature search, and wrote a first draft. AR and RL reviewed the manuscript. BC took care of the patient, reviewed the manuscript, and provided relevant intellectual contribution. All authors have read and agreed to the published version of the 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.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1178348/full#supplementary-material

## References


1. Sentner CP, Hoogeveen IJ, Weinstein DA, Santer R, Murphy E, McKiernan PJ, et al. Glycogen storage disease type III: diagnosis, genotype, management, clinical course and outcome. *J Inherit Metab Dis.* (2016) 39:697–704. doi: 10.1007/s10545-016-9932-2

2. Schreuder AB, Rossi A, Grünert SC, Derks TGJ. "Glycogen storage disease type III. 2010 Mar 9 [updated 2022 Jan 6]," In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, eds. *GeneReviews® [Internet]*. Seattle (WA): University of Washington, Seattle, p. 1993–2022.

3. Hijazi G, Paschall A, Young SP, Smith B, Case LE, Boggs T, et al. A retrospective longitudinal study and comprehensive review of adult patients with glycogen storage disease type III. *Mol Genet Metab Rep.* (2021) 29:100821. doi: 10.1016/j.ymgmr.2021.100821

4. Kishnani PS, Austin SL, Arn P, Bali DS, Boney A, Case LE, et al. Glycogen storage disease type III diagnosis and management guidelines. *Genet Med.* (2010) 12:446–63. doi: 10.1097/GIM.0b013e3181e655b6

5. Rossi A, Hoogeveen IJ, Bastek VB, de Boer F, Montanari C, Meyer U, et al. Dietary lipids in glycogen storage disease type III: a systematic literature study, case studies, and future recommendations. *J Inherit Metab Dis.* (2020) 43:770–7. doi: 10.1002/jimd.12224

6. Derks TG, Van Rijn M. Lipids in hepatic glycogen storage diseases: pathophysiology, monitoring of dietary management and future directions. *J Inherit Metab Dis.* (2015) 38:537–43. doi: 10.1007/s10545-015-9811-2

7. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* (1985) 28:412–9. doi: 10.1007/BF00280883

8. Valayannopoulos V, Bajolle F, Arnoux JB, Dubois S, Sannier N, Baussan C, et al. Successful treatment of severe cardiomyopathy in glycogen storage disease type III With D,L-3-hydroxybutyrate, ketogenic and high-protein diet. *Pediatr Res.* (2011) 70:638–41. doi: 10.1203/PDR.0b013e318232154f

9. Kiechl S, Willeit J, Vogel W, Kohlendorfer U, Poewe W. Reversible severe myopathy of respiratory muscles due to adult-onset type III glycogenosis. *Neuromuscul Disord.* (1999) 9:408–10. doi: 10.1016/S0960-8966(99)00038-3

10. Slonim AE, Weisberg C, Benke P, Evans OB, Burr IM. Reversal of debrancher deficiency myopathy by the use of high-protein nutrition. *Ann Neurol.* (1982) 11:420–2. doi: 10.1002/ana.410110417

11. Dagli,AI, Zori RT, McCune H, Ivsic T, Maisenbacher MK, Weinstein DA. Reversal of glycogen storage disease type IIIa-related cardiomyopathy with modification of diet. *J Inherit Metab Dis.* (2009) 32: S103-6. doi: 10.1007/s10545-009-1088-x

12. Sentner CP, Caliskan K, Vletter WB, Smit GP. Heart failure due to severe hypertrophic cardiomyopathy reversed by low calorie, high protein dietary adjustments in a glycogen storage disease type IIIa patient. *JIMD Rep.* (2012) 5:13–6. doi: 10.1007/8904_2011_111

13. Yurista SR, Chong CR, Badimon JJ, Kelly DP, de Boer RA, Westenbrink BD. Therapeutic potential of ketone bodies for patients with cardiovascular disease: JACC state-of-the-art review. *J Am Coll Cardiol.* (2021) 77:1660–9. doi: 10.1016/j.jacc.2020.12.065

14. Van Hove JL, Grünewald S, Jaeken J, Demaerel P, Declercq PE, Bourdoux P, et al. D,L-3-hydroxybutyrate treatment of multiple acyl-CoA dehydrogenase deficiency (MADD). *Lancet.* (2003) 361:1433–5. doi: 10.1016/S0140-6736(03)13105-4

15. Mayorandan S, Meyer U, Hartmann H, Das AM. Glycogen storage disease type III: modified Atkins diet improves myopathy. *Orphanet J Rare Dis.* (2014) 9:196. doi: 10.1186/s13023-014-0196-3

16. Francini-Pesenti F, Tresso S, Vitturi N. Modified Atkins ketogenic diet improves heart and skeletal muscle function in glycogen storage disease type III. *Acta Myol.* (2019) 38: 17–20.

17. Olgac A, Inci A, Okur I, Biberoglu G, Oguz D, Ezgü FS, et al. Beneficial effects of modified atkins diet in glycogen storage disease type IIIa. *Ann Nutr Metab.* (2020) 76:233–41. doi: 10.1159/000509335

18. Fischer T, Njoroge H, Och U, Klawon I, Marquardt T. Ketogenic diet treatment in adults with glycogenosis type IIIa (Morbus Cori). *Clin Nutr Exp.* (2019) 28:83–91. doi: 10.1016/j.yclnex.2019.09.004

19. Brambilla A, Mannarino S, Pretese R, Gasperini S, Galimberti C, Parini R. Improvement of cardiomyopathy after high-fat diet in two siblings with glycogen storage disease type III. *JIMD Rep.* (2014) 17:91–5. doi: 10.1007/8904_2014_343

20. Kumru Akin B, Ozturk Hismi B, Daly A. Improvement in hypertrophic cardiomyopathy after using a high-fat, high-protein and low-carbohydrate diet in a non-adherent child with glycogen storage disease type IIIa. *Mol Genet Metab Rep.* (2022) 32:100904. doi: 10.1016/j.ymgmr.2022.100904

21. Marusic T, Zerjav Tansek M, Sirca Campa A, Mezek A, Berden P, Battelino T, et al. Normalization of obstructive cardiomyopathy and improvement of hepatopathy on ketogenic diet in patient with glycogen storage disease (GSD) type IIIa. *Mol Genet Metab Rep.* (2020) 24:100628. doi: 10.1016/j.ymgmr.2020.100628

22. Venema A, Peeks F, Rossi A, Jager EA, Derks TGJ. Towards values-based healthcare for inherited metabolic disorders: an overview of current practices for persons with liver glycogen storage disease and fatty acid oxidation disorders. *J Inherit Metab Dis.* (2022) 45:1018–27. doi: 10.1002/jimd.12555

23. Kishnani PS, Sun B, Koeberl DD. Gene therapy for glycogen storage diseases. *Hum Mol Genet.* (2019) 28:R31–41. doi: 10.1093/hmg/ddz133

24. Rossi A, Venema A, Haarsma P, Feldbrugge L, Burghard R, Rodriguez-Buritica D, et al. A prospective study on continuous glucose monitoring in glycogen storage disease type Ia: toward glycemic targets. *J Clin Endocrinol Metab.* (2022) 107:e3612–23. doi: 10.1210/clinem/dgac411

25. Feillet F, Bodamer OA, Leonard JV. Increased resting energy expenditure in glycogen storage disease type Ia. *J Inherit Metab Dis.* (1998) 21:80–1. doi: 10.1023/A:1005375717518

26. Buscemi S, Noto D, Buscemi C, Barile AM, Rosafio G, Settipani V, et al. Resting energy expenditure and substrate oxidation in malnourished patients with type 1 glycogenosis. *J Clin Endocrinol Metab.* (2019) 104:5566–72. doi: 10.1210/jc.2019-00585

27. Doneda D, Lopes AL, Oliveira AR, Netto CB, Moulin CC, Schwartz IV. Gaucher disease type I: assessment of basal metabolic rate in patients from southern Brazil. *Blood Cells Mol Dis.* (2011) 46:42–6. doi: 10.1016/j.bcmd.2010.10.008

28. Rovelli V, Zuvadelli J, Piotto M, Scopari A, Dionigi AR, Ercoli V, et al. L-alanine supplementation in Pompe disease (IOPD): a potential therapeutic implementation for patients on ERT? *A case report Ital J Pediatr.* (2022) 48:48. doi: 10.1186/s13052-022-01249-y

frontiers | Frontiers in Nutrition

TYPE Brief Research Report
PUBLISHED 09 June 2023
DOI 10.3389/fnut.2023.1181436



OPEN ACCESS

EDITED BY
Annalisa Noce,
University of Rome Tor Vergata, Italy

REVIEWED BY
Elisa Grazioli,
Foro Italico University of Rome, Italy
Manuela Di Lauro,
University of Rome Tor Vergata, Italy

*CORRESPONDENCE
María Pía de la Maza
✉ mpmaza@inta.uchile.cl

†These authors have contributed equally to this work

RECEIVED 07 March 2023
ACCEPTED 21 April 2023
PUBLISHED 09 June 2023

CITATION
Monsalves-Álvarez M, Jiménez T, Bunout D, Barrera G, Hirsch S, Sepúlveda-Guzman C, Silva C, Rodriguez JM, Troncoso R and de la Maza MP (2023) High-intensity interval training prevents muscle mass loss in overweight Chilean young adults during a hypocaloric-Mediterranean diet: a randomized trial. *Front. Nutr.* 10:1181436. doi: 10.3389/fnut.2023.1181436



# High-intensity interval training prevents muscle mass loss in overweight Chilean young adults during a hypocaloric-Mediterranean diet: a randomized trial

Matías Monsalves-Álvarez[1†], Teresa Jiménez[2†], Daniel Bunout[2], Gladys Barrera[2], Sandra Hirsch[2], Carlos Sepúlveda-Guzman[2,3], Claudio Silva[4], Juan M. Rodriguez[2], Rodrigo Troncoso[2,5] and María Pía de la Maza[2,4]*

[1]Instituto de Ciencias de la Salud, Universidad de O'Higgins, Rancagua, Chile, [2]Laboratorio de Investigación en Nutrición y Actividad Física (LABINAF), Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile, [3]Laboratorio de Ciencias del Ejercicio, Clínica MEDS, Santiago, Chile, [4]Clínica Alemana, Santiago, Chile, [5]Advanced Center for Chronic Diseases, Universidad de Chile, Santiago, Chile

The hypocaloric Mediterranean diet (MD) mainly reduces fat mass but inevitably causes a loss of skeletal muscle mass. High-intensity interval training (HIIT) seems to have advantages in preserving muscle mass during a hypocaloric regime. Our study compares body composition and metabolic changes in overweight and obese Chilean women and men after 3months of weight loss treatment with a Mediterranean-type hypocaloric diet, HIIT, or a combination of both. The study included 83 overweight or obese women and men between the ages of 25 and 50. The subjects were randomly assigned to one of the three intervention groups: (1) MD, (2) EX, and (3) MD+EX. Baseline and post-intervention measurements included: (a) body composition by dual-beam densitometry, muscle, and fat measurements by thigh ultrasound and computed tomography; (b) handgrip and quadriceps muscle strength; (c) exercise performance by peak oxygen consumption, peak load, work efficiency, and exercise energy expenditure; and (d) metabolic parameters. Out of 83 participants, the retention rate was 49% due to low compliance with the interventions. As expected, the MD group resulted in significantly greater weight loss (MD −7%, EX −0.6% and MD+EX −5.3%) and appendicular fat mass loss (MD −11.1%, EX −2.9, MD+EX −10.2%) but was associated with significant lean tissue loss (2.8%), which was prevented by HIIT (EX −0.1 and MD+EX −0.6%). Metabolic and glycoxidative parameters remained unchanged, irrespective of changes in body composition. Hypocaloric diets remain the most effective means to lose weight and body fat. However, it induces a loss of lean body mass when not accompanied by exercise training. This study shows that HIIT prevents the loss of muscle mass caused by a hypocaloric Mediterranean diet.

KEYWORDS

**HIIT, body composition, Mediterranean diet, skeletal muscle, exercise**

## Introduction

Healthy dietary patterns and exercise training have been proposed as primary components in the prevention and treatment of obesity and its comorbidities (1). Weight loss has been found to depend on different factors, such as the degree of energy deficit imposed by the diet, its duration, and macronutrient composition, which may directly influence adherence and treatment success (2–5). Among the diets, the Mediterranean Diet (MD), originally described by Ancel Keys, has been shown to promote a healthy metabolic profile mainly through its nutrient characteristics based on plants, unsaturated fats, fruits, and fiber while focusing on a low energy density, making it palatable and satiating (6). Interestingly, MD with limited amounts of carbohydrates has promoted differential mobilization of visceral fat depots and reduced cardiovascular risk factors in insulin-resistant patients. As with other diets, the weight loss seen with MD or low-calorie diets is mainly fat mass, but the weight loss inevitably affects skeletal muscle mass. It can range from ∼2 to 10%, depending on the age of the subjects (7, 8). This loss of lean muscle mass is likely to potentiate adverse effects on physical function and health (9).

Physical activity has a limited contribution to body fat reduction, even among different types of exercise (10–13). However, it is highly relevant for the preservation of muscle mass (7), weight maintenance (14), and improving cardiorespiratory fitness (15). Combining physical exercise with a hypocaloric diet induces slightly more weight loss in the short term (1.0–11.5 kg) (16). However, caloric restriction plus continuous aerobic training causes greater muscle mass loss than resistance exercise (17), suggesting that exercise modalities may play a role in maintaining muscle mass while dieting.

Although the benefits of even modest weight loss have been reported (18), it is unclear which intervention (diet vs. diet plus exercise or exercise alone) induces more favorable changes in body composition and chronic disease management in the long term. Weight maintenance is challenging due to adherence to behavioral changes and metabolic adaptations after weight loss. Therefore, researchers are looking for more effective and less time-consuming strategies that will hopefully increase commitment to healthy lifestyle changes.

High-intensity interval training (HIIT) has emerged as an alternative training modality that improves cardiorespiratory fitness and cardio-metabolic parameters in the healthy population (19) and in dieting obese patients (20, 21). However, few studies have addressed changes in segmental muscle mass and function and fat mass of different compartments after HIIT training (22–24), leading to potential differences when exercise training modalities are prescribed for the treatment of metabolic diseases.

The aim of the present study was to compare body composition and metabolic changes in overweight and obese men and women after 3 months of behavioral management for weight loss using a Mediterranean-type hypocaloric diet, HIIT, or a combination of both. We employed DEXA, abdominal CT scans, leg CT, and ultrasound (US) to analyze body compartments. Metabolic variables included serum glucose, lipoproteins, and insulin. We measured peak exercise oxygen consumption, workload, and handgrip and quadriceps muscle strength to evaluate functional changes. We hypothesized that adding HIIT to the dietary intervention would enhance its effects on body composition and metabolic profile. Our primary endpoints were the reduction of body fat and the maintenance of muscle mass. Secondary outcomes were the changes in the metabolic variables studied. This study was registered in Clinical Trials (NCT01793896); this manuscript refers to body composition and metabolic endpoints.

## Methods

### Subjects

The present study was a 3-month intervention trial. Inclusion criteria considered overweight (BMI 25 to 29.9 kg/m$^2$) or obese (BMI greater than or equal to 30 kg/m$^2$) men and women between the ages of 25 and 50 years who were interested in losing weight. At the same time, exclusion criteria included weight fluctuations (> 3 kg in the last 3 months), diabetes mellitus, neuromuscular or joint diseases, active smoking (>5 cigarettes/day), alcohol intake >30 g/day, and chronic diseases such as cancer, AIDS, or any organ failure. After signing a written informed consent, volunteers underwent an initial evaluation to rule out possible exclusion criteria and were then randomly assigned (according to a randomized number-based algorithm) to 1 of 3 interventions: a low-calorie Mediterranean-type diet (MD), supervised high-intensity interval training (HIIT) 3 times per week (EX), both interventions simultaneously (MD + EX). The study did not include a control group, but we performed a 3-month control period before randomization in 25 volunteers in which no intervention was prescribed.

### Study design

After the initial assessment, subjects were randomly assigned to receive one of the interventions for 3 months, repeating the same evaluation at the end of this period. A total of 83 participants were included and randomized into three different groups (Figure 1). **MD**: the subjects were prescribed a Mediterranean diet with a caloric intake of 20 Kcal/kg. The recommended foods in the MD, as previously described (25), were non-fat fermented milk, vegetables, legumes, fresh fruit, olive oil, and fish. Subjects were also instructed to avoid highly processed foods (such as breakfast cereals, cookies, cakes, powdered milk, and sausages). Wine consumption, which is typical of MD, was not recommended. Subjects were instructed to maintain physical activity or regular exercise without a supervised exercise plan and were checked weekly by the dietitian in charge. **EX:** High-Intensity Interval Training (HIIT) sessions were performed in three weekly sessions on a static bicycle (Spirit, model CU800, AR, United States), following the protocol of Gillen et al. (26). Subjects performed 10 repetitions of 1 min in each session, at 75% of the peak power achieved in the incremental test, with 1 min of rest cycling at 50 watts. This protocol was designed to achieve 85–90% of the maximum cardiac rate (HRmax) and included a 3-min warm-up and a 2-min recovery period, for a total of 25 min per session. **MED + EX**: subjects were enrolled in both interventions simultaneously.

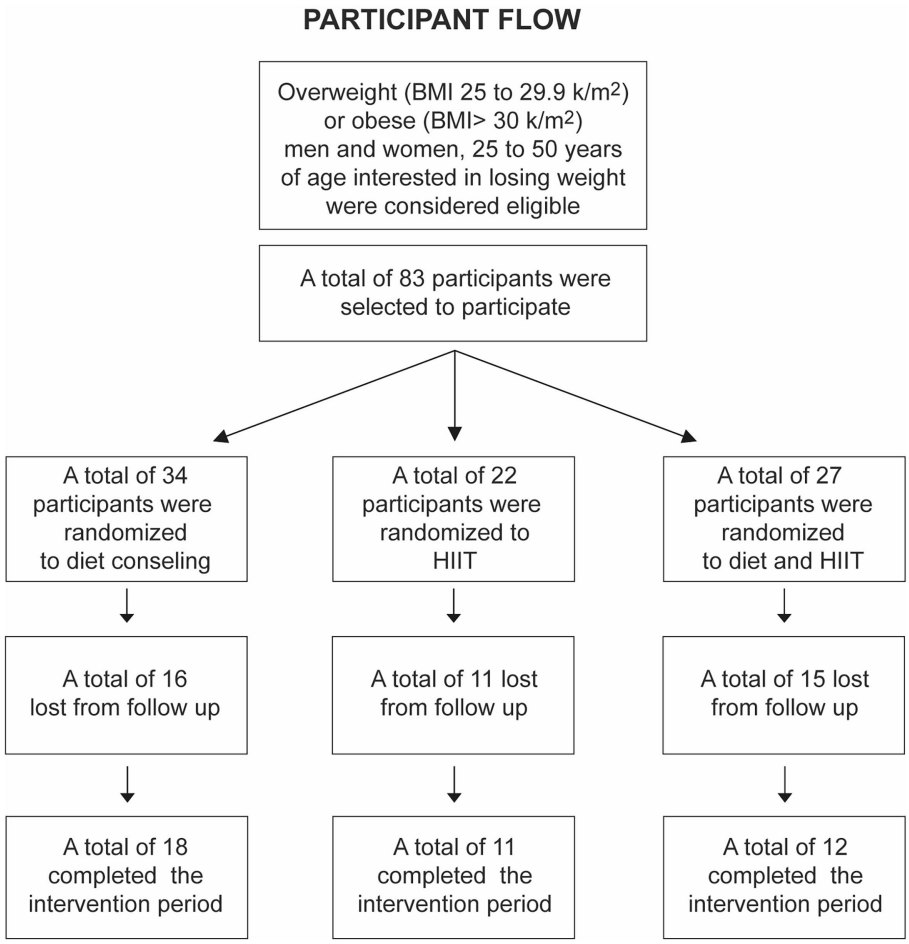



FIGURE 1
Participant flow diagram.

## Dietary intake and Mediterranean score

Habitual dietary intake according to the survey of Uribarri et al. (27) and also the calculation of the Mediterranean Score using the "Aliméntate Sano Dietary Recall," (28) available on the Internet (https://www.mifitbook.cl/que_es_fitbook.php).

## Blood samples

Fasting blood samples were collected by a venous puncture to obtain routine clinical biochemical tests (glucose, insulin, hemoglobin, lipoproteins, creatinine, prothrombin, and thyroid thyrotropin), analyzed in Vidaintegra laboratories.

## Body composition

Subjects were assessed for basic anthropometric measurements (height, weight, and waist circumference). Double-photon densitometry (DEXA) was evaluated for estimation of total body composition on Lunar Encore equipment (software 2011, version 13.60), with a minimal significant variability (MSV) at the 95% confidence level between two measurements of the same object or subject of 0.9% (29).

## Visceral and subcutaneous fat determination by computed tomography

Computed tomography (CT) at the L3 level to measure visceral and subcutaneous fat areas was performed in addition to the measurement of liver density as an indicator of fat infiltration, and CT at the same site of ultrasound measurements to estimate the rectus femoris muscle (RFM) area and density, in a Siemens Definition AS + tomograph with 128 detectors. The images were exported and segmented semi-automatically with the SliceOMatic software (version 5.0, Tomovision, Canada) by the region growth method and manual correction when necessary.

## Muscle mass cross-sectional area

Thigh ultrasound (US) was used to measure the cross-sectional area of the rectus femoris muscle (RFM), at the mid-level between the iliac crest and the patella using a General Electric Logiq

ultrasonographer, with intra- and interrater errors of 3.2 and 7.4%, respectively (30).

## Physical performance

Quadriceps muscle strength was measured using a quadriceps table attached to a transducer, and handgrip strength was measured using a Therapeutic Instruments dynamometer (Clifton, NJ, USA), registering the best of three measurements on each limb (31). Cardiorespiratory fitness was determined by submaximal oxygen consumption ($VO_2$) after an incremental test on a braked cycle ergometer using Sensormedics Vmax Encore 29 equipment. The incremental exercise test was started on a 15-watt ramp with 15-watt increments until volitional exhaustion at 60 rpm to measure $VO_{2peak}$ (32). Gross work efficiency was calculated as the ratio of work rate to energy expenditure in joules multiplied by 100 at submaximal work rates (33).

## Energy expenditure

Actigraph (Actiheart®) was installed on all participants for 72 h for actigraphy and heart rate measurement. The devices were individually calibrated with the heart rate/energy expenditure curve obtained during the exercise calorimetry. Total energy expenditure (TEE), activity energy expenditure (AEE), and physical activity level (PAL) were determined (Actiheart software version 4.0.32, www.camtech.com).

## Statistical analysis

Baseline data distribution was analyzed using the Shapiro–Wilk test, with group comparisons performed using the Student's *T*-test, one-way ANOVA, or Kruskal–Wallis, depending on their distribution. To analyze the effects of the interventions, we transformed the delta changes before and after the intervention into percentages and compared the means using a one-way ANOVA. We also assessed the changes in variables within and between treatment groups. To do this, we performed a multilevel mixed-effects linear regression for repeated measures on raw data. This method has been previously described to detect the time or treatment effect and the interaction between these two conditions (34). All analyses were performed using Stata 13.0 software. Table variables were expressed as mean ± SD, and median (range). Regarding power size calculation, according to our primary endpoint (loss of body fat) and based on a previous study (35), to obtain a 10% reduction in total fat after the intervention, eight participants per group were required to obtain results with an α of less than 0.05 and a power of 0.8 using a paired analysis.

# Results

As mentioned above, a total of 83 patients were initially selected according to the inclusion criteria, but 42 subjects failed to attend the exercise sessions or appointments with the RD, so we could not complete the final evaluation (body composition measurements and blood samples); therefore, the final sample for statistical analysis amounted to 41 (Figure 1). Table 1 shows all the variables studied by the group before the intervention. The groups were homogeneous in every variable at baseline, observing only the expected differences due to gender.

## Brief and intense exercise attenuates appendicular lean body mass loss induced by low-calorie MD

Exercise has been shown to prevent the loss of muscle mass induced by caloric restriction (9). In Table 2, we show that after 3 months of intervention, there was a significant reduction (expressed as %) in body weight, BMI, and waist circumference in MD and MD + EX when compared to EX alone ($p < 0.0001$). Regarding body composition, appendicular LMB, trunk, and total FM were also significantly different in the MD group compared to the other two groups ($p = 0.007$, 0.03, and 0.03, respectively). Regarding biochemical parameters, no statistical differences were observed, despite a decrease in glucose and insulin in the EX and MD + EX groups (−1.5 and − 3.1% reduction, respectively). Interestingly, visceral and subcutaneous fat determined by CT showed that MD alone resulted in a significant reduction in abdominal visceral and subcutaneous fat area ($p = 0.02$ and 0.04), although MD + EX had a − 15.4% and − 15.8% reduction on the same CT measured areas, respectively. We found no difference in exercise testing between groups, but $VO_{2peak}$ and workload showed percentage improvements in EX and MED + EX groups.

## Muscle performance is improved during hypocaloric-MD diets with high-intensity intervals

Preserving skeletal muscle performance is essential to maintaining function at any age (36). Table 3 shows the variables that exhibited significant changes after the intervention according to the mixed-effects linear regression for repeated measures method. Here, we found a lack of weight and total fat loss in the EX group (79.7 to 77 kg and 29.4 to 29.3 kg, respectively), while maintaining lean body mass in MD + EX (~45.6 kg), and an increase in $VO_{2peak}$ and maximal load (W) associated with the inclusion of a HIIT modality in MED + EX (7.4 ml·kg·min and 54 W, respectively). Results did not change significantly when both exercise groups were combined with the calorie-restricted group. In conclusion, caloric restriction-induced greater fat and muscle loss. However, the incorporation of short-interval HIIT prevented muscle mass loss while increasing muscle strength and aerobic capacity as determined by $VO_{2peak}$.

# Discussion

In this study, we used a hypocaloric MD, HIIT 3 times per week, and diet plus training to induce changes in body composition and metabolic parameters. We analyzed the results by protocol, including the 41 compliant men and women. Baseline

TABLE 1 Comparison of baseline variables between study groups.

| Variable | MD (*n*=18) | EX (*n*=11) | MD+ EX (*n*=12) | *p* |
|---|---|---|---|---|
| Sex (M/W) | 9/9 | 4/7 | 4/8 | |
| Age (years) | 38 (25–50) | 39 (26–49) | 31.5 (23–48) | 0.7088 |
| Body composition and dietary recalls | | | | |
| Weight (Kg) | 79.9 (68.7–104.1) | 79.9 (68.3–93.9) | 77.9 (70.4–102.3) | 0.9303 |
| Body mass index (kg/mt$^2$) | 29.9 ± 1.7 | 29.5 ± 1.5 | 29.6 ± 1.6 | 0.7698 |
| Waist circumference (cm) | 98.6 ± 7.6 | 94.2 ± 6.9 | 96.2 ± 7.5 | 0.3088 |
| Total lean body mass (kg) | 42.0 (35.6–62.9) | 40.6 (38.3–62.8) | 45.6 (37.3–69.5) | 0.9081 |
| Appendicular lean body mass (kg) | 18.8 (14.8–30.0) | 18.7 (15.1–29.0) | 21.0 (16.6–34.2) | 0.8196 |
| Total fat mass (kg) | 32.6 ± 3.9 | 31.3 ± 4.6 | 31.1 ± 3.3 | 0.5269 |
| Trunk fat mass (kg) | 18.7 ± 2.8 | 17.7 ± 2.4 | 17.5 ± 2.6 | 0.4380 |
| Appendicular fat mass (kg) | 13.0 ± 2.4 | 12.3 ± 3.3 | 12.2 ± 2.1 | 0.6624 |
| Mediterranean score * | 5.3 ± 2.1 | 5.4 ± 1.7 | 4.7 ± 1.4 | 0.5937 |
| Biochemistry | | | | |
| Glucose (mg/dL) | 90.0 ± 10.1 | 90.9 ± 7.6 | 88.3 ± 9.1 | 0.7977 |
| Insulin (U/mL) | 11.8 (4.8–36.1) | 10.2 (6.6–28.8) | 10.5 (4.5–34.2) | 0.9453 |
| HOMA-IR | 2.7 (1.1–9.3) | 2.2 (1.4–8.7) | 2.2 (1.2–3.9) | 0.3011 |
| Total cholesterol (mg/dL) | 189 (126–362) | 194 (111–223) | 202 (149–223) | 0.5691 |
| HDL cholesterol (mg/dL) | 47 (26–77) | 43 (35–63) | 52 (32–91) | 0.6348 |
| LDL cholesterol (mg/dL) | 107.7 ± 29.4 | 105.9 ± 24.2 | 101.0 ± 43.2 | 0.8601 |
| Tryglicerydes (mg/dL) | 148 (45–1,372) | 134 (34–353) | 139 (62–385) | 0.8722 |
| Muscle strength, ultrasound, and CT scans | | | | |
| Right-hand strength (kg) | 29 (17–52.5) | 25 (16–49.5) | 32.8 (18–48) | 0.5208 |
| Left-hand strength (kg) | 25.5 (15–49.5) | 25 (15–50.5) | 29.5 (15–47.5) | 0.4176 |
| Right quadriceps strength (N) | 350.9 ± 86.8 | 370.0 ± 80.3 | 415.7 ± 128.8 | 0.2248 |
| Left quadriceps strength (N) | 373.6 ± 79.3 | 377.3 ± 95.5 | 424.1 ± 123.7 | 0.3540 |
| US–right RFM area (cm$^2$) | 21.2 ± 3.4 | 23.7 ± 2.6 | 24.2 ± 5.7 | 0.1066 |
| US–left RFM area (cm$^2$) | 21.2 ± 3.5 | 22.6 ± 2.8 | 24.0 ± 5.4 | 0.1540 |
| CT–left RFM area (cm$^2$) | 25.9 ± 3.4 | 27.1 ± 3.4 | 26.8 ± 5.0 | 0.7053 |
| CT–left RFM.Density (HU) | 50.0 ± 3.2 | 52.4 ± 3.0 | 50.8 ± 2.2 | 0.1414 |
| CT–hepatic density (HU) | 54.4 (9.5–66.6) | 61.3 (30.1–71.5) | 54.1 (17.5–62.2) | 0.0624 |
| CT–total Abdominal Fat area (cm$^2$) | 492.5 ± 79.0 | 454.7 ± 58.2 | 433.0 ± 65.2 | 0.0849 |
| CT–abdominal visceral fat area (cm$^2$) | 118.8 (51.1–310.4) | 134.4 (59.6–234.6) | 106.5 (73.8–237.1) | 0.9270 |
| CT–abdominal subcutaneous fat area (cm$^2$) | 348.3 ± 66.9 | 309.6 ± 86.3 | 296.5 ± 43.9 | 0.1137 |
| *Exercise tests* | | | | |
| $VO_2$peak (ml/Kg/min) | 17.3 (10.8–32.1) | 17.2 (11.3–34.3) | 20.3 (14.6–31.2) | 0.2839 |
| Maximal load (W) | 143.2 ± 56.5 | 155.3 ± 45.4 | 169.0 ± 55.7 | 0.4467 |
| Exercise time (Min) | 7.7 (4.4–14.6) | 7.9 (4.5–19.7) | 7.0 (5.2–13.9) | 0.7361 |
| Gross work efficiency (W/J) | 7.7 ± 1.5 | 8.0 ± 1.4 | 7.8 ± 1.0 | 0.9093 |
| Activity energy expenditure (KCal) | 706 (176–2,931) | 521 (194–2,171) | 776 (196–1773) | 0.7726 |
| Physical activity level (METS) | 1.5 (1.2–2.8) | 1.4 (1.3–2.4) | 1.6 (1.3–2.1) | 0.8941 |

DEXA, dual-energy x-ray absorptiometry/RFM, rectus femoris muscle/CT, computed tomography/US, ultrasound / W, watts/J, joules. Values are mean ± SD or median (range) according to the distribution of the variables. Analysis performed by Kruskall–Wallis 2 or one-way ANOVA, depending on the distribution of variables. Significant when $p \leq 0.05$. *Mediterranean score according to the "Aliméntate Sano" dietary survey, where scores 0–4 = low, 4–9 = moderate, and 9–14 = high (14 points being optimal). **Dietary AGEs according to a semiquantitative dietary recall, where < 10 kU/d = low AGE intake, >10 < 16 kU/d = moderate AGE intake and > 16 kU/d = elevated AGE intake.

TABLE 2 Changes in study variables after 3-months of intervention.

| | MD (*n*=18) | EX (*n*=11) | MD+EX (*n*=12) | *p* |
|---|---|---|---|---|
| Body composition and dietary recalls | | | | |
| Weight (%) | −7.2 ± 3.6**α** | −0.6 ± 3.2 | −5.3 ± 2.7**α** | < **0.0001** |
| Body mass index (%) | −7.5 ± 3.6**α** | −0.4 ± 3.4 | −6.1 ± 2.9**α** | < **0.0001** |
| Waist circumference (%) | −7.8 ± 4.5**α** | −1.9 ± 3.3 | −7.4 ± 3.6**α** | **0.0009** |
| DEXA-total lean body mass (%) | −2.8 ± 2.8**α** | −0.1 ± 2.3 | −0.6 ± 2.1 | 0.0153 |
| DEXA-appendicular lean body mass (%) | −3.4 ± 4.4**α** | 5.8 ± 12.5 | −1.6 ± 2.7 | **0.0070** |
| DEXA–total fat mass (%) | −11.7 ± 13.3 | −3.8 ± 4.7 | −13.5 ± 8.0 | 0.0770 |
| DEXA–trunk fat mass (%) | −14.6 ± 13.8 | −4.0 ± 4.9 | −15.2 ± 8.9 | **0.0317** |
| DEXA–appendicular fat mass (%) | −11.1 ± 10.3**α** | −2.9 ± 5.3 | −10.2 ± 7.3 | **0.0375** |
| Mediterranean score | 71.5 ± 84.3 | 32.4 ± 64.6 | 69.6 ± 60.4 | 0.3397 |
| *Biochemistry* | | | | |
| Glucose (%) | 1.4 ± 8.1 | −1.5 ± 7.0 | −3.1 ± 4.8 | 0.2224 |
| Insulin (%) | 16.7 ± 76.8 | −2.8 ± 34.9 | −12.8 ± 33.9 | 0.3691 |
| Homa–IR (%) | 21.7 ± 108.5 | 30.6 ± 140 | 5.3 ± 64.5 | 0.8474 |
| Total cholesterol (%) | −0.6 ± 18.8 | −1.1 ± 19.3 | −6.1 ± 8.5 | 0.6472 |
| HDL cholesterol (%) | 2.5 ± 16.4 | 3.4 ± 15.6 | 0.4 ± 10.6 | 0.8830 |
| LDL cholesterol (%) | 15.8 ± 54 | 2.2 ± 53.0 | 12.6 ± 43.2 | 0.7816 |
| Tryglicerydes (%) | 2.2 ± 67.1 | −4.3 ± 36.2 | −1.6 ± 34.4 | 0.9456 |
| Muscle strength, ultrasound, and CT scans | | | | |
| Right-hand strength (%) | 1.6 ± 19.8 | 1.9 ± 6.6 | 6.3 ± 11.5 | 0.6789 |
| Left-hand strength (%) | 6.9 ± 17.1 | 7.0 ± 22.9 | 3.2 ± 22.8 | 0.8689 |
| Right quadriceps strength (%) | 5.0 ± 16.2 | 7.4 ± 21.2 | 3.8 ± 27.3 | 0.9224 |
| Left quadriceps strength (%) | −0.8 ± 11.4 | 6.1 ± 17.1 | 1.0 ± 16.5 | 0.4871 |
| US–right RFM area (%) | 5.2 ± 14.0 | 10.6 ± 12.0 | 6.9 ± 9.4 | 0.5239 |
| US–left RFM area (%) | 3.1 ± 13.7 | 7.9 ± 9.6 | 7.9 ± 10.5 | 0.4503 |
| CT–left RFM area (%) | −0.1 ± 12.5 | 4.6 ± 9.9 | 5.5 ± 14.4 | 0.4338 |
| CT–left RFM.DENSITY (%) | 2.2 ± 5.2 | −1.1 ± 6.2 | 1.2 ± 7.6 | 0.4428 |
| CT–Hepatic density (%) | 25.0 ± 45.9 | 1.8 ± 15.6 | 17.4 ± 44.4 | 0.3508 |
| CT–total abdominal fat area (%) | −18.1 ± 13.7**α** | −4.1 ± 6.7 | −15.4 ± 13.6 | **0.0215** |
| CT–abdominal visceral fat area (%) | −15.5 ± 25.0 | 1.7 ± 15.7 | −12.5 ± 28.2 | 0.1969 |
| CT–abdominal subcutaneous fat area (%) | −18.0 ± 13.0**α** | −6.9 ± 6.2 | −15.8 ± 10.6 | **0.0432** |
| Exercise tests | | | | |
| VO2 peak (%) | 8.2 ± 28.4 | 20.0 ± 32.3 | 24.6 ± 29.9 | 0.3110 |
| Maximal load (%) | 9.9 ± 37.0 | 14.9 ± 24.4 | 22.3 ± 26.8 | 0.5939 |
| Exercise time (%) | 8.6 ± 43.8 | 13.6 ± 41.4 | 9.3 ± 26.5 | 0.9435 |
| Gross work efficiency (%) | 1.5 ± 21.9 | −5.6 ± 30.4 | 6.7 ± 27.8 | 0.5328 |
| Activity energy expenditure (%) | 11.9 ± 35.6 | −4.3 ± 52.5 | 20.9 ± 45.4 | 0.5466 |
| Physical activity level (%) | 3.5 ± 7.6 | 0.9 ± 22.2 | 4.1 ± 14.8 | 0.8744 |

DEXA, dual-energy X-ray absorptiometry / RFM, rectus femoris muscle/CT, computed tomography/US, ultrasound/W, watts/J, joules. Values represent % change from baseline (means ± SD), analyzed by one-way ANOVA. Significant when $p < 0.05$–for post-hoc analysis, **α** = significantly different from EX Group.

variables did not differ between compliant and 42 excluded subjects (data not shown). As in previous studies, we observed that three short weekly sessions of HIIT attenuated calorie restriction-induced fat and lean body mass loss. Exercise is often included in lifestyle intervention programs to promote metabolic flexibility and prevent and treat metabolic diseases (37). Muscle loss associated with even a small and short-term caloric restriction must be emphasized because intermittent dieting is frequent in sedentary obese patients, increasing the risk of future sarcopenia and weight regain (38). These findings are relevant because

TABLE 3 Intergroup differences before and after the interventions.

| | MD (*n*=18) | | EX (*n*=11) | | MD+EX (*n*=12) | | *Post-hoc* |
|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | |
| Weight (Kg) | 79.9 (74.6–85.3) | 72.6 (67.5–77.8) | 79.9 (71.8–87.7) | 77 (73.7–86.2) | 77.9 (72–93.6) | 73.7 (67.8–86.4) | **α** |
| DEXA–total lean body mass (Kg) | 42 (38.1–52.3) | 40.6 (37.2–50.7) | 40.6 (39.7–57.2) | 42.8 (39.1–56.9) | 45.6 (38.6–57.2) | 45.6 (38.8–56) | # |
| DEXA–total fat mass (Kg) | 32.7 (30.6–35.7) | 29.5 (26.9–31.4) | 29.4 (28.2–33.2) | 29.3 (26.7–30.8) | 31.6 (29.4–33) | 27.2 (25.5–28.6) | **α** |
| DEXA–trunk fat mass (Kg) | 18.1 (16.2–20.2) | 16.5 (14.5–17.4) | 18.2 (14.9–18.9) | 16.5 (15.3–17.6) | 17.4 (15.9–18.9) | 14.9 (13.2–15.8) | **α** |
| DEXA-appendicular lean body mass (Kg) | 18.8 (16.7–25) | 17.9 (16.7–23.6) | 18.7 (18–22.8) | 20.1 (18–26.5) | 21 (17.2–26.4) | 20.5 (17.3–25.9) | **α** |
| DEXA-appendicular fat mass (Kg) | 12.9 (11.3–14.7) | 12.3 (9.4–13.5) | 12.5 (8.8–14.6) | 10.9 (9–13.7) | 12.7 (10.1–13.9) | 11.2 (9.3–12.3) | **α** |
| CT-total Abdominal fat area ($cm^2$) | 456.6 (442.6–550.5) | 421.3 (339.4–452.8) | 440.7 (408.1–509.5) | 402.1 (381.2–459.8) | 415.9 (381.9–483.3) | 373 (324.1–419.4) | **α** |
| CT-abdominal subcutaneous fat area ($cm^2$) | 333.5 (308.3–414.6) | 293 (239.8–351.7) | 298.2 (239.5–382.7) | 269.3 (214–329) | 303.3 (256.6–327.3) | 256.9 (199.9–292.8) | **α** |
| VO2 peak (mL/Kg/min) | 17.3 (13–21.5) | 16.9 (15.1–20.2) | 17.2 (13.8–25.6) | 24.8 (18.8–27) | 20.3 (17.2–25.5) | 27.7 (20.3–31.7) | & |
| Maximal load (W) | 135 (100–181) | 130 (110–184) | 156 (118–195) | 156 (132–183) | 160 (120.5–207) | 217 (166–243.5) | & |

DEXA, dual-energy X-ray absorptiometry / RFM, rectus femoris muscle / CT, computed tomography/ US, ultrasound / W, watts / J, joules. Values represent the median (p25-p75) at baseline and after the interventions, analyzed by mixed-effects linear regression for repeated measures method. Significant when $p < 0.05$–For *post-hoc* analysis, **α** = EX group significantly different, # = MD group significantly different, and & = MD + EX group significantly different.

we have previously detected lower muscle mass and strength in our population, even in young adults (39).

One of the main issues regarding the effectiveness of exercise and dietary treatments in patients with chronic conditions is adherence, which in our experience is around 50% (35, 40, 41). We assessed compliance with exercise by registering attendance at scheduled training sessions. As patients usually justify their lack of time, HIIT seemed to be a more time-efficient and less time-consuming strategy. However, in this new study, we observed similar retention rates, which puts pressure on the development of new strategies to achieve adherence in studies that involve efforts such as dietary changes and exercise.

Recently, the randomized PERIMED-plus trial showed that in men and women with metabolic syndrome who followed a Mediterranean diet for 12 months, 40% of the participants reported reductions in weight, waist circumference, blood pressure, and cholesterol with a low dropout rate (42), showing the effectiveness of this dietary pattern in the long term. When analyzing body composition changes by DEXA, it is evident that the hypocaloric diet (MD) induced a significantly greater loss of fat mass and a decrease in lean body mass (especially appendicular). These results were not confirmed by US or CT measurements of femoral muscle mass, suggesting that these measurements were less sensitive because they were localized to only one muscle area compared to the four extremities in DEXA. As expected, the training method employed was well tolerated. However, it did not induce changes in handgrip or quadriceps strength, as can be observed with strength training modalities mediated by mechanical loading and increased muscle protein synthesis pathways (43, 44). Exercise alone did not induce any changes in body composition or muscle parameters, unlike those reported by Blue et al. who demonstrated that 3 weeks of HIIT increased leg vastus lateralis cross-sectional area (CSA) by 14% in obese and overweight participants, but without any caloric restriction (45). However, exercise is essential for preserving muscle mass during hypocaloric diets.

Our results confirm that HIIT can preserve lean mass during a negative energy balance. Interestingly, it has been shown that the mitochondrial and myofibrillar protein synthesis responses are increased after high intensity (above 60% $watt_{max}$) when compared to low-intensity bouts of aerobic training (30% $watt_{max}$) in young men (46). This may explain why our subjects (trained at 75% $watt_{max}$) maintained their lean mass despite a diet-induced energy deficit. It is worth mentioning that the protein intake in our protocol was set at 1 g/kg body weight, which seems to be sufficient, at least for these young obese patients, to preserve muscle in this type of exercise regime with a hypocaloric MD (47).

We observed a barely significant but clinically relevant change in $VO_{2peak}$ that could be explained by factors such as an inadequate training dose (frequency, intensity, volume), insufficient to promote changes in fitness, and interindividual variability. Some investigations have demonstrated significant interindividual variability without improvements in cardiorespiratory fitness in response to typical exercise doses, attributed partly to genetic variants and exercise prescriptions (48, 49). A growing body of evidence indicates that changes following a regular training

program vary among individuals, particularly depending on initial fitness levels, which in the present study were low. Hence, not all subjects will respond in the same way to a given dose of physical training. These subjects are known as non-responders. It is likely that our prescribed amount of HIIT (a fixed workload of 75% $watt_{max}$) was insufficient to induce changes in cardiovascular fitness in most of our volunteers. It may have been more convenient to prescribe training based on respiratory thresholds, as suggested by several authors (40, 41). Thus, non-responders to training should be considered more trained according to inadequate physiological parameters (40, 41, 50) and not as refractory to exercise programs.

Surprisingly, despite a more than 5% change in body weight, a nearly 12% decrease in fat mass, and a significant reduction in abdominal fat after hypocaloric dieting, no differences were observed in metabolic and oxidative stress indicators, contradicting clinical and scientific results. It is possible that adherence to the Mediterranean dietary pattern allowed a loss in total body fat and liver fat. However, no correlation was observed between an increase in the Mediterranean Score and changes in metabolic parameters such as glucose, insulin, HOMA-IR, and lipoprotein levels. Subjects who started the intervention with two or more features of the metabolic syndrome (waist circumference, elevated triglycerides, low HDL cholesterol, or elevated HOMA-IR) behaved similarly. We do not explain these results except for the high variability of laboratory parameters (especially HOMA-IR), which could have been amplified by the small sample size. A recent meta-analysis showed that only aerobic exercise reduced insulin levels and HOMA-IR (51). Another multicenter study using HIIT showed significant changes in these parameters in prediabetic men and women (52), although employing a lower-volume, higher-intensity protocol (5-by-1 min HIT, at ~125% $VO_{2max}$ cycling intensity). In a small sample of healthy subjects, long-term endurance training reduced triglyceride, glucose, and creatinine levels and increased superoxide dismutase activity (53). Lipoprotein profiles have improved through high-intensity aerobic and moderate resistance training (54, 55). Some studies have demonstrated that HIIT induces favorable metabolic changes in overweight and obese adults, while others have shown no apparent changes (56). These differences may be attributed to patient selection and specific training modalities, among other variables.

This study has limitations: a small sample size, due to low adherence to the three interventions, despite every effort to increase it, and a short intervention period. The reduced sample also precluded the analysis of men separately from women, who may have responded differently to diet and training. Our results may not apply to other age groups or groups with a wider gender distribution. With respect to dietary prescriptions, we could not ascertain these subjects' actual protein intake because dietary recall proved limited. However, this study also has significant strengths, such as the various methods used to ensure proper body composition assessment and the measurement of several metabolic and functional parameters to detect changes induced by the interventions. Also, the exclusion of non-compliant volunteers allows for the determination of whether positive changes or a lack of effects occurred and were not a consequence of statistical error.

In conclusion, this study confirms that the best way to reduce body fat is to reduce energy intake. However, this can induce rapid muscle mass loss, which can be avoided by short sessions of HIIT. According to our results, fat mass loss seems to have a less relevant role in metabolic parameters than expected.

## 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 the Institute of Nutrition and Food Technology (INTA), Universidad de Chile. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

MM, MM-Á, TJ, and DB conceived and designed the study. MM-Á, TJ, JR, GB, CS-G, CS, and DB performed the experiments. MM-Á, DB, MM, SH, and RT analyzed the data. MM-Á, DB, RT, and MM interpreted the data. MM-Á, TJ, DB, RT, and MM drafted the manuscript, which was reviewed by all authors. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by the Chilean Agencia Nacional de Investigación y Desarrollo (ANID): MM #1130284 and MM-A #11230186.

## Acknowledgments

To Nancy Cruz, for collaborating in the recruitment of volunteers and part of the procedures carried out in the study.

## Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships construed as a potential conflict of interest.

## Publisher's note

# References


1. Mikkelsen B, Williams J, Rakovac I, Wickramasinghe K, Hennis A, Shin H-R, et al. Life course approach to prevention and control of non-communicable diseases. *BMJ*. (2019) 364:l257. doi: 10.1136/bmj.l257

2. Finkler E, Heymsfield SB, St-Onge M-P. Rate of weight loss can be predicted by patient characteristics and intervention strategies. *J Acad Nutr Diet*. (2012) 112:75–80. doi: 10.1016/j.jada.2011.08.034

3. Del Corral P, Bryan DR, Garvey WT, Gower BA, Hunter GR. Dietary adherence during weight loss predicts weight regain. *Obesity (Silver Spring)*. (2011) 19:1177–81. doi: 10.1038/oby.2010.298

4. Greenberg I, Stampfer MJ, Schwarzfuchs D, Shai IDIRECT Group. Adherence and success in long-term weight loss diets: the dietary intervention randomized controlled trial (DIRECT). *J Am Coll Nutr*. (2009) 28:159–8. doi: 10.1080/07315724.2009.10719767

5. Hall KD, Chen KY, Guo J, Lam YY, Leibel RL, Mayer LE, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Am J Clin Nutr*. (2016) 104:324–3. doi: 10.3945/ajcn.116.133561

6. Keys A. Mediterranean diet and public health: personal reflections. *Am J Clin Nutr*. (1995) 61:1321S–3S. doi: 10.1093/ajcn/61.6.1321S

7. Cava E, Yeat NC, Mittendorfer B. Preserving healthy muscle during weight loss. *Adv Nutr*. (2017) 8:511–9. doi: 10.3945/an.116.014506

8. Elia M, Stubbs RJ, Henry CJK. Differences in fat, carbohydrate, and protein metabolism between lean and obese subjects undergoing Total starvation. *Obes Res*. (1999) 7:597–4. doi: 10.1002/j.1550-8528.1999.tb00720.x

9. McCarthy D, Berg A. Weight loss strategies and the risk of skeletal muscle mass loss. *Nutrients*. (2021) 13:2473. doi: 10.3390/nu13072473

10. Willis LH, Slentz CA, Bateman LA, Shields AT, Piner LW, Bales CW, et al. Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults. *J Appl Physiol (1985)*. (2012) 113:1831–7. doi: 10.1152/japplphysiol.01370.2011

11. Miller W, Koceja D, Hamilton E. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int J Obes*. (1997) 21:941–7. doi: 10.1038/sj.ijo.0800499

12. Pontzer H, Durazo-Arvizu R, Dugas LR, Plange-Rhule J, Bovet P, Forrester TE, et al. Constrained Total energy expenditure and metabolic adaptation to physical activity in adult humans. *Curr Biol*. (2016) 26:410–7. doi: 10.1016/j.cub.2015.12.046

13. Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The role of exercise and physical activity in weight loss and maintenance. *Prog Cardiovasc Dis*. (2014) 56:441–7. doi: 10.1016/j.pcad.2013.09.012

14. Melby CL, Paris HL, Foright RM, Peth J. Attenuating the biologic drive for weight regain following weight loss: must what Goes down always go Back up? *Nutrients*. (2017) 9:E468. doi: 10.3390/nu9050468

15. Mandsager K, Harb S, Cremer P, Phelan D, Nissen SE, Jaber W. Association of Cardiorespiratory Fitness with Long-term Mortality among Adults Undergoing Exercise Treadmill Testing. *JAMA Netw Open*. (2018) 1:e183605. doi: 10.1001/jamanetworkopen.2018.3605

16. Hassan Y, Head V, Jacob D, Bachmann MO, Diu S, Ford J. Lifestyle interventions for weight loss in adults with severe obesity: a systematic review. *Clin Obes*. (2016) 6:395–3. doi: 10.1111/cob.12161

17. Hunter GR, Plaisance EP, Carter SJ, Fisher G. Why intensity is not a bad word: optimizing health status at any age. *Clin Nutr*. (2018) 37:56–60. doi: 10.1016/j.clnu.2017.02.004

18. Ross R, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, et al. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med*. (2000) 133:92–3. doi: 10.7326/0003-4819-133-2-200007180-00008

19. Tan R, Nederveen JP, Gillen JB, Joanisse S, Parise G, Tarnopolsky MA, et al. Skeletal muscle fiber-type-specific changes in markers of capillary and mitochondrial content after low-volume interval training in overweight women. *Physiol Rep*. (2018) 6:e13597. doi: 10.14814/phy2.13597

20. Kong Z, Fan X, Sun S, Song L, Shi Q, Nie J. Comparison of high-intensity interval training and moderate-to-vigorous continuous training for Cardiometabolic health and exercise enjoyment in obese young women: a randomized controlled trial. *PLoS One*. (2016) 11:e0158589. doi: 10.1371/journal.pone.0158589

21. Mangiamarchi P, Caniuqueo A, Ramírez-Campillo R, Cárdenas P, Morales S, Cano-Montoya J, et al. Effects of high-intensity interval training and nutritional education in patients with type 2 diabetes. *Rev Med Chil*. (2017) 145:845–3. doi: 10.4067/s0034-98872017000700845

22. Wewege M, van den Berg R, Ward RE, Keech A. The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. *Obes Rev*. (2017) 18:635–6. doi: 10.1111/obr.12532

23. Honkala SM, Motiani KK, Eskelinen J-J, Savolainen A, Saunavaara V, Virtanen KA, et al. Exercise training reduces Intrathoracic fat regardless of defective glucose tolerance. *Med Sci Sports Exerc*. (2017) 49:1313–22. doi: 10.1249/MSS.0000000000001232

24. Schmitt J, Lindner N, Reuss-Borst M, Holmberg H-C, Sperlich B. A 3-week multimodal intervention involving high-intensity interval training in female cancer survivors: a randomized controlled trial. *Physiol Rep*. (2016) 4:e12693. doi: 10.14814/phy2.12693

25. De la Maza Cave MP. Reducción Sérica De Productos Finales De Glicación Avanzada Séricos. *Nutricion Hospitalaria*. (2015) 6:2511–7. doi: 10.3305/nh.2015.31.6.8936

26. Gillen JB, Percival ME, Ludzki A, Tarnopolsky MA, Gibala MJ. Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women. *Obesity (Silver Spring)*. (2013) 21:2249–55. doi: 10.1002/oby.20379

27. Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc*. (2010) 110:911–916.e12. doi: 10.1016/j.jada.2010.03.018

28. Echeverría G, Urquiaga I, Concha MJ, Dussaillant C, Villarroel L, Velasco N, et al. Validación de cuestionario autoaplicable para un índice de alimentación mediterránea en Chile. *Rev Méd Chile*. (2016) 144:1531–43. doi: 10.4067/S0034-98872016001200004

29. Gajardo H, Barrera G. Quality control of bone densitometry: precision, reproducibility, and clinical application. *Rev Med Chil*. (1998) 126:56–62.

30. Berger J, Bunout D, Barrera G, de la Maza MP, Henriquez S, Leiva L, et al. Rectus femoris (RF) ultrasound for the assessment of muscle mass in older people. *Arch Gerontol Geriatr*. (2015) 61:33–8. doi: 10.1016/j.archger.2015.03.006

31. Bunout D, Barrera G, Leiva L, Gattas V, de la Maza MP, Avendaño M, et al. Effects of vitamin D supplementation and exercise training on physical performance in Chilean vitamin D deficient elderly subjects. *Exp Gerontol*. (2006) 41:746–52. doi: 10.1016/j.exger.2006.05.001

32. Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, et al. Clinician's guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation*. (2010) 122:191–5. doi: 10.1161/CIR.0b013e3181e52e69

33. Moseley L, Jeukendrup AE. The reliability of cycling efficiency. *Med Sci Sports Exerc*. (2001) 33:621–7. doi: 10.1097/00005768-200104000-00017

34. Davidian M, Giltinan DM. Analysis of repeated measurement data using the nonlinear mixed effects model. *Chemom Intell Lab Syst*. (1993) 20:1–24. doi: 10.1016/0169-7439(93)80017-C

35. Henríquez S, Monsalves-Alvarez M, Jimenez T, Barrera G, Hirsch S, de la Maza MP, et al. Effects of two training modalities on body fat and insulin resistance in postmenopausal women. *J Strength Cond Res*. (2017) 31:2955–64. doi: 10.1519/JSC.0000000000002089

36. Tieland M, Trouwborst I, Clark BC. Skeletal muscle performance and ageing: skeletal muscle performance and ageing. *J Cachexia Sarcopenia Muscle*. (2018) 9:3–19. doi: 10.1002/jcsm.12238

37. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metabol*. (2001) 86:5755–61. doi: 10.1210/jcem.86.12.8075

38. Johnstone AM. Fasting – the ultimate diet? *Obes Rev*. (2007) 8:211–2. doi: 10.1111/j.1467-789X.2006.00266.x

39. Wigodski S, Carrasco F, Bunout D, Barrera G, Hirsch S, de la Maza MP. Sarcopenia: the need to establish different cutoff points of fat-free mass for the Chilean population. *Nutrition*. (2019) 57:217–4. doi: 10.1016/j.nut.2018.05.031

40. Bunout D, Barrera G, Avendaño M, de la Maza P, Gattas V, Leiva L, et al. Results of a community-based weight-bearing resistance training programme for healthy Chilean elderly subjects. *Age Ageing*. (2005) 34:80–3. doi: 10.1093/ageing/afi005

41. Bonafiglia JT, Rotundo MP, Whittall JP, Scribbans TD, Graham RB, Gurd BJ. Inter-individual variability in the adaptive responses to endurance and Sprint interval training: a randomized crossover study. *PLoS One*. (2016) 11:e0167790. doi: 10.1371/journal.pone.0167790

42. Sayón-Orea C, Razquin C, Bulló M, Corella D, Fitó M, Romaguera D, et al. Effect of a nutritional and behavioral intervention on energy-reduced Mediterranean diet adherence among patients with metabolic syndrome: interim analysis of the PREDIMED-plus randomized clinical trial. *JAMA*. (2019) 322:1486–99. doi: 10.1001/jama.2019.14630

43. Schoenfeld BJ. The mechanisms of muscle hypertrophy and their application to resistance training. *J Strength Cond Res*. (2010) 24:2857–72. doi: 10.1519/JSC.0b013e3181e840f3

44. Blue MNM, Smith-Ryan AE, Trexler ET, Hirsch KR. The effects of high intensity interval training on muscle size and quality in overweight and obese adults. *J Sci Med Sport*. (2018) 21:207–2. doi: 10.1016/j.jsams.2017.06.001

45. Di Donato DM, West DWD, Churchward-Venne TA, Breen L, Baker SK, Phillips SM. Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery. *Am J Physiol Endocrinol Metab*. (2014) 306:E1025–32. doi: 10.1152/ajpendo.00487.2013

46. Weatherwax RM, Harris NK, Kilding AE, Dalleck LC. The incidence of training responsiveness to cardiorespiratory fitness and cardiometabolic measurements following individualized and standardized exercise prescription: study protocol for a randomized controlled trial. *Trials*. (2016) 17:601. doi: 10.1186/s13063-016-1735-0

47. Campbell WW, Kim JE, Amankwaah AF, Gordon SL, Weinheimer-Haus EM. Higher Total protein intake and change in Total protein intake affect body composition but not metabolic syndrome indexes in middle-aged overweight and obese adults who perform resistance and aerobic exercise for 36 weeks. *J Nutr*. (2015) 145:2076–83. doi: 10.3945/jn.115.213595

48. Pickering C, Kiely J. Do non-responders to exercise exist-and if so, what should we do about them? *Sports Med*. (2019) 49:1–7. doi: 10.1007/s40279-018-01041-1

49. Zapata-Lamana R, Henríquez-Olguín C, Burgos C, Meneses-Valdés R, Cigarroa I, Soto C, et al. Effects of polarized training on Cardiometabolic risk factors in young overweight and obese women: a randomized-controlled trial. *Front Physiol*. (2018) 9:1287. doi: 10.3389/fphys.2018.01287

50. Marson EC, Delevatti RS, Prado AKG, Netto N, Kruel LFM. Effects of aerobic, resistance, and combined exercise training on insulin resistance markers in overweight or obese children and adolescents: a systematic review and meta-analysis. *Prev Med*. (2016) 93:211–8. doi: 10.1016/j.ypmed.2016.10.020

51. Phillips BE, Kelly BM, Lilja M, Ponce-González JG, Brogan RJ, Morris DL, et al. A practical and time-efficient high-intensity interval training program modifies cardio-metabolic risk factors in adults with risk factors for type II diabetes. *Front Endocrinol (Lausanne)*. (2017) 8:229. doi: 10.3389/fendo.2017.00229

52. Gagnon DD, Dorman S, Ritchie S, Mutt SJ, Stenbäck V, Walkowiak J, et al. Multi-day prolonged low- to moderate-intensity endurance exercise mimics training improvements in metabolic and oxidative profiles without concurrent chromosomal changes in healthy adults. *Front Physiol*. (2019) 10:1123. doi: 10.3389/fphys.2019.01123

53. Ghamarchehreh ME, Shamsoddini A, Alavian SM. Investigating the impact of eight weeks of aerobic and resistance training on blood lipid profile in elderly with non-alcoholic fatty liver disease: a randomized clinical trial. *Gastroenterol Hepatol Bed Bench*. (2019) 12:190–6.

54. Mann S, Beedie C, Jimenez A. Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendations. *Sports Med*. (2014) 44:211–1. doi: 10.1007/s40279-013-0110-5

55. Vella CA, Taylor K, Drummer D. High-intensity interval and moderate-intensity continuous training elicit similar enjoyment and adherence levels in overweight and obese adults. *Eur J Sport Sci*. (2017) 17:1203–11. doi: 10.1080/17461391.2017.1359679

56. Batacan RB, Duncan MJ, Dalbo VJ, Tucker PS, Fenning AS. Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies. *Br J Sports Med*. (2017) 51:494–3. doi: 10.1136/bjsports-2015-095841

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 27 July 2023
DOI 10.3389/fnut.2023.1203669



OPEN ACCESS

EDITED BY
Mauro Serafini,
University of Teramo, Italy

REVIEWED BY
Manuela Di Lauro,
University of Rome Tor Vergata, Italy
Lina Ilaras D'Apolito,
University of Rome Tor Vergata, Italy

*CORRESPONDENCE
Kunio Miyake
✉ kmiyake@yamanashi.ac.jp

RECEIVED 11 April 2023
ACCEPTED 20 June 2023
PUBLISHED 27 July 2023

CITATION
Miyake K, Horiuchi S, Shinohara R, Kushima M, Otawa S, Yui H, Akiyama Y, Ooka T, Kojima R, Yokomichi H, Mochizuki K, Yamagata Z and The Japan Environment Children's Study Group (2023) Maternal dietary fiber intake during pregnancy and child development: the Japan Environment and Children's Study.
*Front. Nutr.* 10:1203669.
doi: 10.3389/fnut.2023.1203669



# Maternal dietary fiber intake during pregnancy and child development: the Japan Environment and Children's Study

Kunio Miyake[1]*, Sayaka Horiuchi[2], Ryoji Shinohara[2], Megumi Kushima[2], Sanae Otawa[2], Hideki Yui[1], Yuka Akiyama[1], Tadao Ooka[1], Reiji Kojima[1], Hiroshi Yokomichi[1], Kazuki Mochizuki[3], Zentaro Yamagata[1,2] and The Japan Environment Children's Study Group

[1]Department of Health Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan, [2]Center for Birth Cohort Studies, University of Yamanashi, Chuo, Japan, [3]Department of Local Produce and Food Sciences, Faculty of Life and Environmental Sciences, University of Yamanashi, Kofu, Japan

**Background:** Animal studies have shown that maternal low-fiber diets during pregnancy may impair brain development and function in offspring, but this has not been validated by epidemiological studies. The aim of this study was to investigate the link between maternal dietary fiber intake during pregnancy and neurodevelopmental delay in offspring using a large birth cohort.

**Methods:** A total of 76,207 mother-infant pairs were analyzed using data from the Japan Environment and Children's Study, a nationwide prospective cohort study. Maternal dietary fiber intake was estimated using the food frequency questionnaire in mid-pregnancy. Maternal dietary fiber intake was adjusted for energy and classified into quintiles. Developmental delay was assessed in five domains using the Japanese version of the Ages and Stages Questionnaire, Third Edition at the age of 3 years. The logistic regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (CI) for the link between dietary fiber intake during pregnancy and developmental delay at the age of 3 years.

**Results:** The lowest intake group of total dietary fiber had a higher risk of delayed communication [adjusted OR (aOR), 1.51; 95% CI, 1.32–1.74], fine motor (aOR, 1.45; 95% CI, 1.32–1.61), problem-solving (aOR, 1.46; 95% CI, 1.32–1.61), and personal-social skills (aOR, 1.30; 95% CI, 1.12–1.50) than did the highest intake group. An analysis that excluded the effects of insufficient folic acid intake during pregnancy also showed a similar trend.

**Conclusion:** This study showed that maternal dietary fiber deficiency during pregnancy might influence an increased risk of neurodevelopmental delay in offspring.

KEYWORDS

**DOHaD (Developmental Origins of Health and Disease), dietary fiber, birth cohort, FFQ food frequency questionnaire, ASQ-3, gut microbiome**

# 1. Introduction

The Developmental Origins of Health and Disease concept (DOHaD) has proposed that various environmental factors (e.g., undernutrition, stress, and chemical exposures) during the fetal period and infancy are linked with increased risk of several diseases (e.g., obesity, diabetes, allergic diseases, and neurodevelopmental diseases) later in life (1, 2). The first 1,000 days of life, from conception to a child's second birthday, are considered particularly important for child development. Intervention programs are being implemented in developing countries to improve malnutrition (3). Maternal malnutrition during pregnancy is a serious problem even in developed countries. In Japan, according to the Dietary Reference Intakes for Japanese (2020 version), the intake of several nutrients (e.g., dietary fiber, vitamin C, folic acid, and iron) is well-below the requirements for pregnant women (4). In young women, underweight, diet orientation, low-protein diet, and high-fat diet are also problems (5).

Dietary fiber is defined as edible carbohydrate polymers that are not decomposed by human digestive enzymes, and are classified into soluble and insoluble fiber depending on the difference in water solubility. Epidemiological studies have shown that increasing dietary fiber consumption is linked to a lower risk of various chronic diseases, including type 2 diabetes (6), cancer (7), and cardiovascular disease (8). Animal studies have reported that a high-fiber diet during pregnancy is linked to improved immune system development, decreased metabolic syndrome, and decreased cognitive and social dysfunction caused by maternal obesity in offspring (9–11). Another animal study also showed that a low-fiber diet during pregnancy impairs brain nerve function in offspring (12). However, no epidemiological studies have confirmed a link between maternal dietary fiber intake during pregnancy and neurodevelopment in offspring. Therefore, we investigated whether maternal dietary fiber intake during pregnancy was linked to child developmental delay at the age of 3 years.

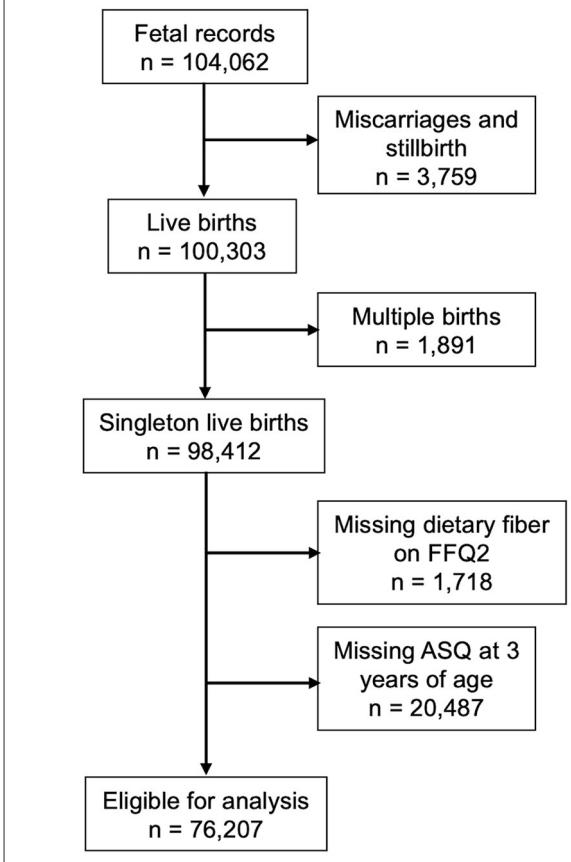



FIGURE 1
Flow chart of the participants.

# 2. Materials and methods

## 2.1. Study setting and population

The Japan Environment and Children's Study (JECS) is an ongoing nationwide prospective birth cohort study. The detailed protocols have been published elsewhere (13, 14). Participants were recruited between January 2011 and March 2014 in 15 Regional Centers covering 19 prefectures across Japan. We used the dataset jecs-ta-20190930-qsn, which was first released in October 2019 and completed in March 2022. We eliminated 3,759 miscarriages or stillbirths and 1,891 multiple pregnancies from a total of 104,062 fetal records. In addition, 1,718 and 20,487 were not included in this study because Food Frequency Questionnaire (FFQ) in mid-pregnancy and the Japanese translations of Ages and Stages Questionnaires, Third Edition (J-ASQ-3) at 3 years, respectively, had missing data. Finally, 76,207 mother-child pairs were analyzed in this study (Figure 1).

The JECS protocol was reviewed and approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies and the Ethics Committees of all participating institutions (ethical approval number: 100910001). The JECS was conducted following the Declaration of Helsinki, and written informed consent was obtained from all the participants.

## 2.2. Dietary information

Dietary information was obtained using the FFQ, which is a validated self-administered dietary questionnaire evaluated in previous studies (15). Respondents were asked about their dietary status from conception to the answering date Respondents were asked about their dietary status during the second/third trimester. The food and nutrient intakes were calculated using the FFQ items based on the 2010 edition of the Standardized Tables of Food Composition in Japan. Energy adjustment for nutrients was conducted using the nutrient density model (per 1,000 kcal) (16). Participants were categorized into quintiles based on dietary fiber intake. The lowest and highest quintiles were Q1 and Q5, respectively.

To analyze the effects of folic acid intake, we used data on dietary folic acid intake and the frequency of folic acid supplement use during the second and third trimesters. Based on the estimated mean folic acid requirement for pregnant Japanese women (400 μg/day) (4), we divided the population into two

groups. Mothers answered about folate supplement intake during pregnancy by choosing frequency from never, once a month, 2–3 times a month, 1–3 times a week, 4–6 times a week, once a day, and twice or more times a day. Furthermore, we recategorized it into three groups: none, occasional (less than six times per week), and daily (more than once a day).

## 2.3. Outcome definitions

Developmental delays at the age of 3 years were assessed using the ASQ-3, which was included in the questionnaire sent when the child was 3 years old. Parents or caregivers filled out the ASQ-3 developmental screening questionnaire (17). The ASQ-3 comprised 30 questions across the following five domains: communication, gross motor, fine motor, problem-solving, and personal-social skills. A respondent chose yes, sometimes, or no for each question and scored 10 for yes, 5 for sometimes, and 0 for no. The J-ASQ-3, which was used in this study, has been validated using benchmark scores for Japanese children (17). The child was determined to have a developmental delay in that domain if their score fell below the cutoff in each domain.

## 2.4. Covariates

Covariates were selected a priori based on previous studies (18–20). Information on maternal smoking and drinking during pregnancy, annual household income (million yen), maternal educational level, maternal age at birth, and older siblings was collected from mothers in the first and second/third trimesters of pregnancy using a self-administered questionnaire. Child sex, pre-pregnancy body mass index (BMI), mode of delivery, birth weight, and the gestational week at birth were extracted from medical record transcripts. Preterm birth was defined as delivery at <37 weeks gestation. Maternal age at birth was classified as <25, 25–29, 30–34, and ≥35 years. Pre-pregnancy BMI was categorized into three groups: underweight (<18.5 kg/m$^2$), normal (18.5–24.9 kg/m$^2$), and overweight (≥25 kg/m$^2$). Information on childcare facility attendance, caretaker other than the child's mother, and breastfeeding was collected from the self-questionnaire administered to mothers when their children were 1 year old. In this questionnaire, the mother was asked, "I love this child", to which she chose "Strongly agree"; "Somewhat agree"; "Somewhat disagree"; or "Strongly disagree". We re-categorized the responses into two categories: agree and disagree.

The Kessler Psychological Distress Scale (K6) score was extracted from a self-questionnaire during the second trimester and when the child reached 1 year of age. Scores above the cutoff points of K6 (≥5 points) have been reported to indicate poor mental health in Japanese (21).

## 2.5. Statistical analyses

The logistic regression analysis was used to estimate the crude and adjusted odds ratios (cOR and aOR, respectively), as well as

TABLE 1 Characteristics of the study population.

| Variable | Mean ± SD or *N* (%) |
|---|---|
| **Intake of dietary fiber during mid-pregnancy (g/1,000 kcal/day)** | |
| Total dietary fiber | 6.18 ± 2.04 |
| Soluble dietary fiber | 1.50 ± 0.58 |
| Insoluble dietary fiber | 4.45 ± 1.40 |
| **Folic acid diet during mid-pregnancy (μg/day)** | 259.48 ± 158.09 |
| <400 | 67,257 (88.3) |
| ≥400 | 8,950 (11.7) |
| **Folic acid supplement during pregnancy** | |
| None | 39,116 (51.3) |
| Occasional | 15,318 (20.1) |
| Daily | 15,976 (21.0) |
| Missing data | 5,797 (7.6) |
| **Maternal age at birth (years)** | |
| <25 | 6,277 (8.2) |
| 25–29 | 20,681 (27.1) |
| 30–34 | 27,674 (36.3) |
| ≥35 | 21,574 (28.3) |
| Missing data | 1 (0.0) |
| **Maternal smoking status during pregnancy** | |
| No | 67,717 (88.9) |
| Yes | 2,457 (3.2) |
| Missing data | 6,033 (7.9) |
| **Maternal drinking during pregnancy** | |
| No | 67,561 (88.7) |
| Yes | 1,834 (2.4) |
| Missing data | 6,812 (8.9) |
| **Pre-pregnancy BMI (kg/m$^2$)** | |
| <18.5 | 12,392 (16.3) |
| 18.5–25 | 56,307 (73.9) |
| >25 | 7,470 (9.8) |
| Missing data | 38 (0.0) |
| **Maternal education level (years)** | |
| ≤12 | 23,374 (30.7) |
| >12 | 46,942 (61.6) |
| Missing data | 5,930 (7.8) |
| **Annual household income (millions of yen)** | |
| <4 | 24,895 (32.7) |
| ≥4 | 41,240 (54.1) |
| Missing data | 10,072 (13.2) |
| **Birth weight (g)** | |
| <2,500 | 5,966 (7.8) |

*(Continued)*

10.3389/fnut.2023.1203669

TABLE 1 (Continued)

| Variable | Mean ± SD or *N* (%) |
|---|---|
| ≥2,500 | 70,033 (91.9) |
| Missing data | 208 (0.3) |
| **Gestational age at birth (week)** | |
| ≥37 | 72,667 (95.4) |
| <37 | 3,378 (4.4) |
| Missing data | 162 (0.2) |
| **Mode of delivery** | |
| Vaginal | 61,877 (81.2) |
| Cesarean section | 14,011 (18.4) |
| Missing data | 319 (0.4) |
| **Child sex** | |
| Male | 39,053 (51.2) |
| Female | 37,154 (48.8) |
| **Older siblings** | |
| No | 35,211 (46.2) |
| Yes | 40,736 (53.5) |
| Missing data | 260 (0.3) |
| **Mother's K6 scores during pregnancy** | |
| 0–4 points | 55,215 (72.5) |
| ≥5 points | 20,832 (27.3) |
| Missing data | 160 (0.2) |
| **Mother's K6 scores when her child was 1 year of age** | |
| 0–4 points | 58,645 (77.0) |
| ≥5 points | 16,091 (21.1) |
| Missing data | 1,471 (1.9) |
| **Attendance to nursery school at 1 year of age** | |
| No | 55,268 (72.5) |
| Yes | 19,296 (25.3) |
| Missing data | 1,643 (2.2) |
| **Breastfeeding at 1 year of age** | |
| No | 28,799 (37.8) |
| Yes | 45,625 (59.9) |
| Missing data | 1,783(2.3) |
| **Caretaker other than the child's mother when her child was 1 year of age** | |
| No | 2,953 (3.9) |
| Yes | 68,400 (89.8) |
| Missing data | 4,854 (6.4) |
| **Attachment bond when her child was 1 year of age** | |
| Strongly agree | 71,271 (93.5) |
| Somewhat agree | 3,324 (4.4) |
| Somewhat disagree | 87 (0.1) |
| Strongly disagree | 30 (0.0) |
| Missing data | 1,495(2.0) |
| **ASQ score at age 3 years** | |
| **Communication skill** | |
| ≥29.95 | 73,082 (95.9) |
| <29.95 | 3,125 (4.1) |
| **Gross motor skill** | |
| ≥39.26 | 72,680 (95.4) |
| <39.26 | 3,527 (4.6) |
| **Fine motor skill** | |
| ≥27.91 | 70,341 (92.3) |
| <27.91 | 5,866 (7.7) |
| **Problem-solving skill** | |
| ≥30.03 | 70,511 (92.5) |
| <30.03 | 5,696 (7.5) |
| **Personal-social skill** | |
| ≥29.89 | 73,613 (96.6) |
| <29.89 | 2,594 (3.4) |

*(Continued)*

TABLE 1 (Continued)

ASQ, Ages and Stages Questionnaires; BMI, body mass index; SD, standard deviation.

the 95% confidence interval (CI), for the link between dietary fiber intake during pregnancy and developmental delay in children at the age of 3 years. Multivariable models were adjusted for pre-pregnancy BMI, maternal education level, maternal smoking and drinking during pregnancy, annual household income, older siblings, maternal age at birth, gestational age at birth, birth weight, child's sex, attendance at a childcare facility at the age of 1 year, and breastfeeding until the age of 1 year, maternal mental health during pregnancy and when her child was 1 year of age, caretaker other than the child's mother at 1 year of age, and attachment bond when her child was 1 year of age. Missing values were excluded from the multivariate analysis. A population with sufficient folic acid intake was used for sensitivity analysis. Furthermore, the median value of each intake quintile was used to calculate P for the trend. A $P = 0.05$ (two-sided) was considered significant. All statistical analyses were performed using the Statistical Package for the Social Sciences software version 27.

## 3. Results

The characteristics of the mothers and their children are presented in Table 1. Mean [± standard deviation (SD)] intakes of total, soluble, and insoluble dietary fiber (g/1,000 kcal/day) during mid-pregnancy were 6.18 ± 2.04, 1.50 ± 0.58, and 4.45 ± 1.40, respectively. The numbers of children aged 3 years, whose scores fell below the threshold for each J-ASQ-3 category were as follows:

10.3389/fnut.2023.1203669

3,125 (4.1%) for communication, 3,527 (4.6%) for gross motor, 5,866 (7.7%) for fine motor, 5,696 (7.5%) for problem-solving, and 2,594 (3.4%) for personal-social skills.

Table 2 shows the association between maternal total dietary fiber intake during mid-pregnancy and developmental delay in children at the age of 3 years. In comparison to the highest intake group, the lower intake groups of total dietary fiber had a higher risk of children showing developmental delays in four domains. For instance, the lowest intake group of total dietary fiber had the following aOR and 95% CI: communication (aOR, 1.51; 95% CI, 1.32–1.74; $P$ for trend <0.001), fine motor (aOR, 1.45; 95% CI, 1.32–1.61; $P$ for trend <0.001), problem-solving (aOR, 1.46; 95% CI, 1.32–1.61; $P$ for trend <0.001), and personal-social skills (aOR, 1.30; 95% CI, 1.12–1.50; $P$ for trend <0.001).

Table 3 shows the association between maternal intake of soluble and insoluble dietary fiber during mid-pregnancy and child developmental delay at age 3. Similar to the results for total dietary fiber, both low-fiber groups were linked to a higher risk of child developmental delay in four domains, excluding gross motor skill. For gross motor skills, the lower soluble dietary fiber groups had a higher risk of child developmental delay than did the groups with highest soluble dietary fiber. (Q1: aOR, 1.15; 95% CI, 1.02–1.31; Q2: aOR, 1.18; 95% CI, 1.04–1.33; Q3: aOR, 1.16; 95% CI, 1.02–1.31; Q4: aOR, 1.16; 95% CI, 1.02–1.31; $P$ for trend = 0.016).

We also analyzed the association between maternal total dietary fiber intake during mid-pregnancy and child developmental delay at the age of 3 years, stratified by pre-pregnancy BMI (Table 4). In the normal BMI group, similar to the results presented in Table 2, a lower total dietary fiber intake was associated with a significantly higher risk of developmental delay at the age of 3 years in four domains (communication, fine motor, problem-solving, and personal-social skills). In the underweight BMI group, the lowest intake groups of total dietary fiber had a significantly higher risk of developmental delay in communication (Q1: aOR, 1.12; 95% CI, 1.07–2.15) and problem-solving (Q1: aOR, 1.50; 95% CI, 1.14–1.97) compared that of the group with the highest intake. In the overweight BMI group, the lowest intake groups of total dietary fiber had a significantly higher risk of developmental delay in communication (Q1: aOR, 1.64; 95% CI, 1.12–2.38), fine motor (Q1: aOR, 1.51; 95% CI, 1.15–2.00), and problem-solving skills (Q1: aOR, 1.59; 95% CI, 1.20–2.11) compared with that of group with the highest intake.

To exclude the effects of folic acid intake during pregnancy, we selected mothers who consumed more than 400 μg/day of dietary folic acid ($n$ = 8,950) or used folic acid supplements daily ($n$ = 15,976) during pregnancy. Table 5 shows the results of mothers who consumed more than 400 μg/day of dietary folic acid at mid-pregnancy. For communication, the lower intake groups of total dietary fiber had a higher risk of child developmental delay at the age of 3 years (Q1: aOR, 1.62; 95% CI, 1.05–2.50; $P$ for trend = 0.004). For personal social skills, the second highest dietary fiber group had a lower risk of child developmental delay than did the highest dietary fiber group (Q4: aOR, 0.59; 95% CI, 0.36–0.96). Table 6 shows the results of mothers who used folic acid supplements daily during pregnancy. The group with the lowest total dietary fiber had a higher risk of child developmental delay at the age of 3 years in four domains than did the highest intake group: communication (Q1: aOR, 1.65; 95% CI, 1.23–2.19; $P$ for

TABLE 2 Association between intake of total dietary fiber on mid-pregnancy and development delay at the age of 3 years.

| Subscales in ASQ-3 | Communication | | Gross motor | | Fine motor | | Problem solving | | Personal-social | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) |
| Total dietary fiber (g/1,000 kcal/day) | | | | | | | | | | |
| Q5 (≥7.56) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (6.39–<7.56) | **1.14 (1.01–1.29)** | **1.19 (1.04–1.37)** | 0.99 (0.89–1.10) | 1.03 (0.91–1.17) | 1.09 (1.00–1.19) | **1.11 (1.01–1.23)** | 1.04 (0.95–1.14) | 1.07 (0.97–1.19) | 1.11 (0.98–1.27) | 1.13 (0.97–1.30) |
| Q3 (5.51–<6.39) | **1.26 (1.12–1.42)** | **1.31 (1.15–1.51)** | 1.05 (0.94–1.16) | 1.10 (0.97–1.24) | **1.17 (1.07–1.28)** | **1.17 (1.06–1.29)** | **1.21 (1.11–1.32)** | **1.23 (1.12–1.36)** | 1.12 (0.98–1.27) | 1.11 (0.96–1.29) |
| Q2 (4.62–<5.51) | **1.37 (1.22–1.54)** | **1.41 (1.23–1.61)** | 1.03 (0.93–1.15) | 1.07 (0.95–1.21) | **1.25 (1.15–1.37)** | **1.24 (1.12–1.37)** | **1.26 (1.16–1.38)** | **1.30 (1.17–1.44)** | **1.21 (1.07–1.37)** | **1.26 (1.09–1.46)** |
| Q1 (<4.62) | **1.48 (1.32–1.66)** | **1.51 (1.32–1.74)** | 1.07 (0.96–1.19) | 1.13 (1.00–1.28) | **1.50 (1.38–1.64)** | **1.45 (1.32–1.61)** | **1.43 (1.31–1.55)** | **1.46 (1.32–1.61)** | **1.33 (1.17–1.51)** | **1.30 (1.12–1.50)** |
| $P$ for trend | **<0.001** | **<0.001** | 0.186 | **0.043** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |

cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; ASQ-3, Ages and Stages Questionnaires, Third Edition. Boldface indicates significance ($p < 0.05$).
Adjusted for maternal smoking and drinking during pregnancy, maternal age at birth, pre-pregnancy BMI, maternal and paternal education levels, annual household income, child sex, mode of delivery, birthweight, gestational age at birth, older siblings, attendance a childcare facility at 1 year of age, breastfeeding until 1 year of age, mother's K6 scores during pregnancy and when her child was 1 year of age, caretaker other than the child's mother at 1 year of age, and attachment bond when her child was 1 year of age.

10.3389/fnut.2023.1203669

TABLE 3 Association between intake of soluble and insoluble dietary fiber on mid-pregnancy and development delay at the age of 3 years.

| Subscales in ASQ-3 | Communication | Gross motor | Fine motor | Problem solving | Personal-social |
|---|---|---|---|---|---|
| | aOR (95% CI) | aOR (95% CI) | aOR (95% CI) | aOR (95% CI) | aOR (95% CI) |
| Soluble dietary fiber (g/1,000 kcal/day) | | | | | |
| Q5 (≥1.89) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (1.55–<1.89) | **1.17 (1.02–1.34)** | **1.16 (1.02–1.31)** | 1.07 (0.96–1.18) | 1.05 (0.95–1.17) | 1.11 (0.96–1.28) |
| Q3 (1.31–<1.55) | **1.24 (1.08–1.42)** | **1.16 (1.02–1.31)** | **1.11 (1.00–1.23)** | **1.21 (1.09–1.34)** | 1.15 (1.00–1.33) |
| Q2 (1.06–<1.31) | **1.43 (1.26–1.64)** | **1.18 (1.04–1.33)** | **1.23 (1.11–1.36)** | **1.32 (1.19–1.46)** | **1.26 (1.09–1.45)** |
| Q1 (<1.06) | **1.49 (1.30–1.70)** | **1.15 (1.02–1.31)** | **1.36 (1.23–1.50)** | **1.40 (1.27–1.55)** | **1.22 (1.05–1.41)** |
| *P* for trend | **<0.001** | **0.016** | **<0.001** | **<0.001** | **0.002** |
| Insoluble dietary fiber (g/1,000 kcal/day) | | | | | |
| Q5 (≥5.41) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (4.60–<5.41) | **1.17 (1.02–1.35)** | 0.99 (0.88–1.12) | 1.10 (0.99–1.21) | 1.03 (0.93–1.14) | 1.07 (0.92–1.24) |
| Q3 (4.00–<4.60) | **1.33 (1.16–1.53)** | **1.15 (1.02–1.29)** | **1.16 (1.05–1.29)** | **1.22 (1.11–1.35)** | 1.12 (0.96–1.29) |
| Q2 (3.38–<4.00) | **1.41 (1.23–1.61)** | 1.04 (0.92–1.17) | **1.24 (1.12–1.37)** | **1.30 (1.17–1.43)** | **1.22 (1.06–1.41)** |
| Q1 (<3.38) | **1.51 (1.31–1.73)** | 1.12 (0.99–1.27) | **1.45 (1.32–1.61)** | **1.45 (1.31–1.60)** | **1.29 (1.11–1.49)** |
| *P* for trend | **<0.001** | 0.055 | **<0.001** | **<0.001** | **<0.001** |

aOR, adjusted odds ratio; CI, confidence interval; ASQ-3, Ages and Stages Questionnaires, Third Edition. Boldface indicates significance ($p < 0.05$).
Adjusted for maternal smoking and drinking during pregnancy, maternal age at birth, pre-pregnancy BMI, maternal and paternal education levels, annual household income, child sex, mode of delivery, birthweight, gestational age at birth, older siblings, attendance a childcare facility at 1 year of age, breastfeeding until 1 year of age, mother's K6 scores during pregnancy and when her child was 1 year of age, caretaker other than the child's mother at 1 year of age, and attachment bond when her child was 1 year of age.

trend <0.001), fine motor skill (Q1: aOR, 1.54; 95% CI, 1.25–1.91; *P* for trend <0.001), problem-solving (Q1: aOR, 1.54; 95% CI, 1.25–1.90; *P* for trend <0.001), and personal-social skills (Q1: aOR, 1.44; 95% CI, 1.07–1.93; *P* for trend = 0.008).

## 4. Discussion

Using a large birth cohort, we clarified for the first time the relationship between reduced maternal dietary fiber intake during pregnancy and increased neurodevelopmental risk in offspring, which had previously been reported in animal studies. Even after taking folic acid intake into account, there was a significant link between maternal dietary fiber intake during pregnancy and child developmental delay at the age of 3 years. Assessments at baseline and 1 year after study began showed that the study population was representative of the general population in Japan (14, 22), suggesting that the results of this study may be generalized.

The dietary reference intakes of the United States and Canada indicate that 28 g of total dietary fiber per day (14 g/1,000 kcal/day) is adequate for pregnant women (23). According to the dietary reference intake for Japanese (2020), the tentative dietary fiber intake goal for pregnant women is calculated to be 18 g or more per day (4). The Dietary Intake Standards for Japanese (2020 edition) recommend that pregnant women consume at least 18 g of dietary fiber per day. In this study, the mean (± SD) of maternal dietary fiber intake during pregnancy was 10.77 (± 6.30) g per day, with only 6,391 mothers (8.4%) consuming more than 18 g per day. Therefore, we used the group with the highest dietary fiber intake as a reference. The lower the dietary fiber intake, the higher the odds ratio for child developmental delay at the age of 3 years.

Animal studies have shown that offspring born to mothers fed a low-fiber diet during pregnancy are at a higher risk for metabolic syndrome, characterized by obesity and impaired glucose tolerance (9). In contrast, maternal high-fiber diets have been demonstrated to reduce cognitive and social-behavioral dysfunction in offspring caused by maternal obesity (10). This study corroborates findings from animal studies, and extends them by demonstrating the link between maternal dietary fiber intake during pregnancy and the risk of neurodevelopmental delay in offspring.

The primary mechanism by which dietary fiber acts is by regulating the gut microbiome. Murine studies have demonstrated a link between the dysbiosis of the maternal gut microbiome during pregnancy and abnormal neurodevelopmental and behavior in offspring (24, 25). In addition, an Australian birth cohort study recently found a link between maternal gut microbiota composition during pregnancy and behavioral abnormalities at age two (26). Specifically, recent studies have demonstrated that short-chain fatty acids (SCFAs) produced by bacterial fermentation of dietary fiber are important for gut microbiota-brain crosstalk (27, 28). Short-chain fatty acids such as acetate, propionate, and butyrate are known to modulate sympathetic nervous system activation (9) and affect brain function and behavior (12, 29). Our results suggest that maternal inadequate dietary fiber intake during pregnancy affected child neurodevelopmental delay through decreased production of SCFAs by gut bacterial fermentation of dietary fiber. Soluble and insoluble dietary fiber had similar effects on the four ASQ-3 subscales (communication, fine motor, problem-solving, and personal-social). However, only soluble dietary fiber was

10.3389/fnut.2023.1203669

TABLE 4 Association between total dietary fiber intake on mid-pregnancy and development delay at the age of 3 years, stratified by pre-pregnancy BMI.

| Subscales in ASQ-3 | Communication | Gross motor | Fine motor | Problem solving | Personal-social |
|---|---|---|---|---|---|
| | aOR (95% CI) | aOR (95% CI) | aOR (95% CI) | aOR (95% CI) | aOR (95% CI) |
| Pre-pregnancy BMI <18.5 (kg/m$^2$) | | | | | |
| Q5 (≥7.56) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (6.39–<7.56) | 1.10 (0.77–1.57) | 0.90 (0.67–1.22) | 0.96 (0.75–1.24) | 1.25 (0.95–1.65) | 0.96 (0.66–1.40) |
| Q3 (5.51–<6.39) | 1.07 (0.75–1.53) | 1.14 (0.86–1.51) | 0.93 (0.72–1.20) | 1.35 (1.03–1.76) | 0.91 (0.63–1.32) |
| Q2 (4.62–<5.51) | 1.20 (0.85–1.71) | 1.11 (0.83–1.48) | 1.08 (0.84–1.38) | 1.52 (1.16–1.98) | 0.98 (0.67–1.42) |
| Q1 (<4.62) | **1.52 (1.07–2.15)** | 1.03 (0.76–1.40) | 1.09 (0.84–1.40) | **1.50 (1.14–1.97)** | 1.16 (0.80–1.68) |
| *P* for trend | **0.022** | 0.467 | 0.419 | **0.001** | 0.564 |
| Pre-pregnancy BMI 18.5–25 (kg/m$^2$) | | | | | |
| Q5 (≥7.56) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (6.39–<7.56) | **1.21 (1.03–1.42)** | 1.05 (0.91–1.21) | **1.15 (1.02–1.30)** | 1.04 (0.92–1.17) | 1.19 (1.00–1.41) |
| Q3 (5.51–<6.39) | **1.32 (1.13–1.55)** | 1.06 (0.92–1.22) | **1.23 (1.09–1.39)** | **1.23 (1.09–1.38)** | **1.22 (1.03–1.45)** |
| Q2 (4.62–<5.51) | **1.43 (1.22–1.68)** | 1.04 (0.90–1.21) | **1.30 (1.15–1.47)** | **1.28 (1.14–1.44)** | **1.39 (1.17–1.65)** |
| Q1 (<4.62) | **1.50 (1.28–1.77)** | 1.11 (0.96–1.29) | **1.53 (1.36–1.72)** | **1.42 (1.26–1.60)** | **1.39 (1.16–1.65)** |
| *P* for trend | **<0.001** | 0.199 | **<0.001** | **<0.001** | **<0.001** |
| Pre-pregnancy BMI >25 (kg/m$^2$) | | | | | |
| Q5 (≥7.56) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (6.39–<7.56) | 1.20 (0.80–1.80) | 1.15 (0.77–1.71) | 1.10 (0.81–1.49) | 1.08 (0.80–1.48) | 1.03 (0.69–1.54) |
| Q3 (5.51–<6.39) | **1.66 (1.13–2.45)** | 1.36 (0.92–2.00) | 1.19 (0.88–1.60) | 1.14 (0.84–1.55) | 0.83 (0.54–1.26) |
| Q2 (4.62–<5.51) | **1.57 (1.07–2.31)** | 1.24 (0.84–1.82) | 1.09 (0.81–1.47) | 1.16 (0.86–1.56) | 1.02 (0.69–1.52) |
| Q1 (<4.62) | **1.64 (1.12–2.38)** | 1.39 (0.96–2.02) | **1.51 (1.15–2.00)** | **1.59 (1.20–2.11)** | 1.08 (0.73–1.58) |
| *P* for trend | **0.004** | 0.080 | **0.005** | **0.001** | 0.745 |

aOR, adjusted odds ratio; CI, confidence interval; ASQ-3, Ages and Stages Questionnaires, Third Edition. Boldface indicates significance ($p < 0.05$).
Adjusted for maternal smoking and drinking during pregnancy, maternal age at birth, maternal and paternal education levels, annual household income, child sex, mode of delivery, birthweight, gestational age at birth, older siblings, attendance a childcare facility at 1 year of age, breastfeeding until 1 year of age, mother's K6 scores during pregnancy and when her child was 1 year of age, caretaker other than the child's mother at 1 year of age, and attachment bond when her child was 1 year of age.

found to significantly increase the risk of child developmental delay in gross motor. Soluble dietary fiber is known to affect the regulation of gut microbiota and the production of SCFAs compared to that by insoluble dietary fiber (30). Thus, maternal soluble fiber intake during pregnancy may have significantly impacted child development.

The gut microbiota composition of pregnant women with overweight is known to differ from that of pregnant women with normal weight (31, 32). The gut microbiota of women with maternal pre-pregnancy overweight has also been shown to influence the gut microbiota composition of their offspring (33, 34). In recent years, it has been clarified that the gut microbiota may influence the association between maternal overweight and neurodevelopmental abnormalities in children (35–37). Therefore, to exclude the effects of maternal overweight, we investigated the association between total dietary fiber intake and developmental delay at the age of 3 years, stratified by pre-pregnancy BMI. Our results showed that even in the normal weight group, lower dietary fiber intake during pregnancy was associated with an increased risk of child developmental delay in four ASQ-3 subscales (communication, fine motor, problem-solving, and personal-social). These results strengthen the evidence that inadequate intake of dietary fiber during pregnancy affects neurodevelopmental delay in offspring. Therefore, future studies should elucidate the molecular mechanisms associated with maternal dietary fiber intake during pregnancy and neurodevelopment of the offspring by analyzing the gut microbiota and its metabolites.

Grains, beans, potatoes, vegetables, fruits, mushrooms, and seaweed are known as food groups rich in dietary fiber (38). Therefore, our results may reflect the effects of other nutrients that are highly correlated with dietary fiber intake. Folic acid deficiency during early pregnancy is known to be associated with an increased risk of neural tube defects and neurodevelopmental disorders, such as autism (39, 40). A JECS study found a link between dietary folic acid intake by pregnant women and child's verbal cognitive development at 2 years of age (41). According to the dietary reference intakes for Japanese (2020), the estimated average folic acid requirement for pregnant women is 400 μg per day (4). However, only 8,950 (11.7%) pregnant women in this study satisfied the requirements for dietary folic acid intake. In fact, when examining the association between intake of dietary fiber

TABLE 5 Association between intake of total dietary fiber and development delay at the age of 3 years in mothers with folic acid intake above the reference value (400 μg/day) during pregnancy.

| Subscales in ASQ-3 | Communication | | Gross motor | | Fine motor | | Problem solving | | Personal-social | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) |
| Total dietary fiber (g/1,000 kcal/day) | | | | | | | | | | |
| Q5 (≥9.93) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (8.33–<9.93) | 1.14 (0.76–1.73) | 0.97 (0.60–1.56) | 0.93 (0.67–1.30) | 0.97 (0.66–1.42) | **0.76 (0.57–0.99)** | 0.74 (0.54–1.02) | 1.05 (0.80–1.39) | 1.03 (0.75–1.43) | 0.71 (0.47–1.08) | **0.59 (0.36–0.96)** |
| Q3 (7.11–<8.33) | 1.43 (0.97–2.13) | 1.38 (0.89–2.15) | 1.14 (0.83–1.56) | 1.22 (0.84–1.76) | 0.88 (0.68–1.15) | 0.88 (0.65–1.21) | 1.11 (0.85–1.46) | 1.15 (0.84–1.58) | 1.06 (0.72–1.55) | 0.94 (0.61–1.45) |
| Q2 (5.78–<7.11) | **1.68 (1.14–2.46)** | **1.66 (1.08–2.54)** | 1.11 (0.81–1.52) | 1.30 (0.91–1.87) | 1.22 (0.95–1.56) | 1.23 (0.92–1.64) | 1.26 (0.96–1.64) | **1.43 (1.05–1.94)** | 1.02 (0.69–1.50) | 0.95 (0.61–1.45) |
| Q1 (<5.78) | **1.73 (1.18–2.53)** | **1.62 (1.05–2.50)** | 1.14 (0.83–1.56) | 1.25 (0.86–1.81) | **1.34 (1.05–1.70)** | 1.33 (1.00–1.78) | **1.34 (1.03–1.75)** | 1.31 (0.95–1.79) | 1.14 (0.78–1.66) | 1.07 (0.69–1.64) |
| *P* for trend | **0.001** | **0.004** | 0.264 | 0.096 | **0.001** | **0.005** | **0.012** | **0.021** | 0.257 | 0.442 |

cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; ASQ-3, Ages and Stages Questionnaires, Third Edition. Boldface indicates significance ($p < 0.05$).
Adjusted for maternal smoking and drinking during pregnancy, maternal age at birth, pre-pregnancy BMI, maternal and paternal education levels, annual household income, child sex, mode of delivery, birthweight, gestational age at birth, older siblings, attendance a childcare facility at 1 year of age, breastfeeding until 1 year of age, mother's K6 scores during pregnancy and when her child was 1 year of age, caretaker other than the child's mother at 1 year of age, and attachment bond when her child was 1 year of age.

TABLE 6 Association between intake of total dietary fiber and development delay at the age of 3 years in mothers who took daily folic acid supplements during pregnancy.

| Subscales in ASQ-3 | Communication | | Gross motor | | Fine motor | | Problem solving | | Personal-social | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) | cOR (95% CI) | aOR (95% CI) |
| Total dietary fiber (g/1,000 kcal/day) | | | | | | | | | | |
| Q5 (≥7.86) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q4 (6.62–<7.86) | 1.25 (0.96–1.62) | 1.32 (0.98–1.72) | 1.02 (0.81–1.29) | 1.00 (0.76–1.26) | 1.09 (0.90–1.33) | 1.09 (0.88–1.36) | 1.16 (0.95–1.41) | 1.16 (0.94–1.44) | 1.17 (0.90–1.54) | 1.15 (0.85–1.56) |
| Q3 (5.74–<6.62) | **1.40 (1.08–1.81)** | **1.45 (1.09–1.94)** | **1.29 (1.03–1.61)** | **1.34 (1.05–1.72)** | **1.40 (1.16–1.68)** | **1.39 (1.12–1.72)** | **1.31 (1.06–1.55)** | **1.25 (1.01–1.55)** | 1.24 (0.95–1.61) | 1.23 (0.91–1.66) |
| Q2 (4.84–<5.74) | **1.53 (1.19–1.97)** | **1.63 (1.23–2.17)** | 1.12 (0.89–1.40) | 1.15 (0.89–1.48) | **1.32 (1.09–1.59)** | **1.37 (1.10–1.70)** | **1.30 (1.09–1.60)** | **1.31 (1.05–1.62)** | 1.30 (1.00–1.69) | **1.35 (1.00–1.82)** |
| Q1 (<4.84) | **1.47 (1.14–1.89)** | **1.65 (1.23–2.19)** | 1.09 (0.87–1.38) | 1.12 (0.86–1.45) | **1.52 (1.26–1.83)** | **1.54 (1.25–1.91)** | **1.52 (1.26–1.83)** | **1.54 (1.25–1.90)** | **1.45 (1.12–1.88)** | **1.44 (1.07–1.93)** |
| *P* for trend | **0.001** | **<0.001** | 0.264 | 0.192 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.004** | **0.008** |

cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; ASQ-3, Ages and Stages Questionnaires, Third Edition. Boldface indicates significance ($p < 0.05$).
Adjusted for maternal smoking and drinking during pregnancy, maternal age at birth, pre-pregnancy BMI, maternal and paternal education levels, annual household income, child sex, mode of delivery, birthweight, gestational age at birth, older siblings, attendance a childcare facility at 1 year of age, breastfeeding until 1 year of age, mother's K6 scores during pregnancy and when her child was 1 year of age, caretaker other than the child's mother at 1 year of age, and attachment bond when her child was 1 year of age.



10.3389/fnut.2023.1203669

and various nutrients, folic acid showed the highest correlation ($r = 0.865$). To eliminate the effect of folic acid, we analyzed the link between maternal dietary fiber intake during pregnancy and child developmental delay at the age of 3 years in a group of pregnant women with folic acid intake above the reference value. As a result, similar effects were observed in communication, fine motor, and problem-solving. In fine motor and personal-social, the group with the second highest dietary fiber intake (Q2) had a reduced odds ratio compared with the group with the highest dietary fiber intake (Q1), which may reflect the effect of excessive dietary fiber intake. Therefore, supplementation is recommended since it is difficult to obtain sufficient folic acid from food (4). Our results also showed a link between maternal dietary fiber intake during pregnancy and child developmental delay, in an analysis of a population of mothers taking daily folic acid supplements. Many of the folic acid supplements for pregnant women sold in Japan also contain various vitamins, minerals, iron, and calcium. Therefore, it is conceivable that mothers who take folic acid supplements appropriately also take sufficient nutrients necessary for pregnant women. The results of these sensitivity analyses strengthen the evidence that lower maternal dietary fiber intake during pregnancy is linked to a higher risk of child neurodevelopmental delay. Therefore, it may be necessary to recommend dietary fiber intake through supplementation in pregnant women.

The strength of this study is its large sample size and adjustment for many potential confounders. Our results provided reinforcing evidence for the DOHaD concept that undernutrition during pregnancy is associated with an increased risk of neurodevelopmental delay in children. Nevertheless, this study had some limitations. First, human studies cannot assess the effects of dietary fiber alone. Although this study considered the impact of folic acid intake during pregnancy, the possibility of other nutrients having an impact cannot be completely ruled out. In addition, dietary fiber intake from supplements could not be investigated. Therefore, further intervention studies are needed to clarify the direct impact. Second, because this study used a self-administered questionnaire, it is possible that personal understanding of the questions influenced responses. Third, the FFQ we used has not been validated in pregnant women and may be misclassified. Finally, regarding the nutritional environment after childbirth, breastfeeding was adjusted; however, baby food and early childhood diet were not considered.

This study indicates that maternal dietary fiber deficiency during pregnancy may contribute to a higher risk of neurodevelopmental delay in offspring. Furthermore, most pregnant women in Japan consume far less dietary fiber than what is the recommended intake; thereby, this maternal nutritional imbalance during pregnancy may adversely affect the neurodevelopment of their offspring. Therefore, nutritional guidance for pregnant mothers is crucial to reduce the risk of future health problems for their children.

## Data availability statement

The datasets presented in this article are not readily available because data are unsuitable for public deposition due to ethical restrictions and legal framework of Japan. It is prohibited by the Act on the Protection of Personal Information (Act No. 57 of 30 May 2003, amendment on 9 September 2015) to publicly deposit the data containing personal information. Ethical Guidelines for Medical and Health Research Involving Human Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare also restricts the open sharing of the epidemiologic data. All inquiries about access to data should be sent to: jecs-en@nies.go.jp. The person responsible for handling enquiries sent to this e-mail address is Dr. Shoji F. Nakayama, JECS Programme Office, National Institute for Environmental Studies. Requests to access the datasets should be directed to Dr. Shoji F. Nakayama, jecs-en@nies.go.jp/b.

## Ethics statement

The studies involving human participants were reviewed and approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies and the Ethics Committees. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## Author contributions

KMi conceived and designed the study, drafted the initial manuscript, and revised the manuscript. SH, RS, MK, SO, ZY, and the JECS Group collected data and critically reviewed and revised the manuscript. HYu, YA, TO, RK, HYo, and KMo critically reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

## Group members of The Japan Environment and Children's Study Group

Members of the JECS Group as of 2022: Michihiro Kamijima (principal investigator, Nagoya City University, Nagoya, Japan); Shin Yamazaki (National Institute for Environmental Studies, Tsukuba, Japan); Yukihiro Ohya (National Center for Child Health and Development, Tokyo, Japan); Reiko Kishi (Hokkaido University, Sapporo, Japan); Nobuo Yaegashi (Tohoku University, Sendai, Japan); Koichi Hashimoto (Fukushima Medical University, Fukushima, Japan); Chisato Mori (Chiba University, Chiba, Japan); Shuichi Ito (Yokohama City University, Yokohama, Japan); Zentaro Yamagata (Universit y of Yamanashi, Chuo, Japan); Hidekuni Inadera (University of Toyama, Toyama, Japan); Takeo Nakayama (Kyoto University, Kyoto, Japan); Tomotaka Sobue (Osaka University, Suita, Japan); Masayuki Shima (Hyogo Medical University, Nishinomiya, Japan); Hiroshige Nakamura (Tottori University, Yonago, Japan); Narufumi Suganuma (Kochi University, Nankoku, Japan); Koichi Kusuhara (University of Occupational and Environmental

Health, Kitakyushu, Japan); and Takahiko Katoh (Kumamoto University, Kumamoto, Japan).

## Funding

This study was funded by the Ministry of the Environment, Japan. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the above government.

## Acknowledgments

We thank all the participants and co-operating health care providers for their contribution to the JECS.

## 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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

1. Hoffman DJ, Powell TL, Barrett ES, Hardy DB. Developmental origins of metabolic diseases. *Physiol Rev.* (2021) 101:739–95. doi: 10.1152/physrev.00002.2020

2. Tran NQV, Miyake K. Neurodevelopmental disorders and environmental toxicants: Epigenetics as an underlying mechanism. *Int J Genom.* (2017) 2017:7526592. doi: 10.1155/2017/7526592

3. Mameli C, Mazzantini S, Zuccotti GV. Nutrition in the first 1000 days: the origin of childhood obesity. *Int J Environ Res Public Health.* (2016) 13:838. doi: 10.3390/ijerph13090838

4. Ministry of Health, Labour and Welfare. *Dietary Reference Intakes for Japanese.* (2020). Available online at: https://www.mhlw.go.jp/content/10900000/000862500.pdf (accessed October 21, 2022).

5. Gluckman PD, Seng CY, Fukuoka H, Beedle AS, Hanson MA. Low birthweight and subsequent obesity in Japan. *Lancet.* (2007) 369:1081–2. doi: 10.1016/S0140-6736(07)60524-8

6. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med.* (2000) 342:1392–8. doi: 10.1056/NEJM200005113421903

7. Chen K, Zhao Q, Li X, Zhao J, Li P, Lin S, et al. Dietary fiber intake and endometrial cancer risk: a systematic review and meta-analysis. *Nutrients.* (2018) 10:945. doi: 10.3390/nu10070945

8. Veronese N, Solmi M, Caruso MG, Giannelli G, Osella AR, Evangelou E, et al. Dietary fiber and health outcomes: an umbrella review of systematic reviews and meta-analyses. *Am J Clin Nutr.* (2018) 107:436–44. doi: 10.1093/ajcn/nqx082

9. Kimura I, Miyamoto J, Ohue-Kitano R, Watanabe K, Yamada T, Onuki M, et al. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science.* (2020) 367:eaaw8429. doi: 10.1126/science.aaw8429

10. Liu X, Li X, Xia B, Jin X, Zou Q, Zeng Z, et al. High-fiber diet mitigates maternal obesity-induced cognitive and social dysfunction in the offspring via gut-brain axis. *Cell Metab.* (2021) 33:923–38.e6. doi: 10.1016/j.cmet.2021.02.002

11. Nakajima A, Habu S, Kasai M, Okumura K, Ishikawa D, Shibuya T, et al. Impact of maternal dietary gut microbial metabolites on an offspring's systemic immune response in mouse models. *Biosci Microbiota Food Health.* (2020) 39:33–8. doi: 10.12938/bmfh.19-013

12. Yu L, Zhong X, He Y, Shi Y. Butyrate, but not propionate, reverses maternal diet-induced neurocognitive deficits in offspring. *Pharmacol Res.* (2020) 160:105082. doi: 10.1016/j.phrs.2020.105082

13. Kawamoto T, Nitta H, Murata K, Toda E, Tsukamoto N, Hasegawa M, et al. Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health.* (2014) 14:25. doi: 10.1186/1471-2458-14-25

14. Michikawa T, Nitta H, Nakayama SF, Yamazaki S, Isobe T, Tamura K, et al. Baseline profile of participants in the Japan Environment and Children's Study (JECS). *J Epidemiol.* (2018) 28:99–104. doi: 10.2188/jea.JE20170018

15. Yokoyama Y, Takachi R, Ishihara J, Ishii Y, Sasazuki S, Sawada N, et al. Validity of short and long self-administered food frequency questionnaires in ranking dietary intake in middle-aged and elderly Japanese in the Japan public health center-based prospective study for the next generation (JPHC-NEXT) protocol area. *J Epidemiol.* (2016) 26:420–32. doi: 10.2188/jea.JE20150064

16. Tomova GD, Arnold KF, Gilthorpe MS, Tennant PWG. Adjustment for energy intake in nutritional research: a causal inference perspective. *Am J Clin Nutr.* (2022) 115:189–98. doi: 10.1093/ajcn/nqab266

17. Mezawa H, Aoki S, Nakayama SF, Nitta H, Ikeda N, Kato K, et al. Psychometric profile of the Ages and Stages Questionnaires, Japanese translation. *Pediatr Int.* (2019) 61:1086–95. doi: 10.1111/ped.13990

18. Black MM, Walker SP, Fernald LCH, Andersen CT, DiGirolamo AM, Lu C, et al. Early childhood development coming of age: science through the life course. *Lancet.* (2017) 389:77–90. doi: 10.1016/S0140-6736(16)31389-7

19. Kushima M, Kojima R, Shinohara R, Horiuchi S, Otawa S, Ooka T, et al. Association between screen time exposure in children at 1 year of age and autism spectrum disorder at 3 years of age: the Japan environment and children's study. *JAMA Pediatr.* (2022) 176:384–91. doi: 10.1001/jamapediatrics.2021.5778

20. Kobayashi S, Itoh S, Miyashita C, Ait Bamai Y, Yamaguchi T, Masuda H, et al. Impact of prenatal exposure to mercury and selenium on neurodevelopmental delay in children in the Japan environment and Children's study using the ASQ-3 questionnaire: a prospective birth cohort. *Environ Int.* (2022) 168:107448. doi: 10.1016/j.envint.2022.107448

21. Furukawa TA, Kawakami N, Saitoh M, Ono Y, Nakane Y, Nakamura Y, et al. The performance of the Japanese version of the K6 and K10 in the World Mental Health Survey Japan. *Int J Methods Psychiatr Res.* (2008) 17:152–8. doi: 10.1002/mpr.257

22. Michikawa T, Nitta H, Nakayama SF, Ono M, Yonemoto J, Tamura K, et al. The Japan Environment and Children's Study (JECS): a preliminary report on selected characteristics of approximately 10 000 pregnant women recruited during the first year of the study. *J Epidemiol.* (2015) 25:452–8. doi: 10.2188/jea.JE20140186

23. Turner ND, Lupton JR. Dietary fiber. *Adv Nutr.* (2011) 2:151–2. doi: 10.3945/an.110.000281

24. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature.* (2017) 549:528–32. doi: 10.1038/nature23910

25. Kim E, Paik D, Ramirez RN, Biggs DG, Park Y, Kwon HK, et al. Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4+ T cells. *Immunity.* (2022) 55:145–58.e7. doi: 10.1016/j.immuni.2021.11.005

26. Dawson SL, O'Hely M, Jacka FN, Ponsonby AL, Symeonides C, Loughman A, et al. Maternal prenatal gut microbiota composition predicts child behaviour. *Ebiomedicine.* (2021) 68:103400. doi: 10.1016/j.ebiom.2021.103400

27. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The microbiota-gut-brain axis. *Physiol Rev.* (2019) 99:1877–2013. doi: 10.1152/physrev.00018.2018

28. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol.* (2019) 16:461–78. doi: 10.1038/s41575-019-0157-3

29. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol.* (2020) 11:25. doi: 10.3389/fendo.2020.00025

30. Guan ZW Yu EZ, Feng Q. Soluble dietary fiber, one of the most important nutrients for the gut microbiota. *Molecules.* (2021) 26:6802. doi: 10.3390/molecules26226802

31. Santacruz A, Collado MC, García-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr.* (2010) 104:83–92. doi: 10.1017/S0007114510000176

32. Comert TK, Akpinar F, Erkaya S, Durmaz B, Durmaz R. The effect of pre-pregnancy obesity on gut and meconium microbiome and relationship with fetal growth. *Matern Fetal Neonatal Med.* (2022) 35:10629–37. doi: 10.1080/14767058.2022.2148098

33. Dreisbach C, Prescott S, Alhusen J. Influence of maternal prepregnancy obesity and excessive gestational weight gain on maternal and child gastrointestinal microbiome composition: A systematic review. *Biol Res Nurs.* (2020) 22:114–25. doi: 10.1177/1099800419880615

34. Singh SB, Madan J, Coker M, Hoen A, Baker ER, Karagas MR, et al. Does birth mode modify associations of maternal pre-pregnancy BMI and gestational weight gain with the infant gut microbiome? *Int J Obes.* (2020) 44:23–32. doi: 10.1038/s41366-018-0273-0

35. Guzzardi MA, Ederveen THA, Rizzo F, Weisz A, Collado MC, Muratori F, et al. Maternal pre-pregnancy overweight and neonatal gut bacterial colonization are associated with cognitive development and gut microbiota composition in pre-school-age offspring. *Brain Behav Immun.* (2022) 100:311–20. doi: 10.1016/j.bbi.2021.12.009

36. Basak S, Das RK, Banerjee A, Paul S, Pathak S, Duttaroy AK. Maternal obesity and gut microbiota are associated with fetal brain development. *Nutrients.* (2022) 14:4515. doi: 10.3390/nu14214515

37. Nieto-Ruiz A, Cerdo T, Jordano B, Torres-Espinola FJ, Escudero-Marín M, Garcia-Ricobaraza M, et al. Maternal weight, gut microbiota, and the association with early childhood behavior: the PREOBE follow-up study. *Child Adolesc Psychiatry Ment Health.* (2023) 17:41. doi: 10.1186/s13034-023-00589-9

38. Dhingra D, Michael M, Rajput H, Patil RT. Dietary fibre in foods: a review. *J Food Sci Technol.* (2012) 49:255–66. doi: 10.1007/s13197-011-0365-5

39. Czeizel AE, Dudas I, Vereczkey A, Bánhidy F. Folate deficiency and folic acid supplementation: the prevention of neural-tube defects and congenital heart defects. *Nutrients.* (2013) 5:4760–75. doi: 10.3390/nu5114760

40. Hoxha B, Hoxha M, Domi E, Gervasoni J, Persichilli S, Malaj V, et al. Folic acid and autism: a systematic review of the current state of knowledge. *Cells.* (2021) 10:1976. doi: 10.3390/cells10081976

41. Suzuki T, Nishigori T, Obara T, Masumoto T, Mori M, Murata T, et al. Maternal folic acid supplement use/dietary folate intake from preconception to early pregnancy and neurodevelopment in 2-year-old offspring: The Japan Environment and Children's Study. *Br J Nutr.* (2022) 128:2480–9. doi: 10.1017/S000711452200037X

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 10 August 2023
DOI 10.3389/fnut.2023.1226891



OPEN ACCESS

EDITED BY
Giulia Marrone,
University of Rome Tor Vergata, Italy

REVIEWED BY
Alicia Ruiz,
University of Granada, Spain
Giulia Cafiero,
Bambino Gesù Children's Hospital (IRCCS), Italy

*CORRESPONDENCE
Pasqualina Buono
✉ pasqualina.buono@uniparthenope.it

RECEIVED 26 May 2023
ACCEPTED 28 July 2023
PUBLISHED 10 August 2023

CITATION
Mancini A, Cerulli C, Vitucci D, Lasorsa VA, Parente D, Di Credico A, Orrù S, Brustio PR, Lupo C, Rainoldi A, Schena F, Capasso M and Buono P (2023) Impact of active lifestyle on the primary school children saliva microbiota composition.
*Front. Nutr.* 10:1226891.
doi: 10.3389/fnut.2023.1226891



# Impact of active lifestyle on the primary school children saliva microbiota composition

Annamaria Mancini[1,2], Claudia Cerulli[3], Daniela Vitucci[1,2], Vito Alessandro Lasorsa[2], Daniela Parente[2,4], Andrea Di Credico[5,6], Stefania Orrù[1,2], Paolo Riccardo Brustio[7], Corrado Lupo[8], Alberto Rainoldi[8], Federico Schena[9], Mario Capasso[2,4] and Pasqualina Buono[1,2]*

[1]Department of Movement Sciences and Wellness, University Parthenope, Naples, Italy, [2]CEINGE-Biotecnologie Avanzate "Franco Salvatore", Napoli, Italy, [3]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy, [4]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy, [5]Reprogramming and Cell Differentiation Lab, Center for Advanced Studies and Technology (CAST), Chieti, Italy, [6]Department of Medicine and Aging Sciences, University "G. D'Annunzio" of Chieti-Pescara, Chieti, Italy, [7]Department of Clinical and Biological Sciences, University of Torino, Turin, Italy, [8]Department of Medical Sciences, University of Torino, Turin, Italy, [9]Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona, Italy

The aim of the study was to evaluate the effects of Active or Sedentary lifestyle on saliva microbiota composition in Italian schoolchildren.

**Methods:** Male (114) and female children (8–10 years) belonging to five primary schools in the neighborhoods of Turin were classified as active (A) or sedentary (S) based on PAQ-C-It questionnaire. PCR amplification of salivary DNA targeted the hypervariable V3–V4 regions of the 16S rRNA bacterial genes. DADA2 workflow was used to infer the Amplicon Sequence Variants and the taxonomic assignments; the beta-diversity was obtained by PCoA with the UniFrac method; LEfSe algorithm, threshold at 5%, and Log LDA cutoff at ±0.5 were used to identify differently abundant species in A compared to S saliva sample. Daily food intake was assessed by 3-Days food record. The metabolic potential of microbial communities was assessed by PICRUSt.

**Results:** No significant differences were found in individual's gender distribution ($p = 0.411$), anthropometry, BMI ($p > 0.05$), and all diet composition between A and S groups ($p > 0.05$). Eight species were differently abundant: *Prevotella nigrescens* (LDA score = –3.76; FDR = 1.5×10–03), *Collinsella aerofaciens* (LDA score = –3.17; FDR = 7.45×10–03), *Simonsiella muelleri* (LDA score = –2.96; FDR = 2.76×10–05), *Parabacteroides merdae* (LDA score = –2.43; FDR = 1.3×10–02) are enriched in the A group; *Gemella parahaemolysans*, *Prevotella aurantiaca* (LDA score = –3.9; FDR = 5.27×10–04), *Prevotella pallens* (LDA score = 4.23; FDR = 1.93×10–02), *Neisseria mucosa* (LDA score = 4.43; FDR = 1.31×10–02; LDA score = 2.94; FDR = 7.45×10–03) are enriched in the S group. A prevalence of superpathway of fatty acid biosynthesis initiation (*E. coli*) and catechol degradation II (meta-cleavage pathway) was found in saliva from A compared to S children.

**Conclusion:** Our results showed that active children had an enrichment of species and genera mainly associated with a healthier profile. By contrast, the genera and the species enriched in the sedentary group could be linked to human diseases.

KEYWORDS

**lifestyle, exercise, saliva, microbiota, children**

# 1. Introduction

The human gut microbiota is intimately associated with different aspects of human health and disease. Its characterization could help diagnosis, prognosis, and therapy settings by giving over 150 times more genetic information than the human genome alone (1). The microbiota composition depends on spatial distribution and age; in general, the microbiota diversity increases over the time and decreases in elderly (2, 3). In children of about 3 years, gut microbiota becomes similar to that of adults, with five predominant bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia (4). Recent findings established the role of environmental factors on microbiota composition suggesting a close cross-talk between the lifestyle and the diversity of microorganisms populating the intestine (5). To date, the effects of exercise on human health have been thoroughly studied. In the last decade, many evidences supported a dynamic relationship between the composition of gut microbiota (GM) and physical activity levels in animal models (6–10) and in human (11–14). In particular, the theory that exercise is able to enrich the diversity of the human gut microbiota depending on the volume of training resulting in improved health status of the host, has been supported (12, 15). In particular, GM diversity has been associated to cardiorespiratory fitness (11, 16, 17) and to VO2max in adults (18). Moreover, Barton et al. (18) highlighted in GM, by a metagenomic and metabolomic approach, relative increases in fecal pathways and metabolites, e.g., short-chain fatty acids (SCFAs) produced by microbes, associated with higher muscle turnover and overall health in professional athletes compared with sedentary controls.

While in adults there are some evidences of the influence of physical activity on GM composition, in children or pre-adolescents, very poor results have been provided. Recent reports indicated that the GM profile is associated with the body mass index and could be modulated by exercise training and lifestyle habits in obese children (19–22). Furthermore, several evidences show that the salivary microbiota mirrors the gut microbiota and that some oral bacteria colonize the gut and have been associated both to oral and systemic health. (23–26).

Despite these recent advances, the complete landscape of the association between the saliva profile and lifestyle habits in children is still to be clarified. Further, no data on saliva microbiota composition have been provided in Italian schoolchildren associated to Active compared to Sedentary status, to date. Thus, the principal aim of this study was to analyse the possible association between saliva microbiota compositions and lifestyle in Active compared to Sedentary cohort of 8–10-year-old Italian school-aged children living in the neighborhoods of Turin (northwest Italy).

We conducted this study by hypothesizing that active lifestyle could be associated with saliva microbiota profiles contributing to host health promotion. Indeed, the main aim of our work was to identify the differences in the saliva of Active compared to Sedentary schoolchildren. In order to study the microbiota composition, we sequenced the bacterial 16S rRNA of saliva biospecimens and assessed their differential abundance.

# 2. Materials and methods

## 2.1. Participants

One hundred and thirty children (8–10 years) belonging to five primary schools in the neighborhoods of Turin (northwest Italy) were enrolled. All information on the aim of the study has been provided to children's parents/guardians and teachers as previously described (27).

Children meeting any of the following criteria were excluded from the study: (i) recent infections (1 month prior to sample collection), (ii) having disorders affecting diet or physical activity, and (iii) recent usage of either antibiotic, prebiotics and probiotics supplements (1 month prior to data and sample collection). The enrolled children were classified in two groups: active (A) and sedentary (S) on the basis of Physical Activity Questionnaire for Older Children (PAQ-C-It cut-off score of 2.75), the related procedures are detailed in Lupo et al. (27). Parents/guardians and teachers provided written informed consent for participation to the study, according to the ethical standards provided in the 1964 Declaration of Helsinki. Ethics committee on human research of the University of Turin (9 March 2020: Protocol #134691) and Naples (17 January 2020: Protocol #376/19) approved the study. The procedures used to take anthropometric measures were described in Lupo et al. (27); briefly, stature was measured by a portable stadiometer (Model 214; Seca, Hamburg, Germany), body mass was measured by an electronic scale (Model 876; Seca, Hamburg, Germany), participants' waist circumference was measured in the standing position, midway between the lowest rib and the iliac crest by Ana elastic meter. The Body Mass Index (BMI) was calculated as body mass divided by height squared ($kg/m^2$).

To estimate the daily food intake, all participants filled the questionnaire (3-Days food records). Records were processed using Winfood software (Medimatica S.u.r.l., Colonnella, TE, Italy). Statistical analysis was performed through a one-way ANOVA (Statview software).

## 2.2. Saliva sample collection and genomic DNA extraction

The donor was asked not to eat and not to use oral hygiene products 1 h before saliva collection. At least 2 mL of unstimulated saliva was collected, put on ice and stored at −80°C until the analysis. DNA was extracted from saliva samples using the MagPurix Bacterial DNA Extraction Kit (ZP02006; Zinexts Life Science Corp.) according to the manufacturer's instructions. DNA was quantified using the Qubit dsDNA BR and HS assay kit (Life Technologies, CA, United States).

## 2.3. Preparation of the 16S metagenomic sequencing library

PCR amplification was conducted to target the hypervariable V3–V4 regions of the 16S rRNA bacterial genes. Specific primers with barcodes and high-efficiency enzymes were used to perform PCR. The PCR primers were: forward 341F: CCTAYGGGRBGCASCAG; reverse 806R: GGACTACNNGGGTATCTAAT. The PCR products of 450–500 bp were collected with 2% agarose gel electrophoresis. To build library, same amount of PCR products from each sample is pooled, end-repaired, A-tailed and further ligated with Illumina adapters. The library QC was performed with Qubit and real-time PCR for quantification and with bioanalyzer to check the insert size distribution. Libraries were sequenced on a paired-end Illumina

platform to generate 250 bp paired-end raw reads. The raw sequencing data are available in Zenodo (https://doi.org/10.5281/zenodo.7920752; Publication date: May 10, 2023).

## 2.4. Bioinformatic analysis and statistics

We used the R platform for statistical analysis and for the data processing. We applied the DADA2 workflow (28) to infer the Amplicon Sequence Variants (ASVs) and for the taxonomic assignments.

In brief, we first filtered and trimmed raw sequencing reads in order to remove low quality bases and adapter contamination. Then, we removed identical reads. Moreover, the reads were denoised, merged filtered to remove artifacts (PCR, and PhiX related chimeras). We obtained the ASVs quantifications and assigned taxonomy annotations (including the Species level) using the SILVA database of non-redundant sequences (version: v138, nr99) (29). The data were structured in objects including the ASVs quantifications, the taxonomy annotations, the sample group data and the phylogenetic tree using the phyloseq and the APE packages (30, 31). Finally, based on the initial DNA concentration, we removed possible contaminant ASVs by using the "prevalence" method of the decontam package (32).

Downstream analyses were performed using the MicrobiomeAnalystR package (33, 34) and included data normalization, measures of diversity and differential abundance estimation.

Briefly, we normalized the ASV counts based on their abundance (low count filter: for any ASV to be retained, at least 20% of its values should contain at least 4 counts) and variance (low variance filter: based on Inter-quantile range ± 10%). This, because ASVs with small counts (in few samples) could represent sequencing errors. Moreover, ASVs that are closely constant in all samples could be excluded from the comparative analyses. Finally, we used the total sum scaling in order to bring all the samples to the same scale.

We evaluated the alpha-diversity by calculating the Abundance-based coverage estimator (ACE) and a nonparametric estimator of species richness (Chao1) indices and by the Fisher metrics (to consider both richness and eveness). The degree to which the species composition changes between the two groups (the beta-diversity) was obtained by PCoA (Principal Coordinates Analysis) of the distances calculated with the un-weighted UniFrac method and the statistical significance assessed by the PERMANOVA test.

We also used the rarefaction curves to evaluate whether the samples were sufficiently sampled and sequenced to represent their species richness. We assessed the statistical significance of comparisons between the two groups of samples under study by using the Mann–Whitney test.

The differential abundance was assessed by the LEfSe (Linear Discriminant Analysis Effect Size) algorithm (35) for biomarker discovery and interpretation of metagenomics data. It involves the Kruskal-Wallis rank sum test to identify features (e.g., Species or Genera) with significant differential abundance in the two groups, followed by linear discriminant analysis (LDA) to evaluate the relevance (the effect size) of the selected features. Different abundant features were considered if the FDR adjusted *value of p* was less than or equal to 0.05 and if the Log LDA was greater than or less than 0.5. We used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to assess the metabolic potential of microbial communities (KEGG pathways). In this analysis, we started from the ASVs belonging to the significant genera obtained by LefSE algorithm.

# 3. Results

## 3.1. Cohort characteristics

Anthropometric characteristics and eating habits of the children enrolled in this study are shown in Table 1. No significant differences in individuals' gender (Chi-square 0.6748; $p = 0.411399$). anthropometric characteristics such as height, weight, BMI and the waist/height ratio were observed ($p > 0.05$). Similarly, no significant differences in all diet components analyzed in A and S groups were found (Table 1).

## 3.2. Sequencing reads processing and taxonomic assignments

The Illumina sequencing of the hypervariable V3–V4 regions of the 16S rRNA bacterial genes generated 2 × 250 bp paired-end reads. On average, we obtained 169.124 reads per-sample (Supplementary Figure S1A). Overall, the percentage of bases with quality scores above 20 and 30 (Q20 and Q30, respectively) was of 96.45 and 91.22, respectively (Supplementary Figures S1B,C). The percentage of GC nucleotides was of 52.03 (Supplementary Figure S1D). The set of reads was used to run the DADA2 workflow including the filtering and trimming (median = 169.02), denoising of forward (median = 165.00) and reverse (median = 164.80) reads; merging (median = 149.27) and chimeric reads removal (median = 114.43; Supplementary Figure S1E). After merging, the median length of reads was of 424 bp. Overall, starting from filtered reads, we obtained a merging rate of 88.38% and a final rate of read processing (non-chimera over merged reads) of 67.62% (Supplementary Figure S1F). Details on read processing are reported in Supplementary Table S1.

For taxonomic assignments we used the SILVA database of non-redundant sequences (version: v138, nr99). Overall, we could identify a total of 14.197 taxa (ASVs) that were annotated to the seven taxonomic ranks as follows. All the ASVs were taxonomically assigned to the kingdom of bacteria. The 98.06% of the ASVs was annotated at the phylum level (43 phyla), the 96.97% at the class level (94 classes), the 94.93% at the order level (195 orders), the 88.43% at the family level (231 families), the 81.04% at the genus level (404 genera) and the 4.86% was annotated up to the species level (257 species; Supplementary Figure S2A). From the initial set of annotated ASVs, we discarded a total of 68 taxa as possible contaminants. Moreover, as described in Methods we removed low abundant and low variable ASVs to obtain the final set of 472 ASVs that was normalized and used for downstream analyses.

## 3.3. Diversity estimates

At the genus level, alpha-diversity estimates were significantly different between the two groups, A and S. Indeed, ACE (Figure 1A),

TABLE 1 Anthropometric characteristics and eating habits of Active (A) and Sedentary (S) children.

| | Total | Active (A) | Sedentary (S) |
|---|---|---|---|
| Gender M/F | 69/45 | 33/18 | 36/27 |
| Age (years) | 8–10 | 8–10 | 8–10 |
| *Anthropometric data* | | | |
| Height (cm) | 142.8 ± 7.5 | 143.5 ± 8.0 | 142.2 ± 7.0 |
| Weight (kg) | 38.7 ± 9.8 | 38.2 ± 9.3 | 39.2 ± 10.2 |
| BMI (kg/m$^2$) | 18.9 ± 3.8 | 18.5 ± 3.7 | 19.2 ± 3.9 |
| Waist/Height (cm) | 0.5 ± 0.06 | 0.45 ± 0.05 | 0.5 ± 0.06 |

| Eating habits (Average daily intake) | Active | Sedentary |
|---|---|---|
| Calories (kcal) | 1464.7 ± 284.2 | 1491.1 ± 281.1 |
| Carbohydrates (%) | 48.1 ± 5.8 | 48.9 ± 6.2 |
| Carbohydrates (g) | 188.2 ± 43.8 | 194.0 ± 43.6 |
| Starch (g) | 75.8 ± 27.3 | 70.8 ± 27.0 |
| Oligosaccharides (g) | 50.7 ± 19.6 | 55.9 ± 18.6 |
| Oligosaccharides/ Carbohydrates (%) | 26.7 ± 7.1 | 29.1 ± 8.7 |
| Lipids (%) | 36.1 ± 5.8 | 36.1 ± 5.5 |
| Lipids (g) | 58.5 ± 13.5 | 59.7 ± 13.7 |
| Saturated fatty acids (g) | 14.2 ± 4.6 | 15.3 ± 4.7 |
| Monosaturated fatty acids (g) | 18.7 ± 5.6 | 18.7 ± 6.0 |
| Polysaturated fatty acids (g) | 4.8 ± 1.4 | 5.0 ± 1.9 |
| Saturated/ fatty acids (%) | 37.4 ± 6.5 | 39.5 ± 6.3 |
| Cholesterol (mg) | 135.6 ± 64.7 | 144.9 ± 78.9 |
| Proteins (%) | 15.5 ± 2.0 | 15.0 ± 2.2 |
| Proteins (g) | 56.9 ± 13.8 | 55.9 ± 13.7 |
| Animal proteins/Proteins (%) | 70.6 ± 9.3 | 72.6 ± 14.1 |
| Vegetal proteins/Proteins (%) | 29.4 ± 9.3 | 27.4 ± 14.2 |
| Total fiber/1,000 (kcal) | 7.8 ± 3.2 | 6.8 ± 2.2 |
| Total fiber (g) | 11.7 ± 6.2 | 10.0 ± 3.3 |

Chao1 (Figure 1B) and Fisher (Figure 1C) indices showed $p < 0.01$ (Mann–Whitney test). The beta-diversity analysis, as measured by unweighted UniFrac distances, showed a significantly different microbial composition between the two groups ($r^2 = 0.026$, $p = 0.001$; Figure 1D), the diversity was also confirmed by using the weighted UniFrac distance metric ($r^2 = 0.018$, $p = 0.001$; Figure 1E). As previously reported (36), unweighted and weighted UniFrac distance measures can be considered as quality-based and quantity-based indexes, respectively. Indeed, we can assess that the observed variation between the two groups was due to the different taxa abundances and to the types of taxa in their microbiome. Moreover, the rarefaction analysis clearly evidenced the capacity to capture the species richness from the results of sampling and sequencing in both groups without any statistically significant difference ($p = 0.084$; Supplementary Figure S2B).

## 3.4. Abundance estimates

We evaluated and compared the taxa abundance in the final set of 472 filtered and normalized ASVs. Overall, we identified 8 phyla, 12 classes, 30 orders, 46 families, 84 genera and 96 species.

At the phylum level, on average, the most abundant bacteria were Firmicutes, Bacteroides and Proteobacteria accounting for the 32.08, 26.97% and the 25.58% of the taxa, respectively (Supplementary Figure S3A). The most represented classes were Bacteroides (26.97%), Gammaproteobacteria (25.58%) and Bacilli (21.36%; Supplementary Figure S3B). The most prevalent orders were Bacteroides (26.63%), Lactobacillales (19.58%), Pasteurellales (12.88%) and Burkholderiales (12.12%; Supplementary Figure S3C). Among the most abundant families, we found Prevotellaceae (23.07%), Streptococcaceae (17.66%), Pasteurellaceae (12.88%) and Neisseriaceae (11.77%; Supplementary Figure S3D). At the genus level, we found the *Prevotella* (19.74%), *Streptococcus* (17.66%), *Haemophilus* (11.87%), *Neisseria* (11.57%) and *Veillonella* (6.06%; Figure 2A). Finally, the top abundant species that we were able to classify were *Prevotella melaninogenica* (10.86%), *Fusobacterium periodonticum* (4.44%), *Haemophilus parainfluenzae* (2.27%), *Rothia mucilaginosa* (2.10%) and *Veillonella dispar* (1.55%; Figure 2B).

## 3.5. Differential abundance estimates

As described in Methods, we used the LEfSe algorithm to perform the differential abundance analysis and to identify the taxa that could explain the differences between the two groups A and S. We set the threshold at 5% and the Log LDA cutoff at ± 0.5. Interestingly, using these very stringent criteria, we found *Coriobacteriaceae* family as more abundant in the A compared to the S group (LDA score = −3.17; FDR = 0.021).

Further, at the genus level, we found that ten genera were responsible for the differences between the two groups. In particular, *Agathobacter* (LDA score = −3.40; FDR = 0.015), *Escherichia–Shigella* (LDA score = −3.37; FDR = 7.68×10-04), *Collinsella* (LDA score = −3.17; FDR = 0.012), *Simonsiella* (LDA score = −2.95; FDR = 0.044), *Eubacterium-yurii* group (LDA score = −2.79; FDR = 0.041) and *Parabacteroides* (LDA score = −2.43; FDR = 0.015) were more abundant in the A group. On the contrary, *Mogibacterium* (LDA score = 2.71; FDR = 9.21×10–04), *Stomatobaculum* (LDA score = 3.24; FDR = 0.44), TM7× (also known as *Nanosynbacter lyticus*, LDA score = 3.90; FDR = 0.045) and *Granulicatella* (LDA score = 4.14; FDR = 0.045) were more abundant genera in the S group (Figures 3A,B; Supplementary Table S2). Eight species showed significant differences in the LEfSe analysis. Indeed, *Prevotella nigrescens* (LDA score = −3.76; FDR = 1.5 × 10–03), *Collinsella aerofaciens* (LDA score = −3.17; FDR = 7.45 × 10–03), *Simonsiella muelleri* (LDA score = −2.96; FDR = 2.76 × 10–05), *Parabacteroides merdae* (LDA score = −2.43; FDR = 1.3 × 10–02), were the most represented species in the A group. Conversely, *Gemella parahaemolysans*, *Prevotella aurantiaca* (LDA score = −3.9; FDR = 5.27 × 10–04), *Prevotella pallens* (LDA score = 4.23; FDR = 1.93 × 10–02), *Neisseria mucosa* (LDA score = 4.43;

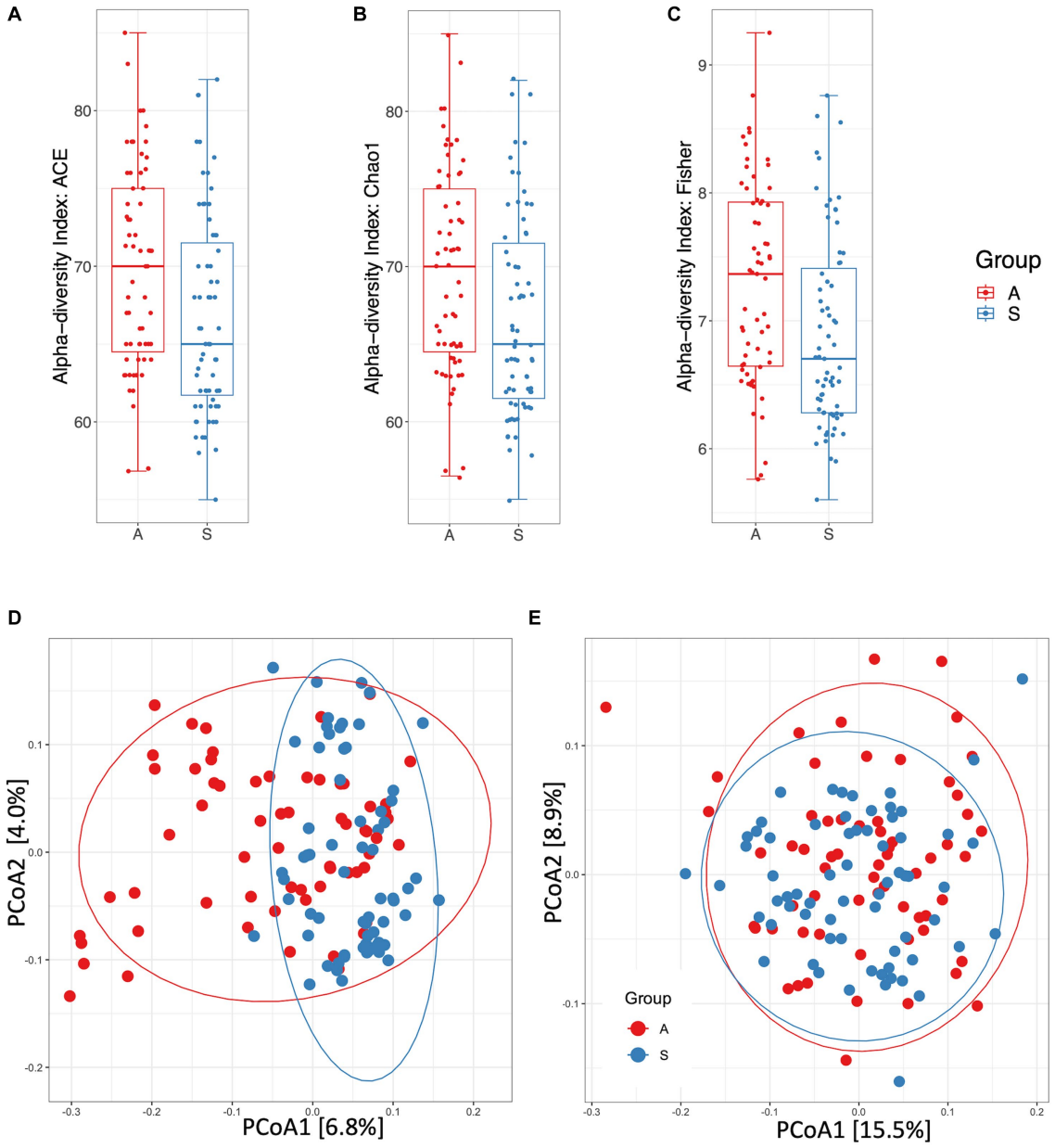



FIGURE 1
Diversity and distance measures between active (A) and sedentary (S). **(A)** Alpha-diversity measured by ACE index ($p = 0.0099$). **(B)** Alpha-diversity measured by Chao1 index ($p = 0.0097$). **(C)** Alpha-diversity measured by Fisher index ($p = 0.0039$). **(D)** Principal Coordinates Analysis plot of beta-diversity index measured by unweighted UniFrac distances ($p = 0.001$). **(E)** Principal Coordinates Analysis plot of beta-diversity index measured by weighted UniFrac distances ($p = 0.001$). (**A**), B, C, F: Mann–Whitney test. (**D**), E: PERMANOVA test.

FDR = 1.31 × 10−02) were more abundant species in the S group (LDA score = 2.94; FDR = 7.45×10-03; Supplementary Table S3; Figures 3C,D).

## 3.6. Metabolic pathways reconstruction

PICRUSt analysis highlighted the predominance of super pathway of hexitol degradation, L-glutamate degradation VII (to propionate), 2-methylcitrate cycle II, tetrapyrrole biosynthesis I, L-histidine degradation II, superpathway of beta-D-gluconide and D-gluconate degradation, biotin biosynthesis I, and L-arginine biosynthesis pathways activation in saliva from S compared to A children. Conversely, we found a prevalence of superpathway of fatty acid biosynthesis initiation (*E. coli*) and catechol degradation II (meta-cleavage pathway) in saliva from A respect to S children (Figure 4).

# 4. Discussion

The aim of the study was to evaluate the effects of Active or Sedentary lifestyle on saliva microbiota composition in Italian

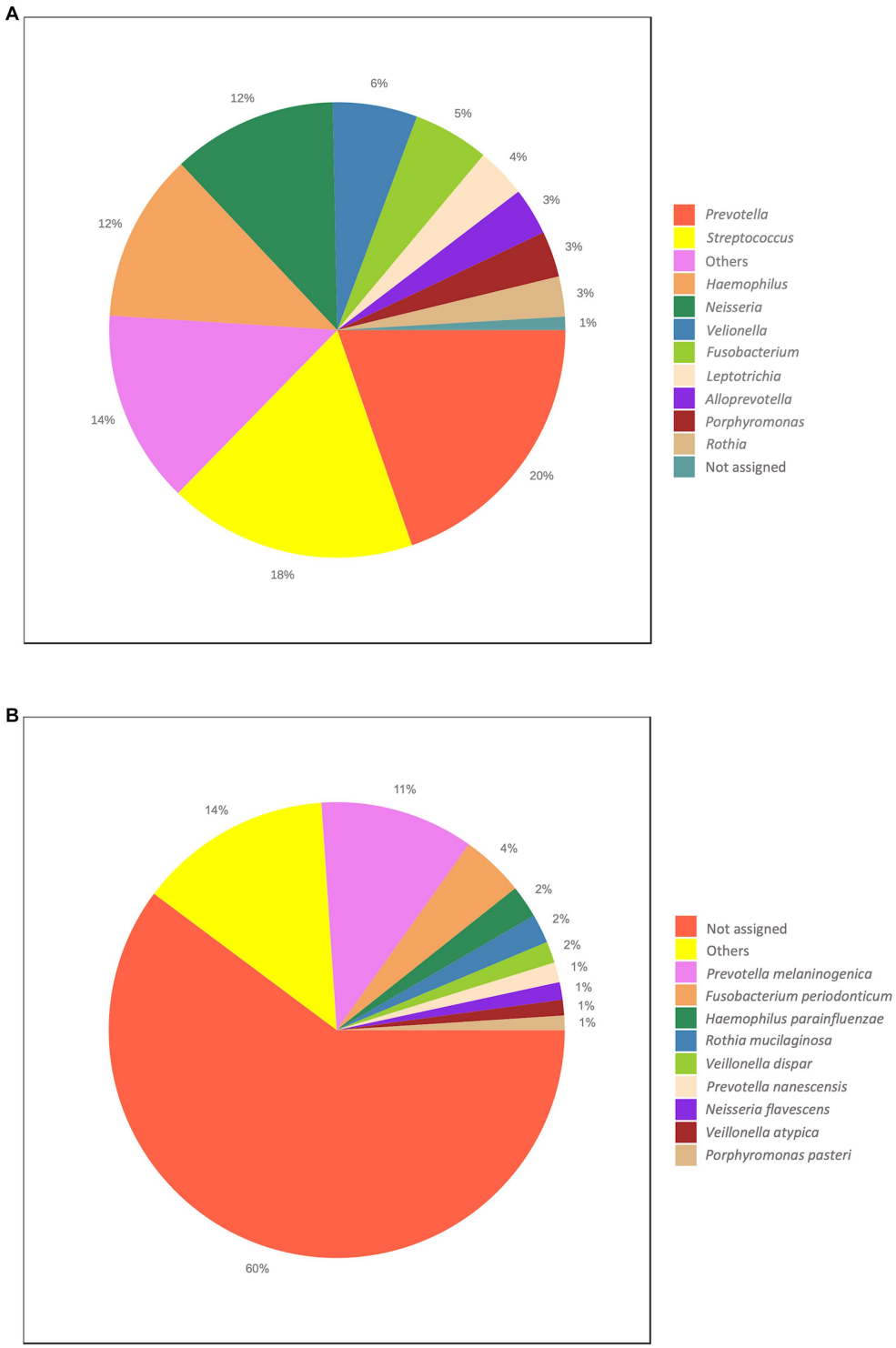



FIGURE 2
Overall taxonomic distribution. The Figure reports the overall abundance of the identified taxa. **(A)** Genus level. **(B)** Species level. Each plot shows the top ten abundant taxa.

schoolchildren living in the outskirts of Turin. The participants were classified as Active (A) or Sedentary (S) according to the cut-off score of 2.75 for PAQ-C-It (27). We evidenced an enrichment of several genera, such as *Agathobacter*, *Collinsella*, *Simonsiella*, and *Parabacteroides* in the children's saliva from A compared to S group. Among these, four species were differentially represented: *Prevotella nigrescens*, *Collinsella aerofaciens*, *Simonsiella muelleri*, and *Parabacteroides merdae*. Increased abundance of *Agathobacter* and *Prevotella* at both genus and species levels, was reported in GM of cross-country and marathon athletes; although an inverse correlation

10.3389/fnut.2023.1226891

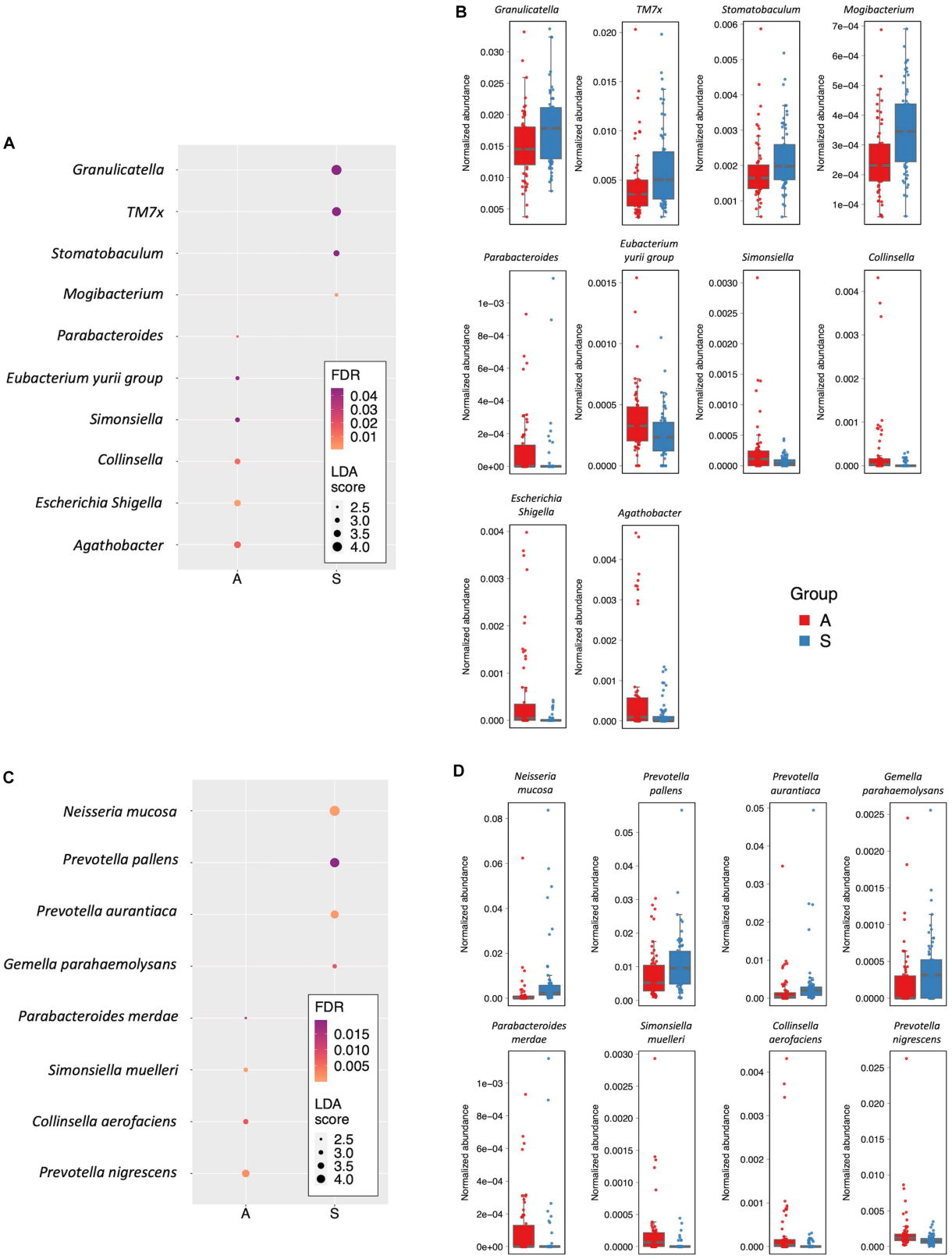



FIGURE 3
Differently abundant taxa. **(A)** Dot plot showing the differently abundant genera. **(B)** Box plots showing the Normalized abundance levels of genera reported in panel A. **(C)** Dot plot reporting the differentially abundant species. **(D)** Box plots showing the normalized abundance levels of species reported in panel C. In panels A and C, the dot size is proportional to the score of the LDA algorithm. The dot graduation color is proportional to the significance level as determined by FDR adjustment of Kruskal-Wallis rank sum test *p* values. A: Active; S: Sedentary.

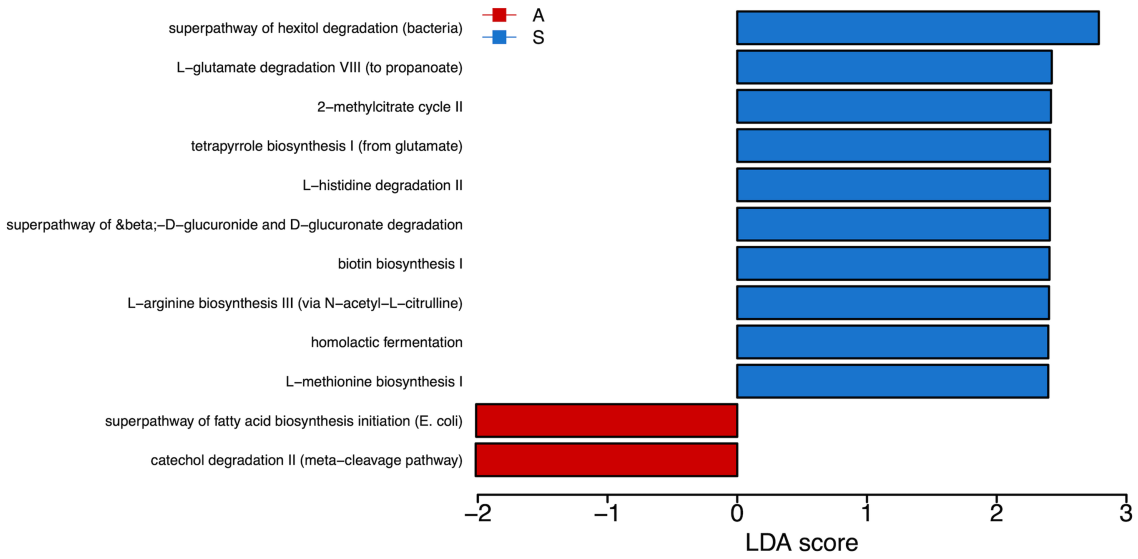



FIGURE 4
Metabolic pathways reconstruction. We used Phylogenetic Inves-tigation of Communities by Reconstruction of Unobserved States (PICRUSt) to predict the activity of metabolic pathways (KEGG) starting from the significant genera obtained by LefSE analysis. In red are the pathways enriched in the A group. In blue are the pathways enriched in the S group. A: Active; S: Sedentary.

was found for Prevotella and sucrose intake and a positive correlation for Agathobacter and dietary fiber content (37), we did not find statistically significant differences in all dietary components, including fiber content in A compared to S children. *Parabacteroides* are involved in host health promotion by regulating different pathways including inflammation, obesity and cancer prevention (38). Moreover, recent data suggest an anti-seizure and anti-cancer functions for *Parabacteroides merdae* (39, 40) and increased abundance have been also found in the GM of centenarians living in East China (41). As no differences in BMI were found between A or S children belonging to our cohort, we speculate that the prevalence of *Parabacteroides merdae* in saliva of group A children could be associated to the higher level of daily Physical Activity Amounts (PAA) when compared to group S. Furthermore, the increased abundance of *Parabacteroides* in group A resulted in the enrichment of the superpathway of fatty acid biosynthesis initiation that we found by metabolic pathway reconstruction. Of note, *Parabacteroides* are also involved in regulating different processes as carbohydrates metabolism and metabolites secretion, including Short Chain Fatty Acids (SCFAs) (42, 43) Among them, acetate, propionate, and butyrate are the main metabolites produced by several anaerobic bacteria from the fermentation of complex starch and dietary fibers. The available mechanistic data strongly suggest that SCFAs exert their powerful anti-inflammatory, antitumorigenic and even antimicrobial effects in the preventing gastro-intestinal dysfunction, obesity and type 2 diabetes mellitus (44, 45). In line with these evidences, several studies several studies conducting in patients with type 1 and type 2 diabetes, liver cirrhosis, inflammatory bowel disorders (IBD) and atherosclerosis have shown a reduction in the abundance of SCFA-producing bacteria gut (46, 47). Gut microbiota of athletes have an enriched profile of SCFAs, previously associated to a healthier status and a lean phenotype (44, 48). In skeletal muscle, SCFAs can be oxidized, incorporated into glucose *via* gluconeogenesis or increase the bioavailability of glucose, glycogen and fatty acids during exercise (49). Similarly, increased abundance in GM of taxa as *Firmicutes* and *Feacalibacterium prausnitzii* together with *Akkermansia,* producing butyrate, have been associated to exercise in athletes and non-athletes' controls with improvement in lipid oxidation, healthier profile and reduced risk for obesity and metabolic diseases, independently from body composition and diet (50–53). Further, similarly to our results, the association of a healthier profile with a reduction in *Bacteroides* species together with an increase in *R. hominis*, *A. Muciniphyla* and *F. prausnitzii* species have been described in GM from Active compared to Sedentary adults (54–56).

In group S we found an increased abundance of *Gemella parahemolysan*, *Prevotella aurantiaca*, *Prevotella pallens* and *Neisseria mucosa* species and of the *TM7x* genus as compared to group A. Notably, previous studies reported the abundance of *Neisseria mucosa* as sixfold higher in obese adolescents compared to normal-weight controls (57). Suggesting that although the Sedentary children are normal-weigh, they present a predictive marker linked to obesity. Moreover, *Prevotella* species, habitually present in the oral microbiome, have constant and direct access to the gastrointestinal tract *via* saliva swallowing. Here, they could act as commensals but also as potentially harmful agents (58). Furhermore, the group S showed an increased abundance of the genus *TM7x* (also known as *Nanosynbacter lyticus*) which is an obligate epibiont parasite of the bacteria *Actinomyces odontolyticus* (not significantly enriched in our data) (59, 60). *TM7x* have been previously associated to different human inflammatory mucosal diseases such as the periodontitis (61). Moreover, *TM7x* have been considered as biomarker of active disease in patients with ulcerative colitis (62).

In group S, the metabolic pathway reconstruction highlighted the enrichment of L-glutamate degradation and L-arginine biosynthesis pathways. Interestingly, the dysregulation of L-glutamate and L-glutamine pathways have been associated with poor survival in

colon cancer patients (63, 64). L-glutamate signaling triggers oxidative and nitrosative stress pathways which are essential for the production of ROS that can induce the activation of oncogenes ensuring the survival of colon cancer cells (64).

Conversely, the analysis performed with PICRuST on A children's saliva revealed an abundance of fatty acid biosynthesis and catechol degradation pathways, in line with previous reports (65). The catecholamines are catabolic intermediates of various aromatic compounds, which contribute to Acetyl-CoA production. Acetyl-CoA, is also crucial for the cross-talk between multiple biological processes including, energy storage, membrane biosynthesis, and generation of signaling molecules that are produced in response to physiological cell processes (66, 67). Consequently, dysregulation of fatty acid synthesis can induce or promote disease development (68, 69).

In conclusion, Our results showed that saliva from active children had an enrichment of species and genera mainly associated with a healthier profile. On the contrary, the genera and the species enriched in the saliva from sedentary group could be linked non-communicable diseases. Nevertheless, our indirect observations need to be clarified by further (and possibly larger) studies aimed at understanding how an active lifestyle can modulate the composition of both oral and gut microbiota. Moreover, the minimum volume of physical exercise required to determine changes in oral microbiota composition remains to be assessed.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics committee on human research of the University of Turin (9 March 2020: Protocol #134691) and Naples (17 January 2020: Protocol #376/19) approved the study. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## Author contributions

AM, SO, and PBu: conceptualization. DV, DP, CC, and AC: methodology. VL and MC: software, validation, and formal analysis. PBr and CL: investigation and resources. AM, DV, and VL: data curation and writing—original draft preparation. AM, SO, MC, and PBu: writing—review and editing. PBu: visualization and supervision. AM, SO, FS, and PBu: project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

## Funding

This study was funded by the grant PRIN 2017_Prot.2017RS5M44 to PBu, AM, SO, and FS.

## 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 GC declared a past co-authorship with the author CC to the handling editor.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1226891/full#supplementary-material

## References

1. Grice EA, Segre JA. The human microbiome: our second genome. *Annu Rev Genom Hum Genet*. (2012) 13:151–70. doi: 10.1146/annurev-genom-090711-163814

2. Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, et al. Microbiota in health and diseases. *Signal Transduct Target Ther*. (2022) 7:135. doi: 10.1038/s41392-022-00974-4

3. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature*. (2012) 486:222–7. doi: 10.1038/nature11053

4. Aya V, Flórez A, Perez L, Ramírez JD. Association between physical activity and changes in intestinal microbiota composition: a systematic review. *PLoS One*. (2021) 16:e0247039. doi: 10.1371/journal.pone.0247039

5. Mailing LJ, Allen JM, Buford TW, Fields CJ, Woods JA. Exercise and the gut microbiome: a review of the evidence, potential mechanisms, and implications for human health. *Exerc Sport Sci Rev*. (2019) 47:75–85. doi: 10.1249/JES.0000000000000183

6. Choi JJ, Eum SY, Rampersaud E, Daunert S, Abreu MT, Toborek M. Exercise attenuates PCB-induced changes in the mouse gut microbiome. *Environ Health Perspect*. (2013) 121:725–30. doi: 10.1289/ehp.1306534

7. Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S, Dougherty J, et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. *PLoS One*. (2014) 9:e92193. doi: 10.1371/journal.pone.0092193

8. Lambert JE, Myslicki JP, Bomhof MR, Belke DD, Shearer J, Reimer RA. Exercise training modifies gut microbiota in normal and diabetic mice. *Appl Physiol Nutr Metab*. (2015) 40:749–52. doi: 10.1139/apnm-2014-0452

9. Petriz BA, Castro AP, Almeida JA, Gomes CP, Fernandes GR, Kruger RH, et al. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC Genomics*. (2014) 15:511. doi: 10.1186/1471-2164-15-511

10. Queipo-Ortuño MI, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM, Cardona F, et al. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One*. (2013) 8:e65465. doi: 10.1371/journal.pone.0065465

11. Estaki M, Pither J, Baumeister P, Little JP, Gill SK, Ghosh S, et al. Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. *Microbiome*. (2016) 4:42. doi: 10.1186/s40168-016-0189-7

12. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*. (2014) 63:1913–20. doi: 10.1136/gutjnl-2013-306541

13. Allen AP, Dinan TG, Clarke G, Cryan JF. A psychology of the human brain-gut-microbiome axis. *Soc Personal Psychol Compass*. (2017) 11:e12309. doi: 10.1111/spc3.12309

14. Petersen LM, Bautista EJ, Nguyen H, Hanson BM, Chen L, Lek SH, et al. Community characteristics of the gut microbiomes of competitive cyclists. *Microbiome*. (2017) 5:98. doi: 10.1186/s40168-017-0320-4

15. Hughes RL, Holscher HD. Fueling gut microbes: a review of the interaction between diet, exercise, and the gut microbiota in athletes. *Adv Nutr*. (2021) 12:2190–215. doi: 10.1093/advances/nmab077

16. Durk RP, Castillo E, Márquez-Magaña L, Grosicki GJ, Bolter ND, Lee CM, et al. Gut microbiota composition is related to cardiorespiratory fitness in healthy young adults. *Int J Sport Nutr Exerc Metab*. (2019) 29:249–53. doi: 10.1123/ijsnem.2018-0024

17. Noce A, Tranchita E, Marrone G, Grazioli E, Di Lauro M, Murri A, et al. The possible role of physical activity in the modulation of gut microbiota in chronic kidney disease and its impact on cardiovascular risk: a narrative review. *Eur Rev Med Pharmacol Sci*. (2023) 27:3733–46. doi: 10.26355/eurrev_202304_32172

18. Barton W, Penney NC, Cronin O, Garcia-Perez I, Molloy MG, Holmes E, et al. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut*. (2018) 67:gutjnl-2016-313627–33. doi: 10.1136/gutjnl-2016-313627

19. Quiroga R, Nistal E, Estébanez B, Porras D, Juárez-Fernández M, Martínez-Flórez S, et al. Exercise training modulates the gut microbiota profile and impairs inflammatory signaling pathways in obese children. *Exp Mol Med*. (2020) 52:1048–61. doi: 10.1038/s12276-020-0459-0

20. Bai J, Hu Y, Bruner D. Composition of gut microbiota and its association with body mass index and lifestyle factors in a cohort of 7–18 years old children from the American gut project. *Pediatric Obes*. (2019) 14:e12480. doi: 10.1111/ijpo.12480

21. Moran-Ramos S, Lopez-Contreras BE, Villarruel-Vazquez R, Ocampo-Medina E, Macias-Kauffer L, Martinez-Medina JN, et al. Environmental and intrinsic factors shaping gut microbiota composition and diversity and its relation to metabolic health in children and early adolescents: a population-based study. *Gut Microbes*. (2020) 11:900–17. doi: 10.1080/19490976.2020.1712985

22. Santarossa S, Sitarik AR, Johnson CC, Li J, Lynch SV, Ownby DR, et al. Associations of physical activity with gut microbiota in pre-adolescent children. *Phys Act Nutr*. (2021) 25:24–37. doi: 10.20463/pan.2021.0023

23. Belstrøm D, Holmstrup P, Nielsen CH, Kirkby N, Twetman S, Heitmann BL, et al. Bacterial profiles of saliva in relation to diet, lifestyle factors, and socioeconomic status. *J Oral Microbiol*. (2014) 6:6. doi: 10.3402/jom.v6.23609

24. Keller MK, Kressirer CA, Belstrøm D, Twetman S, Tanner ACR. Oral microbial profiles of individuals with different levels of sugar intake. *J Oral Microbiol*. (2017) 9:1355207. doi: 10.1080/20002297.2017.1355207

25. Acharya A, Chan Y, Kheur S, Jin LJ, Watt RM, Mattheos N. Salivary microbiome in non-oral disease: a summary of evidence and commentary. *Arch Oral Biol*. (2017) 83:169–73. doi: 10.1016/j.archoralbio.2017.07.019

26. Schmidt TS, Hayward MR, Coelho LP, Li SS, Costea PI, Voigt AY, et al. Extensive transmission of microbes along the gastrointestinal tract. *elife*. (2019) 8:e42693. doi: 10.7554/eLife.42693

27. Lupo C, De Pasquale P, Boccia G, Ungureanu AN, Moisè P, Mulasso A, et al. The Most active child is not always the fittest: physical activity and fitness are weakly correlated. *Sports (Basel)*. (2022) 11:3. doi: 10.3390/sports11010003

28. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. (2016) 13:581–3. doi: 10.1038/nmeth.3869

29. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res*. (2007) 35:7188–96. doi: 10.1093/nar/gkm864

30. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. (2013) 8:e61217. doi: 10.1371/journal.pone.0061217

31. Paradis E, Schliep K. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*. (2019) 35:526–8. doi: 10.1093/bioinformatics/bty633

32. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*. (2018) 6:226. doi: 10.1186/s40168-018-0605-2

33. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. Microbiome analyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Res*. (2017) 45:W180–8. doi: 10.1093/nar/gkx295

34. Chong J, Liu P, Zhou G, Xia J. Using microbiome analyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nat Protoc*. (2020) 15:799–821. doi: 10.1038/s41596-019-0264-1

35. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol*. (2011) 12:R60. doi: 10.1186/gb-2011-12-6-r60

36. Cecarini V, Gogoi O, Bonfili L, Veneruso I, Pacinelli G, De Carlo S, et al. Modulation of gut microbiota and neuroprotective effect of a yeast-enriched beer. *Nutrients*. (2022) 14:2380. doi: 10.3390/nu14122380

37. Kulecka M, Fraczek B, Mikula M, Zeber-Lubecka N, Karczmarski J, Paziewska A, et al. The composition and richness of the gut microbiota differentiate the top polish endurance athletes from sedentary controls. *Gut Microbes*. (2020) 11:1374–84. doi: 10.1080/19490976.2020.1758009

38. Kverka M, Zakostelska Z, Klimesova K, Sokol D, Hudcovic T, Hrncir T, et al. Oral administration of *Parabacteroides distasonis* antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition. *Clin Exp Immunol*. (2011) 163:250–9. doi: 10.1111/j.1365-2249.2010.04286.x

39. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The gut microbiota mediates the anti-seizure effects of the ketogenic diet. *Cells*. (2018) 173:1728–1741.e13. doi: 10.1016/j.cell.2018.04.027

40. Roncase EJ, Moon C, Chatterjee S, González-Páez GE, Craik CS, O'Donoghue AJ, et al. Substrate profiling and high resolution co-complex crystal structure of a secreted C11 protease conserved across commensal Bacteria. *ACS Chem Biol*. (2017) 12:1556–65. doi: 10.1021/acschembio.7b00143

41. Wang N, Li R, Lin H, Fu C, Wang X. Enriched taxa were found among the gut microbiota of centenarians in East China. *PLoS One*. (2019) 14:e0222763. doi: 10.1371/journal.pone.0222763

42. Lei Y, Tang L, Liu S, Hu S, Wu L, Liu Y, et al. Parabacteroides produces acetate to alleviate heparanase-exacerbated acute pancreatitis through reducing neutrophil infiltration. *Microbiome*. (2021) 9:115. doi: 10.1186/s40168-021-01065-2

43. Du X, Xiang Y, Lou F, Tu P, Zhang X, Hu X, et al. Microbial community and short-chain fatty acid mapping in the intestinal tract of quail. *Animals (Basel)*. (2020) 10:1006. doi: 10.3390/ani10061006

44. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol*. (2014) 121:91–119. doi: 10.1016/B978-0-12-800100-4.00003-9

45. Blaak EE, Canfora EE, Theis S, Frost G, Groen AK, Mithieux G, et al. Short chain fatty acids in human gut and metabolic health. *Benef Microbes*. (2020) 11:411–55. doi: 10.3920/BM2020.0057

46. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. (2010) 464:59–65. doi: 10.1038/nature08821

47. Vatanen T, Franzosa EA, Schwager R, Tripathi S, Arthur TD, Vehik K, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature*. (2018) 562:589–94. doi: 10.1038/s41586-018-0620-2

48. Bongiovanni T, Yin MOL, Heaney LM. The Athlete and Gut Microbiome: Short-chain Fatty Acids as Potential Ergogenic Aids for Exercise and Training. *Int J Sports Med*. (2021) 42:1143–1158. doi: 10.1055/a-1524-2095

49. Frampton J, Murphy KG, Frost G, Chambers ES. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nat Metab*. (2020) 2:840–848. doi: 10.1038/s42255-020-0188-7

50. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett*. (2009) 294:1–8. doi: 10.1111/j.1574-6968.2009.01514.x

51. De Filippis F, Pasolli E, Ercolini D. Newly explored Faecalibacterium diversity is connected to age, lifestyle, geography, and disease. *Curr Biol*. (2020) 30:4932–4943.e4. doi: 10.1016/j.cub.2020.09.063

52. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A*. (2013) 110:9066–71. doi: 10.1073/pnas.1219451110

53. Scheepers LE, Penders J, Mbakwa CA, Thijs C, Mommers M, Arts IC. The intestinal microbiota composition and weight development in children: the KOALA birth cohort study. *Int J Obes*. (2015) 39:16–25. doi: 10.1038/ijo.2014.178

54. Cataldi S, Bonavolontà V, Poli L, Clemente FM, De Candia M, Carvutto R, et al. The relationship between physical activity, physical exercise, and human gut microbiota in healthy and unhealthy subjects: a systematic review. *Biology (Basel)*. (2022) 11:479. doi: 10.3390/biology11030479

55. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J Gastroenterol*. (2011) 17:1519–28. doi: 10.3748/wjg.v17.i12.1519

56. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut*. (2014) 63:1275–83. doi: 10.1136/gutjnl-2013-304833

57. Zeigler CC, Persson GR, Wondimu B, Marcus C, Sobko T, Modéer T. Microbiota in the oral subgingival biofilm is associated with obesity in adolescence. *Obesity (Silver Spring)*. (2012) 20:157–64. doi: 10.1038/oby.2011.305

58. Könönen E, Gursoy UK. Oral Prevotella species and their connection to events of clinical relevance in gastrointestinal and respiratory tracts. *Front Microbiol*. (2022) 12:798763. doi: 10.3389/fmicb.2021.798763

59. Bedree JK, Bor B, Cen L, Edlund A, Lux R, McLean JS, et al. Quorum sensing modulates the Epibiotic-parasitic relationship between *Actinomyces odontolyticus* and its *Saccharibacteria epibiont*, a *Nanosynbacter lyticus* strain, TM7x. *Front Microbiol*. (2018) 9:2049. doi: 10.3389/fmicb.2018.02049

60. He X, McLean JS, Edlund A, Yooseph S, Hall AP, Liu SY, et al. Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle. *Proc Natl Acad Sci U S A*. (2015) 112:244–9. doi: 10.1073/pnas.1419038112

61. Brinig MM, Lepp PW, Ouverney CC, Armitage GC, Relman DA. Prevalence of bacteria of division TM7 in human subgingival plaque and their association with disease. *Appl Environ Microbiol*. (2003) 69:1687–94. doi: 10.1128/AEM.69.3.1687-1694.2003

62. Zhu S, Han M, Liu S, Fan L, Shi H, Li P. Composition and diverse differences of intestinal microbiota in ulcerative colitis patients. *Front Cell Infect Microbiol*. (2022) 12:953962. doi: 10.3389/fcimb.2022.953962

63. Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic signaling along the microbiota-gut-brain Axis. *Int J Mol Sci*. (2019) 20:1482. doi: 10.3390/ijms20061482

64. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in Cancer. *Cancer Cell*. (2020) 38:167–97. doi: 10.1016/j.ccell.2020.06.001

65. Cronan JE. Bacterial membrane lipids: where do we stand? *Annu Rev Microbiol*. (2003) 57:203–24. doi: 10.1146/annurev.micro.57.030502.090851

66. Harayama S, Rekik M, Ngai KL, Ornston LN. Physically associated enzymes produce and metabolize 2-hydroxy-2, 4-dienoate, a chemically unstable intermediate formed in catechol metabolism via meta cleavage in *Pseudomonas putida*. *J Bacteriol*. (1989) 171:6251–8. doi: 10.1128/jb.171.11.6251-6258.1989

67. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. *J Lipid Res*. (2009) 50:S138–43. doi: 10.1194/jlr.R800079-JLR200

68. Röhrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. *Nat Rev Cancer*. (2016) 16:732–49. doi: 10.1038/nrc.2016.89

69. Wang Y, Yu W, Li S, Guo D, He J, Wang Y. Acetyl-CoA carboxylases and diseases. *Front Oncol*. (2022) 12:836058. doi: 10.3389/fonc.2022.836058

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 11 August 2023
DOI 10.3389/fnut.2023.1226672



OPEN ACCESS

EDITED BY
Attilio Parisi,
Foro Italico University of Rome, Italy

REVIEWED BY
Rui Poínhos,
University of Porto, Portugal
Hua Jiang,
Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, China

*CORRESPONDENCE
Wei Chen
✉ chenw@pumch.cn
Yan Liu
✉ Shyneeliu@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 22 May 2023
ACCEPTED 25 July 2023
PUBLISHED 11 August 2023

CITATION
Chen M, Xu X, Liu Y, Yao Y, Zhang P, Liu J, Zhang Q, Li R, Li H, Liu Y and Chen W (2023) Association of eating habits with health perception and diseases among Chinese physicians: a cross-sectional study. *Front. Nutr.* 10:1226672. doi: 10.3389/fnut.2023.1226672



# Association of eating habits with health perception and diseases among Chinese physicians: a cross-sectional study

Moxi Chen[1†], Xuan Xu[1†], Yinghua Liu[2], Ying Yao[3], Pianhong Zhang[4], Jingfang Liu[5], Qian Zhang[6], Rongrong Li[1], Hailong Li[1], Yan Liu[1*] and Wei Chen[1*]

[1]Department of Clinical Nutrition, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, [2]The First Medical Center of PLA General Hospital of China, Beijing, China, [3]Department of Clinical Nutrition, Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Shanghai, China, [4]The Second Affiliated Hospital Zhejiang University School of Medicine, Hangzhou, China, [5]Division of Nutrition, National Clinical Research Center for Aging and Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China, [6]Beijing Tongren Hospital, CMU, Beijing, China

**Background:** Some eating habits may be related to the development of gastrointestinal diseases, obesity, and related metabolic dysfunctions. Because of long working hours, and shift schedules, physicians are more likely to form such eating habits and have a high risk of developing these diseases.

**Objectives:** We aimed to investigate the association between physicians' eating habits and their health perception and diseases.

**Methods:** Between 24 June and 5 August 2020, we performed convenience sampling of in-service physicians in hospitals in mainland China. A questionnaire was administered to collect data pertaining to basic sociodemographic characteristics, eating habits, health-related information such as body mass index classification, and prevalence of common diseases. The associations among eating habits and perceived suboptimal health status, micronutrient deficiency-related diseases, obesity, and related metabolic diseases were analysed.

**Results:** The prevalence of unhealthy eating habits was high: more eating out-of-home (53.4% in hospital canteens, 23.0% in restaurants and takeaways), fewer meals at home, irregular meals (30.5%), and eating too fast (the duration <10 min, 34.6%). Among those with the above eating habits, the prevalence rates of sub-optimal health and disease were higher than among those without the above eating habits.

**Conclusion:** Eating habits such as frequent eating out-of-home, irregular meals, and eating too fast were common among physicians, and were significantly related to perceived sub-optimal health status and disease occurrence.

KEYWORDS

**eating habits, health perception, disease, obesity, physicians**

# 1. Introduction

With the development of the social economy and improvement in the living standards, the eating habits of modern people have undergone tremendous changes compared to the past. These changes are not only reflected in the diversification of food sources and the complexity of processing them, but also in the consumption of fast-food and out-of-home meals. Excessive consumption of high-energy density and ultra-processed foods, fast food and takeaways, inadequate and irregular eating times, and snacking between meals are the main characteristics of the modern diet (1). Dietary patterns characterised by the consumption of red and processed meat, fast food, and sugar-sweetened beverages are considered to be important risk factors for chronic non-communicable diseases such as metabolic and cardiovascular diseases in Chinese residents (2), and the pathogenic effects of unhealthy eating habits are gradually attracting increasing attention.

The main manifestations of unhealthy eating habits are short and irregular meal times, and excessive consumption of fast food and takeaways. Eating too fast can affect the levels of gastrointestinal hormones such as ghrelin, YY peptide, and glucagon-like peptide-1 (GLP-1), and therefore disturb the balance between hunger and satiety (3). Thus, it can lead to redundant energy intake, and glucose and lipid metabolism disorders (4), which would in turn lead to an increased incidence of obesity, diabetes, and metabolic syndrome (MetS) (5). Eating too fast is also believed to be associated with gastrointestinal diseases such as indigestion (6). Out-of-home meals (canteens, fast food, takeaways, etc.) have been shown to be a risk factor for high energy and fat intake, and low micronutrient intake (especially vitamin C, calcium, and iron) (7). People with these habits show a higher prevalence of overweight and obesity (8).

Shift work is thought to be an important contributor to irregular meals in modern society. Shift workers exhibit a significant preference for nocturnal activity, higher intake of high-fat diets and sweets such as candy and sweetened beverages, and disordered temporal eating patterns (irregular timing and frequency of meals, night meals) (9). These unhealthy habits are thought to be associated with the elevation of cardiometabolic risk factors [higher triglyceride and lower high-density lipoprotein (HDL) levels] and increased incidence of chronic diseases (including MetS, obesity, diabetes, and hypertension) (10).

Medical personnel are one of the representative populations of shift work, and previous studies have reported that they have irregular diets and excessive intake of fast foods high in salt, fat, and/or sugar after night shifts (11). Because of the COVID-19 pandemic, physicians are confronted with more working pressure and irregular working arrangements (12). With the emerging problems of work-life balance, physicians are more likely to develop unhealthy eating habits such as fast eating and more out-of-home meals. Previous surveys on physicians' the health statuses have found that the prevalence rates of obesity and related metabolic diseases [such as hyperlipidaemia and coronary artery disease (13)], and cardiovascular disease [such as hypertension (14)] were higher among physicians than in the general population. However, previous studies have mainly focused on the effects of physical inactivity (15) and sleep deprivation (16), and there are few studies on the association of physicians' eating habits with their health perception and diseases.

Suboptimal health status (SHS) is a physical state between health and disease, characterised by perceived discomfort, general malaise, chronic fatigue, and low energy (17). Unhealthy eating habits, lack of physical activity, poor sleep quality, and other unhealthy living habits are significantly related to self-perceived SHS (18). We conducted this survey to explore the association between the physicians' eating habits and their perceived SHS status, micronutrient deficiency-related diseases, obesity, and related metabolic diseases.

# 2. Subjects and methods

## 2.1. Subjects

From 24 June to 5 August 2020, we conducted a convenience sampling of in-service doctors in hospitals of all levels in mainland China (31 provinces, municipalities, and autonomous regions). Inclusion criteria were in-service physicians aged 20 years and above. Exclusion criteria were those with a physical or mental illness that affects eating habits (including eating behavior disorders, like bulimia and anorexia) and those who were unable to cooperate with the investigation. The online questionnaire was collected using mini apps in WeChat, and was forwarded to WeChat groups in different hospitals and departments to investigate the corresponding population. Each respondent voluntarily participated in the study and completed the questionnaire.

## 2.2. Questionnaire

The questionnaire we designed collected basic sociodemographic characteristics such as the age, sex and the department of physicians, eating habits, health-related information such as the body mass index (BMI) classification, perceived SHS status, and prevalence of common diseases. To rule out the influence of other lifestyle factors on the study results, we also included self-assessments of working time, sedentary time, exercise time and sleep quality. Smoking habits and alcohol consumption were also assessed through self-reported grading options, as detailed in the supplementary material (Original Questionnaire-Translated Version).

## 2.3. Eating habits

We divided eating habits into different types according to the source of meals, mode, and duration of eating. Specifically, the questionnaire counted the sources of the respondents' working meals (hospital canteen, dining out, or home-made), frequency of meals at home in a week (< 7 times/week, 8–14 times/week, or ≥ 15 times/week), regularity of meals, and average meal duration (< 10 min/meal, 10–30 min/meal, > 30 min/meal).

## 2.4. Health perception and diseases

Participants reported the classification of their BMI (<18.5 $kg/m^2$, 18.5 $kg/m^2 \leq$ BMI < 24.0 $kg/m^2$, 24 $kg/m^2 \leq$ BMI < 28.0 $kg/m^2$, and ≥ 28.0 $kg/m^2$), which was graded according to the cut-off value for the Chinese population (19). And the assessment of their own health perception was recorded, which was graded to very healthy, basic

health, subhealth, and disease state. In order to further evaluate the disease state of the participants, we also asked them to answer whether they often suffered from common diseases related to micronutrient deficiencies (cold, angular stomatitis/oral ulcer, chronic gastroenteritis/diarrhoea, constipation, and vision decrease/asthenopia), and whether they had obesity and metabolic-related chronic diseases (diabetes, cardiovascular and cerebrovascular diseases, dyslipidaemia, and tumours).

## 2.5. Quality control

The questionnaire used in this survey was designed on the basis of the "Report on Nutrition and Health Status of Chinese Physicians (2013)", and its validity has been verified by a previous survey (20). The collected online questionnaires were reviewed in a timely manner. If key variables such as the name, sex, and work unit had logical errors or were missing, and could not be corrected and filled, the questionnaires were regarded as invalid.

## 2.6. Statistical analysis

Previous studies have shown that the prevalence of perceived poor or fair health status in physicians was approximately 21% (21); the type I error α was 0.05, and the permissible error δ was set at 0.02. Thus, at least 1,594 patients should be included in this study. All analyses were conducted with International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, United States). Categorical variables were described as absolute and relative frequencies, and comparisons between groups were performed using the chi-square test. Associations between eating habits and health perception [subhealth/disease compared with very/basically healthy (reference)] were analysed using multivariable logistic regression, while controlling for sociodemographic characteristics and other covariates (Model 1 was adjusted for sex and age; model 2 was adjusted for sex, age, working time, sleep quality, exercise time, sedentary time, smoking habits, and alcohol consumption). All statistical analyses were two-sided tests, and the null hypothesis was rejected when $p < 0.05$.

# 3. Results

## 3.1. General information

In total, 9,624 questionnaires were collected. After excluding 428 non-physicians and questionnaires with incomplete information, 9,196 questionnaires (95.6%) were recovered. The distribution of respondents among various regions was relatively even, comprising mainly of doctors from second-level and above hospitals, covering various clinical and administrative departments. Among all respondents, obstetricians and gynaecologists accounted for the highest proportion (3,710, 40.34%), followed by internal medicine (1,352, 14.7%), and nutrition specialists (1,027, 11.17%). Most were women (7,662, 83.32%), and were aged 30–50 years old (Table 1).

## 3.2. Lifestyle

The prevalence of unhealthy eating and living habits among physicians was high, including eating out-of-home (53.4% in hospital canteens, 23.0% in dining out), less frequent meals at home, irregular meals (30.5%), and eating too fast (<10 min/meal, 34.6%). Those who worked more than 60 h per week accounted for 19.3% of the respondents. Poor sleep quality was reported by 14.6% of the physicians. In addition, physicians also had unhealthy lifestyle habits such as inadequate exercise (<40 min/d, 73.7%) and prolonged sedentary time (>2 h/d, 68.1%) (Table 1).

## 3.3. Health perception and diseases

According to the BMI classification obtained from the survey, 31.5% of the physicians were overweight or obese (BMI ≥ 24 kg/m$^2$), 56.6% thought they had SHS, 3.3% said they had diseases, and only 5.3% thought they were healthy. Among the physicians participating in the survey, the common diseases related to micronutrient deficiency ranked from high to low prevalence were decreased vision or asthenopia (81.3%), frequent colds (62.20%), chronic gastroenteritis or chronic diarrhoea (33.05%), constipation (31.94%), and frequent angular stomatitis/oral ulcer (13.80%); see Figure 1A. The prevalent obesity and metabolic-related chronic diseases were dyslipidaemia (21.50%), cardiovascular and cerebrovascular diseases (8.42%), tumours (5.87%), and diabetes (2.49%), as shown in Figure 1B.

## 3.4. Eating habits, health perception and diseases

Physicians with unhealthy eating habits, including when the working meals were mainly fast food and takeaways, few meals at home, irregular meals, and short meal duration (<10 min) had higher prevalence rates of micronutrient deficiency-related common diseases, obesity, and metabolic-related chronic diseases than other respondents (Table 2). After adjusting for the effects of sex, age, working hours, sleep quality, exercise time, sedentary time, smoking habits, and alcohol consumption, physicians with the above unhealthy eating habits was significantly associated with higher odds of subhealth/disease when compared with those with relatively healthy eating habits (Table 3). We further analyzed the differences in eating habits and their disease prevalence of physicians from different departments, and found that physicians from the department of nutrition had relatively healthy eating habits, that is, they mainly brought their own meals at work, had more meals at home, and had regular and sufficient eating time (Table 4). Correspondingly, nutrition doctors also have a relatively low prevalence of common diseases and chronic diseases than other departments (Table 5).

# 4. Discussion

Our survey results showed that unhealthy eating habits, including more out-of-home eating, fewer meals at home, irregular meals, and eating too fast were common among physicians. These eating habits were significantly related to the perceived sub-optimal health status

TABLE 1 Sociodemographic characteristics of the physicians.

| Characteristics | | *N* | % |
|---|---|---|---|
| Sex | Male | 1,534 | 16.7 |
| | Female | 7,662 | 83.3 |
| Age group (years) | 20–29 | 1,350 | 14.7 |
| | 30–39 | 3,607 | 39.2 |
| | 40–49 | 2,809 | 30.5 |
| | ≥ 50 | 1,430 | 15.6 |
| Hospital level | Tertiary | 5,581 | 60.7 |
| | Secondary | 3,119 | 33.9 |
| | Primary | 496 | 5.4 |
| Department | Gynaecology and obstetrics | 3,710 | 40.3 |
| | Internal medicine | 1,352 | 14.7 |
| | Nutrition | 1,027 | 11.2 |
| | Surgery | 836 | 9.1 |
| | Medical technology and administration | 765 | 8.3 |
| | Pediatrics | 389 | 4.2 |
| | Others | 1,117 | 12.1 |
| Main dining location | Hospital canteen | 4,910 | 53.4 |
| | Dining out | 2,117 | 23.0 |
| | At home | 2,169 | 23.6 |
| Frequency of consuming home-cooked meals | < 7 times/week | 3,063 | 33.3 |
| | 8–14 times/week | 4,019 | 43.7 |
| | ≥ 15 times/week | 2,114 | 23.0 |
| Eat regularly | Yes | 6,395 | 69.5 |
| | No | 2,801 | 30.5 |
| Eating duration | < 10 min/meal | 3,186 | 34.6 |
| | 10–30 min/meal | 5,794 | 63.0 |
| | > 30 min/meal | 216 | 2.3 |
| Working time | < 40 h/week | 1792 | 19.5 |
| | 40–60 h/week | 5,628 | 61.2 |
| | > 60 h/week | 1776 | 19.3 |
| Sleep quality | Good | 2,492 | 27.1 |
| | Average | 5,358 | 58.3 |
| | Poor | 1,346 | 14.6 |
| Exercise time | < 40 min/d | 6,781 | 73.7 |
| | 40–60 min/d | 1912 | 20.8 |
| | > 60 min/d | 503 | 5.5 |
| Sedentary time | < 1 h/d | 1,110 | 12.1 |
| | 1–2 h/d | 1826 | 19.9 |
| | > 2 h/d | 6,260 | 68.1 |
| Smoking habits | Yes | 374 | 4.1 |
| | No | 8,822 | 95.9 |
| Alcohol consumption | 0 g/d | 6,732 | 73.2 |
| | 1–14 g/d | 2,197 | 23.9 |
| | 15–24 g/d | 180 | 2.0 |
| | ≥ 25 g/d | 87 | 0.9 |

TABLE 2 Self-reported prevalence of common and chronic diseases among physicians by the different eating habits.

| Eating habits | Common diseased *N* (%) | | | | | Chronic diseases N(%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cold | Frequent angular stomatitis/ oral ulcer | Chronic gastroenteritis/ diarrhoea | Constipation | Vision decrease/ asthenopia | Overweight/ obesity | Diabetes | Cardiovascular and cerebrovascular diseases | Dyslipidaemia | Tumours |
| Main dining location | | | | | | | | | | |
| Hospital canteen | 3,010 (61.3)*** | 674 (13.7)*** | 1,565 (31.9)*** | 1,522 (31.0)*** | 3,989 (81.2)*** | 1,460 (29.7)*** | 125 (2.5)*** | 418 (8.5)** | 1,059 (21.6)*** | 274 (5.6) |
| Dining out | 1,435 (67.8) | 340 (16.1) | 856 (40.4) | 776 (36.7) | 1788 (84.5) | 834 (39.4) | 44 (2.1) | 165 (7.8) | 491 (23.2) | 130 (6.1) |
| At home | 1,275 (58.8) | 255 (11.8) | 618 (28.5) | 639 (29.5) | 1701 (78.4) | 602 (27.8) | 60 (2.8) | 191 (8.8) | 427 (19.7) | 136 (6.3) |
| Frequency of consuming home-cooked meals | | | | | | | | | | |
| < 7 times/ week | 2004 (65.4)*** | 497 (16.2)*** | 1,158 (37.8)*** | 1,151 (37.6)*** | 2,583 (84.3)*** | 1,025 (33.5)** | 69 (2.3)*** | 232 (7.6)*** | 670 (21.9)*** | 181 (5.9) |
| 8–14 times/ week | 2,543 (63.3) | 563 (14.0) | 1,317 (32.8) | 1,235 (30.7) | 3,272 (81.4) | 1,269 (31.6) | 96 (2.4) | 362 (9.0) | 862 (21.4) | 235 (5.8) |
| ≥15 times/ week | 1,173 (55.5) | 209 (9.9) | 564 (26.7) | 551 (26.1) | 1,623 (76.8) | 602 (28.5) | 64 (3.0) | 180 (8.5) | 445 (21.1) | 124 (5.9) |
| Eat regularly | | | | | | | | | | |
| Yes | 3,790 (59.3)*** | 726 (11.4)*** | 1771 (27.7)*** | 1780 (27.8)*** | 5,019 (78.5)*** | 1827 (28.6)*** | 155 (2.4)*** | 498 (7.8)*** | 1,307 (20.4)*** | 342 (5.3)** |
| No | 1930 (68.9) | 543 (19.4) | 1,268 (45.3) | 1,157 (41.3) | 2,459 (87.8) | 1,069 (38.2) | 74 (2.6) | 276 (9.9) | 670 (23.9) | 198 (7.1) |
| Eating duration | | | | | | | | | | |
| < 10 min/ meal | 2076 (65.2)*** | 555 (17.4)*** | 1,271 (39.9)*** | 1,159 (36.4)*** | 2,740 (86.0)*** | 1,240 (38.9)*** | 96 (3.0)*** | 350 (11.0)*** | 827 (26.0)*** | 223 (7.0)** |
| 10–30 min/ meal | 3,530 (60.9) | 688 (11.9) | 1716 (29.6) | 1719 (29.7) | 4,574 (78.9) | 1,611 (27.8) | 130 (2.2) | 411 (7.1) | 1,114 (19.2) | 309 (5.3) |
| > 30 min/ meal | 114 (52.8) | 26 (12.0) | 52 (24.1) | 59 (27.3) | 164 (75.9) | 45 (20.8) | 3 (1.4) | 13 (6.0) | 36 (16.7) | 8 (3.7) |

Differences across the groups were tested with Pearson's $\chi 2$ test: $^{**}p < 0.01$, $^{***}p < 0.001$.





10.3389/fnut.2023.1226672

10.3389/fnut.2023.1226672

TABLE 3 Association between eating habits and health status.

| Eating habits | Health status | | | | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Very/basically healthy | | Subhealth/disease | | OR | 95% CI | *p*-value | OR | 95% CI | *p*-value |
| Main dining location | | | | | | | | | | |
| At home | 1,011 | 46.6 | 1,158 | 53.4*** | 1.00 | | | 1.00 | | |
| Hospital canteen | 2061 | 42.0 | 2,849 | 58.0 | 1.207 | 1.089–1.337 | <0.001 | 1.117 | 1.001–1.247 | <0.048 |
| Dining out | 618 | 29.2 | 1,499 | 70.8 | 2.057 | 1.807–2.342 | <0.001 | 1.748 | 1.521–2.008 | <0.001 |
| Frequency of consuming home-cooked meals | | | | | | | | | | |
| ≥15 times/week | 1,069 | 50.6 | 1,045 | 49.4*** | 1.00 | | | 1.00 | | |
| 8–14 times/week | 1,627 | 40.5 | 2,392 | 59.5 | 1.471 | 1.322–1.637 | <0.001 | 1.237 | 1.103–1.388 | <0.001 |
| <7 times/week | 994 | 32.5 | 2,069 | 67.5 | 2.038 | 1.813–2.292 | <0.001 | 1.612 | 1.421–1.829 | <0.001 |
| Eat regularly | | | | | | | | | | |
| Yes | 3,072 | 48.0 | 3,323 | 52.0*** | 1.00 | | | 1.00 | | |
| No | 618 | 22.1 | 2,183 | 77.9 | 3.234 | 2.918–3.584 | <0.001 | 2.440 | 2.189–2.720 | <0.001 |
| Eating duration | | | | | | | | | | |
| > 30 min/meal | 131 | 60.6 | 85 | 39.4*** | 1.00 | | | 1.00 | | |
| 10–30 min/meal | 2,593 | 44.8 | 3,201 | 55.2 | 1.851 | 1.404–2.440 | <0.001 | 1.420 | 1.058–1.905 | 0.019 |
| <10 min/meal | 966 | 30.3 | 2,220 | 69.7 | 3.549 | 2.677–4.705 | <0.001 | 2.252 | 1.668–3.041 | <0.001 |

Differences across groups were tested with Pearson's $\chi^2$ test. ***$p < 0.001$. Associations were examined using multivariable logistic regression. Model 1 was adjusted for sex and age; model 2 was adjusted for sex, age, working time, sleep quality, exercise time, sedentary time, smoking habits, and alcohol consumption. subhealth/disease compared with very/basically healthy (reference). OR, odds ratio; CI, confidence interval.

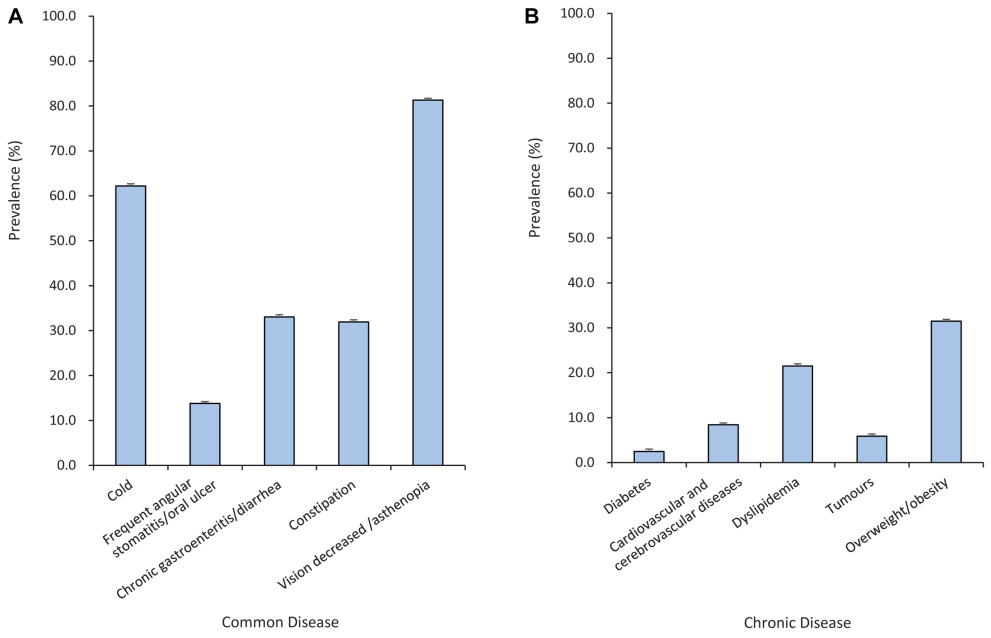



FIGURE 1
Prevalence of physicians' self-reported common diseases **(A)** and chronic diseases **(B)**.

TABLE 4 Eating habits among physicians from different departments.

| Eating habits | Department *N* (%) | | | | | | | *p*-value |
|---|---|---|---|---|---|---|---|---|
| | Gynaecology and obstetrics | Internal medicine | Nutrition | Surgery | Medical technology and administration | Pediatrics | Others | |
| Main dining location | | | | | | | | <0.001 |
| Hospital canteen | 1975 (53.2) | 672 (49.7) | 632 (61.5) | 457 (54.7) | 438 (57.3) | 196 (50.4) | 540 (48.3) | |
| Dining out | 846 (22.8) | 407 (30.1) | 107 (10.4) | 261 (31.2) | 135 (17.6) | 98 (25.2) | 263 (23.5) | |
| At home | 889 (24.0) | 273 (20.2) | 288 (28.0) | 118 (14.1) | 192 (25.1) | 95 (24.4) | 314 (28.1) | |
| Frequency of consuming home-cooked meals | | | | | | | | <0.001 |
| < 7 times/week | 1,332 (35.9) | 492 (36.4) | 222 (21.6) | 351 (42.0) | 213 (27.8) | 120 (30.8) | 333 (29.8) | |
| 8–14 times/week | 1,662 (44.8) | 585 (43.3) | 411 (40.0) | 352 (42.1) | 321 (42.0) | 176 (45.2) | 512 (45.8) | |
| ≥15 times/week | 716 (19.3) | 275 (20.3) | 394 (38.4) | 133 (15.9) | 231 (30.2) | 93 (23.9) | 272 (24.4) | |
| Eat regularly | | | | | | | | <0.001 |
| Yes | 2,286 (61.6) | 922 (68.2) | 984 (95.8) | 478 (57.2) | 653 (85.4) | 252 (64.8) | 820 (73.4) | |
| No | 1,424 (38.4) | 430 (31.8) | 43 (4.2) | 358 (42.8) | 112 (14.6) | 137 (35.2) | 297 (26.6) | |
| Eating duration | | | | | | | | <0.001 |
| < 10 min/meal | 1,573 (42.4) | 449 (33.2) | 157 (15.3) | 353 (42.2) | 206 (26.9) | 103 (26.5) | 341 (30.5) | |
| 10–30 min/meal | 2074 (55.9) | 870 (64.3) | 826 (80.4) | 465 (55.6) | 535 (69.9) | 278 (71.5) | 746 (66.8) | |
| > 30 min/meal | 63 (1.7) | 33 (2.4) | 44 (4.3) | 18 (2.2) | 24 (3.1) | 8 (2.1) | 30 (2.7) | |

Differences across the groups were tested with Pearson's $\chi^2$ test.

and disease occurrence, and even after adjusting for the effects of confounding factors such as sex, age, sleep quality, and exercise on the health perception, the risk of developing SHS and disease among physicians with the above eating habits remained significantly elevated. Nutrition doctors, because of work or life factors, have relatively healthy eating habits and a relatively low prevalence of common and chronic diseases, indicating the importance of healthy eating habits and eating environment for physicians to maintain health.

Previous studies generally believe that physicians have better health awareness and health habits than ordinary people, so they mainly focus on their mental stress and burnout. In recent years, studies have found that the incidence of diseases related to overweight, obesity (16) and dyslipidemia (13) in physicians may be significantly higher than that in the general population. This is significantly related to their long working hours, lack of physical activity and the shift system (21). A study investigating the health habits of police officers, ambulance workers, hospital staffs (doctors and nurses) and office workers found that doctors had significantly higher dining-out rates than those in other jobs, and skipped breakfast more often than office workers (22).

Dining out and fast-food rich diets are often characterised by high-energy density, high-fat, larger food portions, and higher consumption of sugar-sweetened beverages (23), while home-cooked meals involve healthier food preparation methods and more dietary varieties (24). Studies in adolescents and adults have demonstrated that increasing the number of meals at home can improve diet quality and reduce obesity and related diseases (25) due to increased fruit/vegetable and dairy consumption and reduced consumption of sugar-sweetened beverages and unhealthy snacks (26). Our study found that physicians had more meals outside and fewer meals at home, consistent with the results of previous studies (27–29), which demonstrated physicians' high consumption of fast food and alcoholic beverages and less intake of fruits and vegetables.

Due to irregular meals and limited supply time in hospital canteens (30), physicians usually eat faster and tend to consume more snacks at unusual times (mainly at night) (31). These unhealthy eating

TABLE 5 Self-reported prevalence of common and chronic diseases among physicians from different departments.

| Diseases | Department *N* (%) | | | | | | | *p*-value |
|---|---|---|---|---|---|---|---|---|
| | Gynaecology and obstetrics | Internal medicine | Nutrition | Surgery | Medical technology and administration | Pediatrics | Others | |
| Common diseases | | | | | | | | |
| Cold | 2,423 (65.3) | 861 (63.7) | 552 (53.7) | 501 (59.9) | 460 (60.1) | 242 (62.2) | 681 (61.0) | <0.001 |
| Frequent angular stomatitis/oral ulcer | 604 (16.3) | 178 (13.2) | 77 (7.5) | 116 (13.9) | 99 (12.9) | 61 (15.7) | 134 (12.0) | <0.001 |
| Chronic gastroenteritis/diarrhoea | 1,333 (35.9) | 448 (33.1) | 218 (21.2) | 292 (34.9) | 240 (31.4) | 122 (31.4) | 386 (34.6) | <0.001 |
| Constipation | 1,425 (38.4) | 407 (30.1) | 200 (19.5) | 210 (25.1) | 209 (27.3) | 125 (32.1) | 361 (32.3) | <0.001 |
| Vision decrease/asthenopia | 3,189 (86.0) | 1,104 (81.7) | 745 (72.5) | 649 (77.6) | 610 (79.7) | 315 (81.0) | 866 (77.5) | <0.001 |
| Chronic diseases | | | | | | | | |
| Overweight/obesity | 1,192 (32.1) | 423 (31.3) | 185 (18.0) | 329 (39.4) | 257 (33.6) | 128 (32.9) | 382 (34.2) | <0.001 |
| Diabetes | 92 (2.5) | 28 (2.1) | 27 (2.6) | 29 (3.5) | 20 (2.6) | 5 (1.3) | 28 (2.5) | <0.001 |
| Cardiovascular and cerebrovascular diseases | 330 (8.9) | 96 (7.1) | 61 (5.9) | 107 (12.8) | 60 (7.8) | 27 (6.9) | 93 (8.3) | <0.001 |
| Dyslipidaemia | 762 (20.5) | 299 (22.1) | 196 (19.1) | 234 (28.0) | 159 (20.8) | 79 (20.3) | 248 (22.2) | <0.001 |
| Tumours | 296 (8.0) | 58 (4.3) | 35 (3.4) | 41 (4.9) | 36 (4.7) | 17 (4.4) | 57 (5.1) | <0.001 |

Differences across the groups were tested with Pearson's $\chi^2$ test.

habits collectively destroy their energy balance by increasing the serum concentrations of appetite-stimulating hormones (e.g., insulin, glucagon-like peptide 1, and YY peptide) and reducing diet-induced thermogenic effects (32), thus raising the risk of obesity and related metabolic diseases. Circadian rhythm disturbances caused by shift work and excessive intake of ultra-processed foods have also been linked to decreased immune function (33), chronic gastrointestinal dysfunction (34), and increased incidence of breast cancer (35), colorectal cancer (36), and other diseases. These findings are consistent with our results that showed that irregular meals and a short meal duration are associated with an increased prevalence of colds, chronic gastroenteritis/diarrhoea, and tumours.

Our study also found that there were significant differences in eating habits and health status among physicians from different departments. Doctors in nutrition departments had relatively healthy eating habits, and the corresponding prevalence rates of common and chronic diseases were low. This may be related to their relatively regular working hours, day shift work, and relatively comprehensive health knowledge (37). In previous studies, researchers investigated the differences of health habits among people of different occupational types in hospitals, and found that nurses had relatively worse health habits and health status than doctors which was also related to their long working hours, shift work and irregular work schedule (38). Other studies have also shown that the supplying timing of hospital canteens and the quality of meals (30) are important factors hindering physicians' pursuit of healthy diets.

To the best of our knowledge, the present study is the first to investigate the relationship between the eating habits of physicians and their health perception and diseases. The results suggest a relationship between the increased prevalence of SHS and disease among physicians and their work patterns and eating habits, which may be related to the hospitals' eating environment. This relationship indicates the necessity of paying attention to physicians' working pressure and improving the supply of healthy food in hospital canteens to improve the health status and work efficiency of physicians. The "Healthy Canteens" initiative promoted by the Chinese National Health Commission is a meaningful attempt in this direction.

This study also has some limitations. Although the survey covered many departments, obstetrics and gynaecology, internal medicine, and nutrition accounted for more than 60% of the participants, which led to a sex imbalance (women accounted for 83.32% of respondents). Thus, this sample might underrepresent the male-dominated population of surgeons. In addition, Self-reported BMI is somewhat inaccurate, but among physicians it can be calculated more professional and frequent, because doctors usually pay more attention to their health status. Our study was in the form of an online questionnaire, so the participants' eating habits, health perception and diseases were all self-reported. Thus, information bias

cannot be ignored, even if the questionnaires were self-assessments conducted by trained physicians themselves. However, the validity of our questionnaire has been verified by previous survey (20) and can minimize the deficiency of self-reported information.

## 5. Conclusion

Eating habits such as frequent eating out-of-home, irregular meals, and eating too fast were common among physicians. These eating habits were significantly related to the occurrence of perceived SHS, micronutrient deficiency-related diseases, obesity, and related metabolic diseases diseases.

## 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

Ethical approval was not required for the studies involving humans because this is an observational survey study. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements because the questionnaire was filled in anonymously online.

## Author contributions

WC contributed to conception and design. YiL, YY, PZ, JL, and QZ contributed to data collection. RL and HL supervised the study. YaL and XX contributed to statistical analysis and interpretation of data. MC contributed to data interpretation and manuscript drafting. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by the grants from National Key R & D Program of China (grant number: 2020YFC2005000).

## Acknowledgments

The authors would like to thank all participants and friends who helped us forward the questionnaires.

## 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.2023.1226672/full#supplementary-material

## References

1. Sproesser G, Ruby MB, Arbit N, Akotia CS, Alvarenga MS, Bhangaokar R, et al. Understanding traditional and modern eating: the TEP10 framework. *BMC Public Health*. (2019) 19:1606. doi: 10.1186/s12889-019-7844-4

2. Dai SY, Yang F, Hu YF, Xiao X. Research progress on the relationship between dietary patterns and common noninfectious chronic diseases. *Zhonghua Liu Xing Bing Xue Za Zhi*. (2021) 42:1509–17. doi: 10.3760/cma.j.cn112338-20201016-01241

3. Shah M, Copeland J, Dart L, Adams-Huet B, James A, Rhea D. Slower eating speed lowers energy intake in normal-weight but not overweight/obese subjects. *J Acad Nutr Diet*. (2014) 114:393–402. doi: 10.1016/j.jand.2013.11.002

4. Tao L, Yang K, Huang F, Liu X, Li X, Luo Y, et al. Association between self-reported eating speed and metabolic syndrome in a Beijing adult population: a cross-sectional study. *BMC Public Health*. (2018) 18:855. doi: 10.1186/s12889-018-5784-z

5. Kolay E, Bykowska-Derda A, Abdulsamad S, Kaluzna M, Samarzewska K, Ruchala M, et al. Self-reported eating speed is associated with indicators of obesity in adults: a systematic review and meta-analysis. *Healthcare (Basel)*. (2021) 9:1559. doi: 10.3390/healthcare9111559

6. Sinn DH, Shin DH, Lim SW, Kim KM, Son HJ, Kim JJ, et al. The speed of eating and functional dyspepsia in young women. *Gut Liver*. (2010) 4:173–8. doi: 10.5009/gnl.2010.4.2.173

7. Lachat C, Nago E, Verstraeten R, Roberfroid D, van Camp J, Kolsteren P. Eating out of home and its association with dietary intake: a systematic review of the evidence. *Obes Rev*. (2012) 13:329–46. doi: 10.1111/j.1467-789X.2011.00953.x

8. Nago ES, Lachat CK, Dossa RA, Kolsteren PW. Association of out-of-home eating with anthropometric changes: a systematic review of prospective studies. *Crit Rev Food Sci Nutr*. (2014) 54:1103–16. doi: 10.1080/10408398.2011.627095

9. Yoshizaki T, Kawano Y, Noguchi O, Onishi J, Teramoto R, Sunami A, et al. Association of eating behaviours with diurnal preference and rotating shift work in Japanese female nurses: a cross-sectional study. *BMJ Open*. (2016) 6:e011987. doi: 10.1136/bmjopen-2016-011987

10. Phoi YY, Keogh JB. Dietary interventions for night shift workers: a literature review. *Nutrients*. (2019) 11:2276. doi: 10.3390/nu11102276

11. Nicholls R, Perry L, Duffield C, Gallagher R, Pierce H. Barriers and facilitators to healthy eating for nurses in the workplace: an integrative review. *J Adv Nurs*. (2017) 73:1051–65. doi: 10.1111/jan.13185

12. Ayar D, Karaman MA, Karaman R. Work-life balance and mental health needs of health professionals during COVID-19 pandemic in Turkey. *Int J Ment Health Addict*. (2022) 20:639–55. doi: 10.1007/s11469-021-00717-6

13. Unal S, Tanriover MD, Ascioglu S, Demirkazik A, Ertenli I, Eskioglu E, et al. Turkish doctors' cohort: healthy despite low screening. *Postgrad Med*. (2017) 129:393–8. doi: 10.1080/00325481.2017.1272399

14. Kao LT, Chiu YL, Lin HC, Lee HC, Chung SD. Prevalence of chronic diseases among physicians in Taiwan: a population-based cross-sectional study. *BMJ Open*. (2016) 6:e009954. doi: 10.1136/bmjopen-2015-009954

15. Ambakederemo TE, Chikezie EU. Assessment of some traditional cardiovascular risk factors in medical doctors in southern Nigeria. *Vasc Health Risk Manag*. (2018) 14:299–309. doi: 10.2147/VHRM.S176361

16. Borgan SM, Jassim GA, Marhoon ZA, Ibrahim MH. The lifestyle habits and wellbeing of physicians in Bahrain: a cross-sectional study. *BMC Public Health*. (2015) 15:655. doi: 10.1186/s12889-015-1969-x

17. Wang Y, Ge S, Yan Y, Wang A, Zhao Z, Yu X, et al. China suboptimal health cohort study: rationale, design and baseline characteristics. *J Transl Med*. (2016) 14:291. doi: 10.1186/s12967-016-1046-y

18. Xue Y, Huang Z, Liu G, Feng Y, Xu M, Jiang L, et al. Association analysis of suboptimal health status: a cross-sectional study in China. *PeerJ*. (2020) 8:e10508. doi: 10.7717/peerj.10508

19. Zhou BF. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults--study on optimal cut-off points of body mass index and waist circumference in Chinese adults. *Biomed Environ Sci*. (2002) 15:83–96. doi: 10.1046/j.1440-6047.11.s8.9.x

20. Powerpoint of the "Report on Nutrition and Health Status of Chinese Physicians (2013)". (2014). Available at: https://www.slideserve.com/sasha/5211245 (Accessed September 07, 2022).

21. Wilf Miron R, Malatskey L, Rosen LJ. Health-related behaviours and perceptions among physicians: results from a cross-sectional study in Israel. *BMJ Open*. (2019) 9:e031353. doi: 10.1136/bmjopen-2019-031353

22. Tsiga E, Panagopoulou E, Niakas D. Health promotion across occupational groups: one size does not fit all. *Occup Med (Lond)*. (2015) 65:552–7. doi: 10.1093/occmed/kqv097

23. Bezerra IN, Curioni C, Sichieri R. Association between eating out of home and body weight. *Nutr Rev*. (2012) 70:65–79. doi: 10.1111/j.1753-4887.2011.00459.x

24. Mills S, Brown H, Wrieden W, White M, Adams J. Frequency of eating home cooked meals and potential benefits for diet and health: cross-sectional analysis of a population-based cohort study. *Int J Behav Nutr Phys Act*. (2017) 14:109. doi: 10.1186/s12966-017-0567-y

25. Koutras Y, Chrysostomou S, Giannakou K, Yannakoulia M. Weight-related behaviors and weight loss maintenance: a cross-sectional study in Cyprus. *BMC Public Health*. (2021) 21:1115. doi: 10.1186/s12889-021-11153-5

26. Hennessy E, Dwyer L, Oh A, Patrick H. Promoting family meals: a review of existing interventions and opportunities for future research. *Adolesc Health Med Ther*. (2015) 6:115–31. doi: 10.2147/AHMT.S37316

27. Alexandrova-Karamanova A, Todorova I, Montgomery A, Panagopoulou E, Costa P, Baban A, et al. Burnout and health behaviors in health professionals from seven European countries. *Int Arch Occup Environ Health*. (2016) 89:1059–75. doi: 10.1007/s00420-016-1143-5

28. Zeng W, Shang S, Fang Q, He S, Li J, Yao Y. Health promoting lifestyle behaviors and associated predictors among clinical nurses in China: a cross-sectional study. *BMC Nurs*. (2021) 20:230. doi: 10.1186/s12912-021-00752-7

29. Ronda-Pérez E, Campos-Mora J, de Juan A, Gea T, Reid A, Caballero P. Differences in the prevalence of fruit and vegetable consumption in Spanish workers. *Nutrients*. (2020) 12:3848. doi: 10.3390/nu12123848

30. Winston J, Johnson C, Wilson S. Barriers to healthy eating by National Health Service (NHS) hospital doctors in the hospital setting: results of a cross-sectional survey. *BMC Res Notes*. (2008) 1:69. doi: 10.1186/1756-0500-1-69

31. Souza RV, Sarmento RA, de Almeida JC, Canuto R. The effect of shift work on eating habits: a systematic review. *Scand J Work Environ Health*. (2019) 45:7–21. doi: 10.5271/sjweh.3759

32. Ishida Y, Yoshida D, Honda T, Hirakawa Y, Shibata M, Sakata S, et al. Influence of the accumulation of unhealthy eating habits on obesity in a general Japanese population: the Hisayama study. *Nutrients*. (2020) 12:3160. doi: 10.3390/nu12103160

33. Finger AM, Kramer A. Mammalian circadian systems: organization and modern life challenges. *Acta Physiol (Oxf)*. (2021) 231:e13548. doi: 10.1111/apha.13548

34. Chang WP, Peng YX. Differences between fixed day shift workers and rotating shift workers in gastrointestinal problems: a systematic review and meta-analysis. *Ind Health*. (2021) 59:66–77. doi: 10.2486/indhealth.2020-0153

35. Fiolet T, Srour B, Sellem L, Kesse-Guyot E, Allès B, Méjean C, et al. Consumption of ultra-processed foods and cancer risk: results from Nutri net-Santé prospective cohort. *BMJ*. (2018) 360:k322. doi: 10.1136/bmj.k322

36. Romaguera D, Fernández-Barrés S, Gracia-Lavedán E, Vendrell E, Azpiri M, Ruiz-Moreno E, et al. Consumption of ultra-processed foods and drinks and colorectal, breast, and prostate cancer. *Clin Nutr*. (2021) 40:1537–45. doi: 10.1016/j.clnu.2021.02.033

37. Segal L, Opie RS. A nutrition strategy to reduce the burden of diet related disease: access to dietician services must complement population health approaches. *Front Pharmacol*. (2015) 6:160. doi: 10.3389/fphar.2015.00160

38. Kunyahamu MS, Daud A, Jusoh N. Obesity among health-care workers: which occupations are at higher risk of being obese? *Int J Environ Res Public Health*. (2021) 18:4381. doi: 10.3390/ijerph18084381

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 16 November 2023
DOI 10.3389/fnut.2023.1278079



OPEN ACCESS

EDITED BY
Giulia Marrone,
University of Rome Tor Vergata, Italy

REVIEWED BY
Tianlin Gao,
Qingdao University, China
Nicolò Piacentini,
University of Rome Tor Vergata, Italy

*CORRESPONDENCE
Yanguo Qin
✉ qinyg@jlu.edu.cn

RECEIVED 15 August 2023
ACCEPTED 03 November 2023
PUBLISHED 16 November 2023

CITATION
Xie Z and Qin Y (2023) Is diet related to osteoarthritis? A univariable and multivariable Mendelian randomization study that investigates 45 dietary habits and osteoarthritis. *Front. Nutr.* 10:1278079.
doi: 10.3389/fnut.2023.1278079



# Is diet related to osteoarthritis? A univariable and multivariable Mendelian randomization study that investigates 45 dietary habits and osteoarthritis

Zhuoting Xie and Yanguo Qin*

Department of Orthopedics, The Second Hospital of Jilin University, Changchun, China

**Background:** Diet is a safe intervention for many chronic diseases as a modifiable lifestyle. However, the potential causal effect of many dietary intake habits on the risk of osteoarthritis has not been fully understood. The purpose of this study was to reveal the potential causal relationship of 45 genetically predicted dietary intakes with osteoarthritis and its subtypes.

**Methods:** Data on 45 dietary intakes were obtained from the UK Biobank study of approximately 500,000 participants, and data on six osteoarthritis-related phenotypes were obtained from the Genetics of Osteoarthritis Consortium study of 826,690 participants. We performed univariable Mendelian randomization (MR), multivariable MR and linkage disequilibrium score regression (LDSC) analyses.

**Results:** In univariate analyses, 59 potential associations between diet and osteoarthritis were found. After false discovery rate (FDR) correction and sensitivity analyses, 23 reliable causal evidence were identified. In multivariate analyses, controlling separately for the effects of body mass index, total body bone mineral density, and smoking status, eight robust causal relationships remained: Muesli intake was negatively associated with knee osteoarthritis, spine osteoarthritis and total knee replacement. Dried fruit intake had a negative association with osteoarthritis of knee and total knee replacement. Eating cheese may reduce the risk of osteoarthritis in the knee and spine. And alcohol usually taken with meals was associated with a reduced risk of total knee replacement. LDSC analyses showed significant genetic correlations between all exposures and their corresponding outcomes, respectively, in these eight causal relationships.

**Conclusion:** Evidence of dietary effects on osteoarthritis is provided in our study, which has important implications for the prevention, management, and intervention of osteoarthritis in common sites through rational dietary modification.

KEYWORDS

**diet, dietary intake, osteoarthritis, total joint replacement, Mendelian randomization**

## Introduction

Osteoarthritis (OA) is the most common type of arthritis, which can affect one or more different joints throughout the body (1, 2). With the aging of the population and rising rates of obesity in our society, the incidence of osteoarthritis is increasing year by year and has become a major cause of disability in adults. It has a serious impact on the quality of life of patients and

places a heavy burden on society (3). The current mainstay of treatment for osteoarthritis is pain management and total joint replacement surgery at the end stage of osteoarthritis. However, these management options do not stop the progression of the disease and carry the risk of medication side effects and surgical complications. Therefore, early prevention of osteoarthritis is crucial and finding modifiable risk factors other than obesity for early intervention in osteoarthritis is urgent.

Several previous studies have suggested that dietary intake may play an important role in knee OA (4, 5). One study found a significant negative correlation between whole grain consumption and the risk of knee OA (6); whereas, current findings are inconsistent regarding the effect of consumption of dairy products such as milk and cheese on knee OA (7, 8). The most significant limitation of these studies is that they may be affected by other confounding factors that can modify lifestyle, leading to uncertainty in the results. Furthermore, few studies have emphasized the effect of subcategorization of a particular food or drink on osteoarthritis. Moreover, most of the current studies have focused on knee OA, which is the most common form of osteoarthritis, with very little research analyzing the effect of diet on osteoarthritis in other parts of the body and end-stage osteoarthritis.

In this situation, Mendelian randomization (MR) analyses provide us with a suitable method to investigate the causal relationship between specific dietary intake and osteoarthritis, utilizing single nucleotide polymorphisms as instrumental variables (IVs), which are less susceptible to confounding factors because gametes are randomly assigned during gamete formation and are independent of environmental factors (9). Previous MR analyses have explored the causal effects of coffee consumption, tea, and alcohol intake on osteoarthritis of the hip and knee (10–12). However, these studies did not classify drinks in detail and only analyzed the effects of drinks on the most common types of osteoarthritis of the hip and knee. Causal associations of other dietary intake on the risk and severity of osteoarthritis at different sites remain unknown.

In this study, we performed comprehensive univariate MR, multivariate MR and LDSC analyses using the largest known Genome wide association study (GWAS) data on diet and osteoarthritis, and thereby explored the causal relationship between 45 dietary intake habits and six osteoarthritis phenotypes.

# Materials and methods

The flowchart of study design and analytic approach is shown in Figure 1.

## Data source

We obtained summary-level data related to dietary intake habits through the IEU Open GWAS program from the UK Biobank study of approximately 500,000 participants, who were recruited at ages 40–69 years across England, Wales, and Scotland from 2006 to 2010 (13). The participants provided information on lifestyle and physical measurements, donated biomedical samples, and agreed to track their health status. This study used a touchscreen questionnaire to obtain information on participants' frequency of intake of common foods and drinks over the last year. As an example, to investigate cereal intake and the main type of cereals eaten, participants were asked, "How many bowls of cereal do you eat a WEEK? What type of cereal do you mainly eat? If you eat more than one type of cereal, please select the one that you eat the most. And please provide an average considering your intake over the last year." Detailed information on the 45 dietary intake habits is provided in Supplementary Table S1. In addition, we obtained data on body mass index (BMI), total body bone mineral density (TB-BMD) and smoking status in the same way that was used to exclude the effects of these 3 common risk factors for osteoarthritis in the multivariate MR study.

Data on osteoarthritis were from the largest meta-analysis of genome-wide association studies on osteoarthritis to date, which included 13 cohorts with up to 826,690 participants (14). Osteoarthritis was defined by either: self-reported osteoarthritis, clinically diagnosed, ICD10 codes or radiographic depending on the data available in the cohort. Controls were osteoarthritis-free or population-based with or without ICD code exclusions. We selected osteoarthritis phenotypes from different parts of the body, including osteoarthritis of the knee (Knee OA), osteoarthritis of the hip (Hip OA), osteoarthritis of the spine (Spine OA) and osteoarthritis of the hand (Hand OA). Also, since osteoarthritis progression and osteoarthritis development were two distinct etiological endpoints, we chose total knee replacement (TKR) and total hip replacement (THR), which were associated with severe osteoarthritis, to represent end-stage knee and hip OA as a way of exploring whether dietary factors are associated with osteoarthritis progression. Details of the data sources used in this study are shown in Table 1.

## Selection of instrumental variables

In order to include more SNPs as IVs to investigate more relationships between dietary intake habits and osteoarthritis, we used the threshold of $p < 5e\text{-}6$ for IV filtering, and we removed chained unbalanced IVs (clumping: $r^2 = 0.001$, kb = 10,000) to ensure that each IV was independent of each other (15). We also excluded SNPs associated with outcome ($p < 5e\text{-}5$) and retained only strong instrumental variables (F statistics>10) (16). Finally, we dropped outliers using MR-Presso and used the remaining SNPs as the final IVs for the MR analyses.

## Statistical analysis

In the univariate analyses, we mainly used the inverse-variance weighted (IVW) method for estimation. Heterogeneity was firstly tested for using the Cochran Q analysis, and if there was no heterogeneity, fixed-effects IVW (IVW-FE) model was used for the main analyses; otherwise, random-effects IVW (IVW-MRE) model was applied (17). In order to test the robustness and reliability of the results, we also carried out sensitivity analyses for each causal relationship using weighted median, MR Egger, and MR-Presso, and tested the results for pleiotropy using MR Egger intercept. If the results of IVW were statistically significant without pleiotropy and the estimates of the four MR analyses were in the same direction, we considered a potential causal association between the exposure and the outcome. If any other conditions existed, then we considered that no association between the exposure and the outcome was found

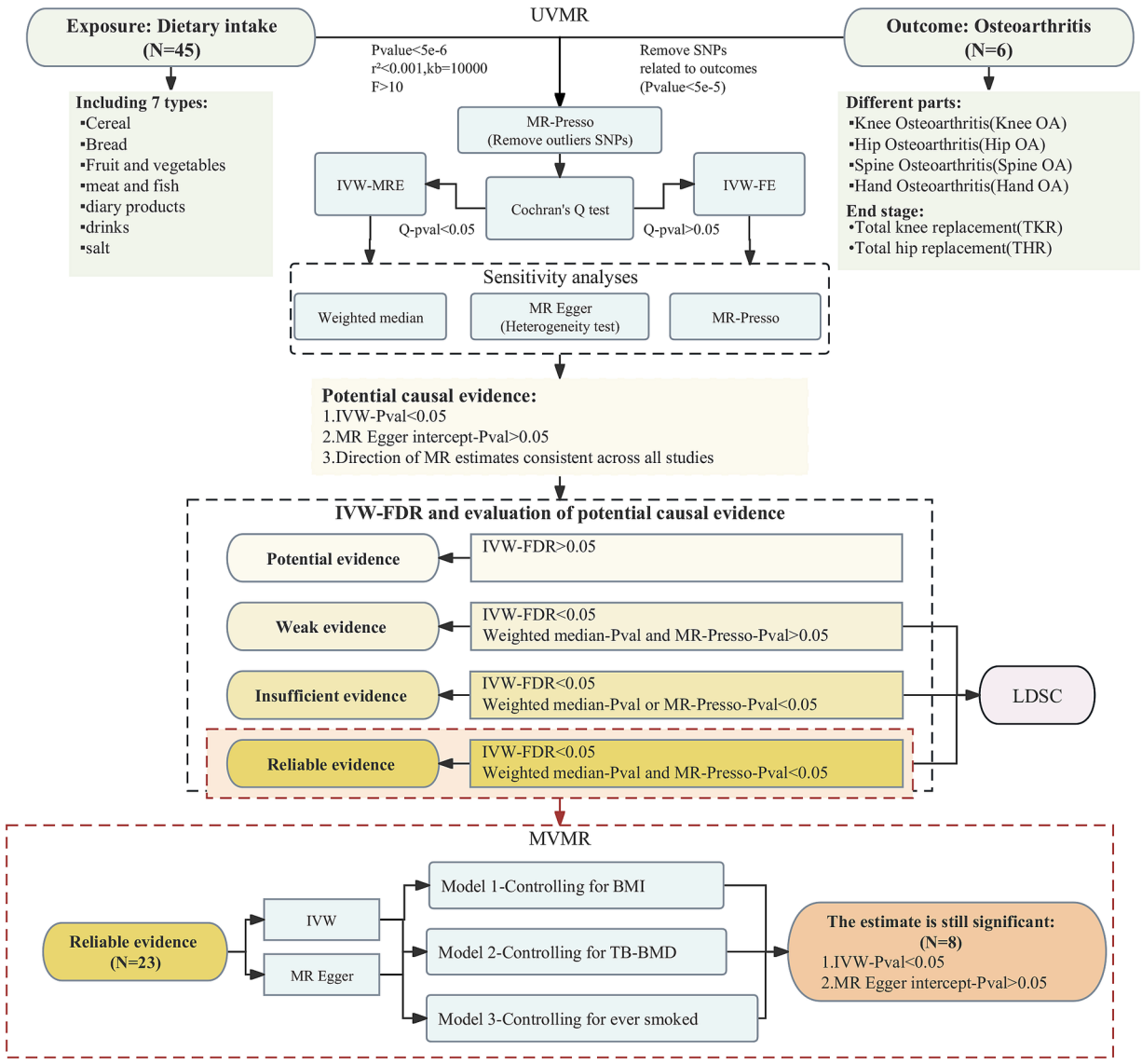



FIGURE 1
The flowchart of study design and analytic approach.

through our study. After adjusting for *p*-values using the FDR approach, further evaluation of the strength of each potential causality evidence was performed based on the following criteria (18): (a) whether the FDR result was <0.05; (b) whether the estimates of weighted median and MR Presso were statistically significant. The degree of evidence strength for each causal relationship is classified as reliable, insufficient, weak, or potential. We screened for reliable evidence of causality for further multivariate MR analyses to explore whether the screened dietary intakes were still associated with outcomes after excluding BMI, TB-BMD and smoking status, independently. Three common risk factors for osteoarthritis were considered as multivariate models: (a) M1: BMI; (b) M2: TB-BMD (c) M3: ever smoked. In the multivariate MR analyses, we mainly applied the IVW method and the MR Egger method was also used to determine whether the results were pleiotropic or not. The statistical analyses in this study were conducted in R 4.3.0 with the packages "TwoSampleMR," "MendelianRandomization" and "MRPRESSO."

## Genetic correlation analysis

Finally, we performed LDSC analyses on the FDR-corrected positive results of univariate analyses to assess the genetic correlation (Rg) of the 2 phenotypes in each causal pair, using LDSC software package[1] (19, 20). The statistically significant association is defined to be $p < 0.05$.

## Results

In univariate analyses, we analyzed the causal association of 45 dietary intake habits with four different sites of osteoarthritis and two

1 https://github.com/bulik/ldsc

TABLE 1 Description of GWAS data sources for each phenotype.

| Dataset type | Variable | GWAS ID | Sample size | Consortium | Journal | Population | Sex |
|---|---|---|---|---|---|---|---|
| Exposure | Dietary habits | See **Supplementary Table S1** | | MRC-IEU | Nature | European | Males and females |
| | Body mass index (BMI) | ukb-b-19953 | 461,460 | MRC-IEU | Nature | European | Males and females |
| | Total body bone mineral density | ebi-a-GCST005348 | 56,284 | NA | Am J Hum Genet. | European | Males and females |
| | Ever smoked | ukb-b-20261 | 461,066 | MRC-IEU | Nature | European | Males and females |
| Outcome | Knee osteoarthritis (Knee OA) | NA | 396,054 | Genetics of Osteoarthritis (GO) | Cell | European (99%) | Males and females |
| | Hip osteoarthritis (Hip OA) | NA | 353,388 | Genetics of Osteoarthritis (GO) | Cell | European | Males and females |
| | Spine osteoarthritis (Spine OA) | NA | 333,950 | Genetics of Osteoarthritis (GO) | Cell | European (99%) | Males and females |
| | Hand osteoarthritis (Hand OA) | NA | 303,782 | Genetics of Osteoarthritis (GO) | Cell | European | Males and females |
| | Total knee replacement (TKR) | NA | 252,041 | Genetics of Osteoarthritis (GO) | Cell | European | Males and females |
| | Total hip replacement (THR) | NA | 319,037 | Genetics of Osteoarthritis (GO) | Cell | European | Males and females |

types of end-stage osteoarthritis. Out of the 270 results analyzed, we identified 59 potential pieces of evidence for causality without pleiotropy. The results of the causal associations between the 45 dietary intakes and the 6 osteoarthritis phenotypes using IVW method were presented in Figure 2; Supplementary Tables S2–S4, and the results of sensitivity analyses showed in Supplementary Tables S5–S10. Using the FDR method to correct the *p*-values, we identified 23 reliable causal evidence, 8 insufficient causal evidence, and 1 weak causal association, as determined by the results of sensitivity analyses. In reliable causal associations, 6 dietary intake habits were associated with the lower risk of Knee OA: cereal (OR = 0.724, 95%CI:0.617–0.848, $p = 6.34E-05$, FDR = 0.001), muesli (OR = 0.335, 95%CI:0.213–0.526, $p = 2.12E-06$, FDR < 0.001), dried fruit (OR = 0.760, 95%CI:0.642–0.900, $p = 1.50E-03$, FDR = 0.015) and cheese intake (OR = 0.713, 95%CI:0.641–0.794, $p = 7.25E-10$, FDR < 0.001), hot drink temperature (OR = 0.649, 95%CI:0.531–0.792, $p = 2.06E-05$, FDR < 0.001) and alcohol usually taken with meals (OR = 0.682, 95%CI:0.556–0.836, $p = 2.40E-4$, FDR = 0.004), while beef intake (OR = 1.450, 95%CI:1.230–1.711, $p = 9.96E-06$, FDR < 0.001) was positively associated with Knee OA. There were 3 dietary habits associated with Hip OA, all of which could increase the risk of Hip OA: cooked vegetable (OR = 1.431, 95%CI:1.134–1.805, $p = 2.55E-03$, FDR = 0.02), coffee (OR = 1.413, 95%CI:1.137–1.754, $p = 1.79E-03$, FDR = 0.017) and tea intake (OR = 1.247, 95%CI:1.081–1.438, $p = 2.46E-03$, FDR = 0.02). For Spine OA, 3 dietary intakes were associated with decreasing its risk: muesli (OR = 0.330, 95%CI:0.199–0.545, $p = 1.53E-05$, FDR < 0.001), cheese (OR = 0.692, 95%CI:0.616–0.779, $p = 8.89E-10$, FDR < 0.001) and average weekly champagne plus white wine intake (OR = 0.654, 95%CI:0.494–0.866, $p = 3.02E-03$, FDR = 0.022). There were 6 dietary intakes linked to the reduced risk for TKR: cereal (OR = 0.701, 95%CI:0.558–0.880, $p = 2.24E-03$, FDR = 0.018), muesli (OR = 0.174, 95%CI:0.095–0.318, $p = 1.27E-08$, FDR < 0.001), dried fruit (OR = 0.640, 95%CI:0.492–0.832, $p = 8.58E-04$, FDR = 0.012) and cheese intake (OR = 0.647, 95%CI:0.552–0.758, $p = 7.69E-08$, FDR < 0.001), hot drink temperature (OR = 0.597, 95%CI:0.447–0.797, $p = 4.69E-04$, FDR = 0.008) and alcohol usually taken with meals (OR = 0.556, 95%CI:0.388–0.796, $p = 1.37E-03$, FDR = 0.015), while beef (OR = 1.956, 95%CI:1.440–2.657, $p = 1.78E-05$, FDR < 0.001) and coffee intake (OR = 1.746, 95%CI:1.328–2.295, $p = 6.50E-05$, FDR = 0.001) were positively associated. Besides, white bread type intake (OR = 0.532, 95%CI:0.333–0.849, $p = 8.19E-03$, FDR = 0.049) and hot drink temperature (OR = 0.645, 95%CI:0.496–0.837, $p = 9.91E-04$, FDR = 0.013) were associated with a decrease in risk of THR. There were 8 insufficient evidence for Knee OA, Spine OA and TKR. Coffee intake (OR = 1.325, 95%CI:1.112–1.577, $p = 1.60E-03$, FDR = 0.015), average weekly beer plus cider intake (OR = 1.385, 95%CI:1.109–1.729, $p = 4.03E-03$, FDR = 0.028) and salt added to food (OR = 1.168, 95%CI:1.039–1.314, $p = 9.56E-03$, FDR = 0.052) were associated with a increase in risk of Knee OA, while cereal intake (OR = 0.751, 95%CI:0.628–0.897, $p = 1.55E-03$, FDR = 0.015), semi-skimmed milk type used (OR = 0.211, 95%CI:0.078–0.573, $p = 2.24E-03$, FDR = 0.018) and alcohol usually taken with meals (OR = 0.690, 95%CI:0.528–0.902, $p = 6.73E-03$, FDR = 0.042) were associated with a decrease in risk of Spine OA. For TKR, wholemeal or wholegrain bread type (OR = 0.472, 95%CI:0.285–0.78, $p = 3.43E-03$, FDR = 0.024) was associated with decreasing its risk, while pork intake (OR = 1.874, 95%CI: 1.174–2.993, $p = 8.51E-03$, FDR = 0.049) might increase the risk. And there was a weak evidence of negative associations between biscuit cereal intake and Hip OA (OR = 0.348, 95%CI: 0.165–0.735, $p = 5.68E-03$, FDR = 0.037). FDR results of associations between dietary habits and subtypes related to osteoarthritis showed in Supplementary Table S11. Figure 3 illustrates the assessment of the strength of potential causal associations after *p*-value correction and sensitivity analysis, showing the results using the IVW method.

The multivariate analyses were further analyzed for the 23 reliable causal relationships screened. After adjusting separately for BMI, TB-BMD, and ever smoked, which were common risk factors for osteoarthritis, 8 causality estimates remained statistically significant: the effects of muesli intake on Knee OA, Spine OA and TKR; the effects of dried fruit intake on Knee OA and TKR; the effects of eating cheese on Knee OA and Spine OA; and the effect of alcohol usually taken with meals on TKR. Consistent with the results of univariate MR analyses, the multivariable MR analysis results supported that the 4 dietary intake habits above were protective against OA without pleiotropy in MR Egger intercept tests. The results of multivariable MR were shown in Figure 4; Supplementary Table S12.

Finally, we performed the LDSC analyses on all positive results corrected for FDR. The LDSC results showed significant genetic correlations for all causal relationships involving the Knee OA, Spine OA and TKR phenotypes, which genetically validated the potential for

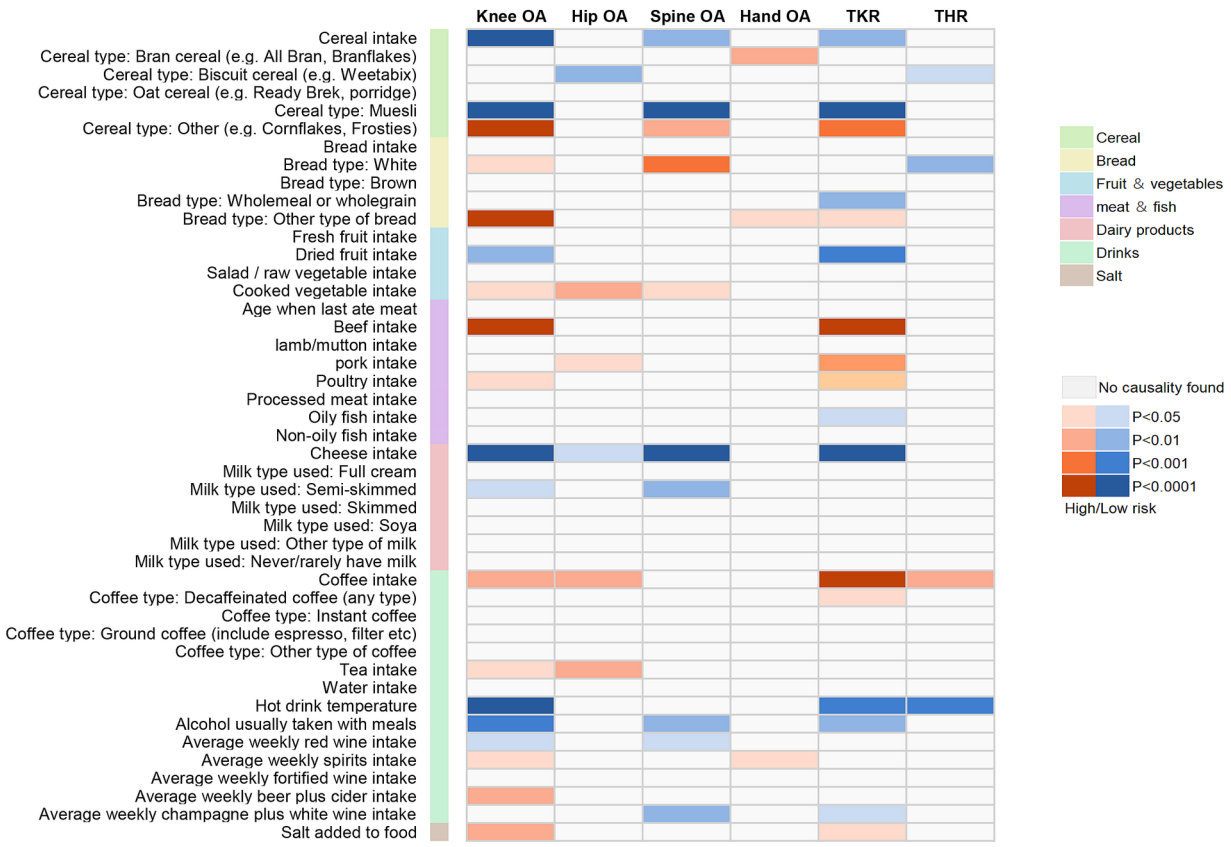



FIGURE 2
Mendelian randomization associations between the 45 dietary intakes and the 6 osteoarthritis phenotypes using IVW method.

dietary management of osteoarthritis of the knee and osteoarthritis of the spine. And we also found that of the 8 causal relationships ultimately screened by multivariate analyses, all exposures were also significantly genetically correlated with their respective corresponding outcomes, as shown in Table 2. All results of LDSC analyses were shown in Supplementary Table S13.

# Discussion

## Principal findings

We analyzed the overall effect of 45 common dietary intake habits on 6 subtypes related to osteoarthritis and identified 59 potential causal associations, including 23 reliable associations, 8 causally insufficient associations, and 1 weak association. We found that cheese intake was negatively associated with the risk of most osteoarthritis phenotypes, whereas coffee intake was positively associated. From the osteoarthritis perspective, we found that the occurrence of Knee OA and TKR (end-stage Knee OA) was influenced by many dietary factors. Some dietary intakes were associated with risk of Spinal OA, while most dietary intake habits were not significantly associated with other osteoarthritis phenotypes. Using multivariate analyses, we identified that 4 dietary intake habits (muesli, dried fruit, cheese intake and alcohol usually taken with meals) were associated with a reduced risk of osteoarthritis and that there might be underlying mechanisms other than those influenced through BMI, TB-BMD and smoking status. And the results of LDSC showed significant genetic correlations in each of the 8 causal pairs.

## Comparisons with other studies

For the effect of coffee and tea intake on OA, our findings are consistent with previous similar univariate MR studies (10, 11). For alcohol intake, a previous MR study found the frequency of alcohol intake to be a risk factor for hip and knee osteoarthritis (12). The exposure of our study regarding alcohol intake was "alcohol usually taken with meals," which was found to be a protective factor for KOA, TKR and THR. Subsequently when we categorized the varieties of alcohol in detail, we discovered that different varieties of alcohol had different effects on osteoarthritis: red wine and champagne plus white wine played a potentially protective role in osteoarthritis, whereas spirits and beer plus cider might play a potentially harmful role in osteoarthritis. The reason for the inconsistency with previous MR analyses regarding alcohol intake is that previous studies did not differentiate between types of alcohol and the outcome phenotype did not differentiate between hip and knee osteoarthritis. In contrast, our study differentiated osteoarthritis at each site in detail and differentiated dietary categories in as much detail as possible while ensuring that IVs were sufficient. We found no other MR studies exploring the causal relationship between dietary factors and osteoarthritis.

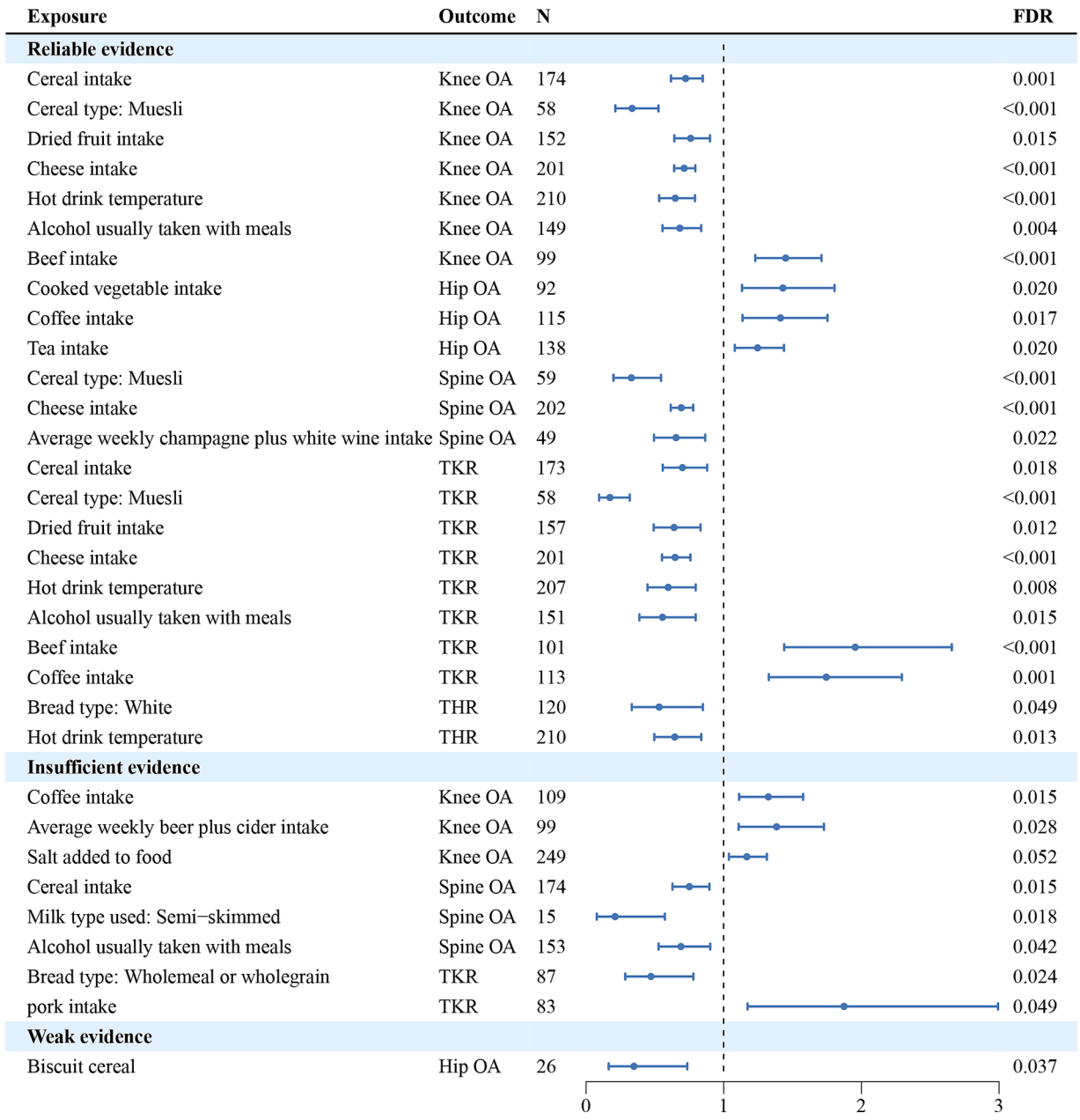



FIGURE 3
The assessment of the strength of potential causal associations after *p*-value correction and sensitivity analysis, showing the results using the IVW method. N denotes the number of SNPs used for each exposure.

In previous observational analyses, higher whole grain intake was found to be related with a lower risk of knee osteoarthritis (6), which is consistent with our findings. In addition, a recent study covering 47 dairy products found a significant negative association between cheese and whole milk intake and Knee OA (7). This is consistent with the results of our analyses of the association between cheese intake and Knee OA, but we did not find a genetically predicted significant association between whole milk intake and Knee OA; instead, the potential effect of semi-skimmed milk on Knee OA and Spine OA was identified. For meat intake, a study found that the high intake of fresh red meat reduced the risk of THR (21), however, the similar association was not found in our study and we found that most of the meat intake might increase the risk of OA, with the positive association between beef intake on Knee OA and TKR being reliable evidence. It is possible that these differences in findings are the result of bias due to the inability of observational studies to completely avoid confounders.

## Potential mechanisms

In previous studies, grains have been shown to be a beneficial dietary factor in the management of chronic diseases, with positive effects on a number of cancers, cardiovascular diseases and overweight

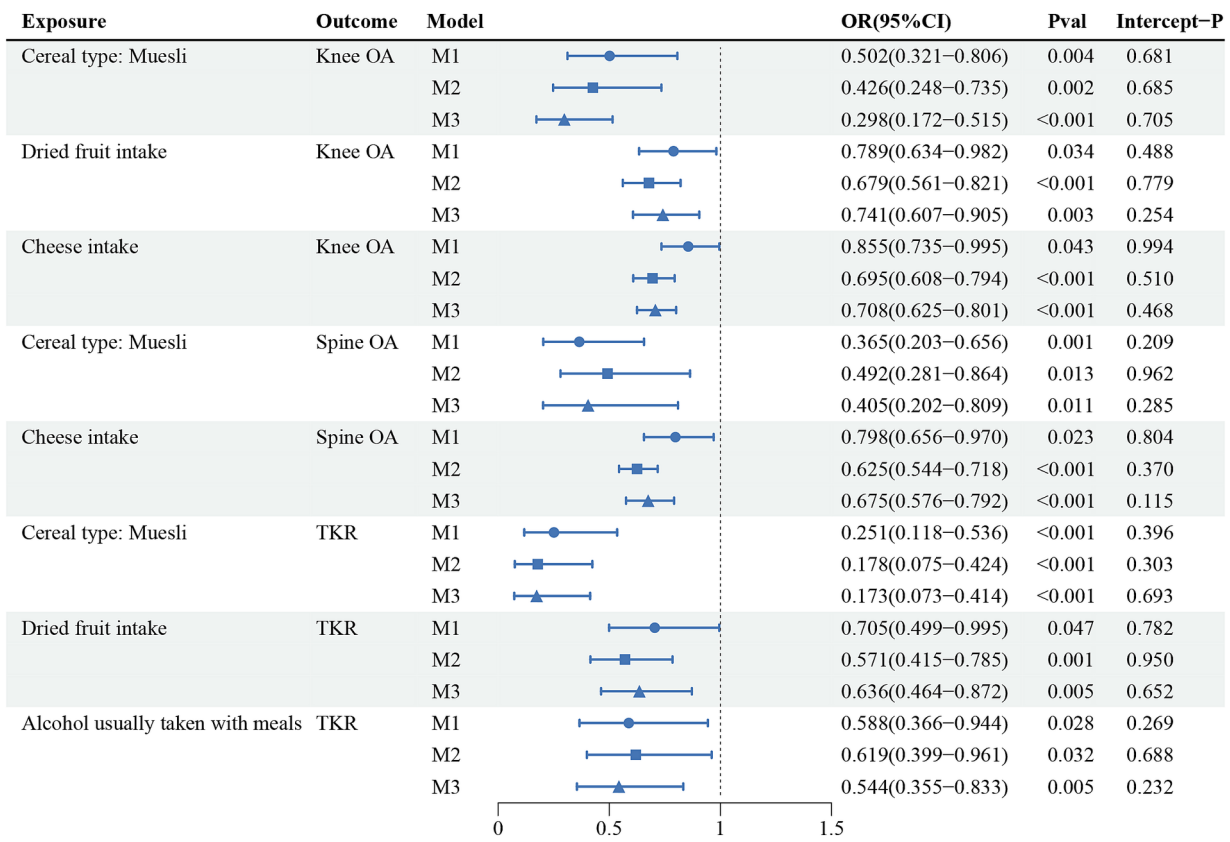



FIGURE 4
The causal relationships that still held after further multivariate analyses of 23 reliable evidence.

TABLE 2 LDSC analysis results of 8 causal associations in Figure 4.

| Phenotype 1 | Phenotype 2 | Rg | Se | Pval |
|---|---|---|---|---|
| Cereal type: Muesli | Knee OA | −0.277 | 0.037 | 4.48E-14 |
| Dried fruit intake | Knee OA | −0.155 | 0.032 | 1.64E-06 |
| Cheese intake | Knee OA | −0.208 | 0.029 | 9.67E-13 |
| Cereal type: Muesli | Spine OA | −0.331 | 0.048 | 5.87E-12 |
| Cheese intake | Spine OA | −0.327 | 0.046 | 7.73E-13 |
| Cereal type: Muesli | TKR | −0.307 | 0.042 | 1.60E-13 |
| Dried fruit intake | TKR | −0.212 | 0.037 | 7.79E-09 |
| Alcohol usually taken with meals | TKR | −0.206 | 0.034 | 1.15E-09 |

Rg, genetic correlation.

and obesity (22–24). Cereals provide the body with soluble fiber b-glucan, which not only reduces serum cholesterol, but also controls blood glucose levels and insulin response and thus indirectly controls BMI (25). In our study total cereal intake was significantly associated with Knee OA and TKR, and the results were found to be non-significant after adjusting for BMI, suggesting that BMI played an important mediating role in this association. However, the protective effect of muesli on Knee OA, Spine OA and TKR remained significant after controlling for the 3 risk factors, suggesting other possible mechanisms for the effect of grains on osteoarthritis. Muesli is rich in phenolic substances as well as flavonoids, which can exert antioxidant properties in the human body through mechanisms such as 2,2-diphenyl-1-trinitrohydrazine free radical scavenging and inhibition of iron chelating activity (26). A study examined total phenols, flavonoids, and antioxidant activity in cereal flakes and found that flakes and muesli made from Dickkopf wheat and red wheat showed the highest total phenolics and flavonoids content as well as antioxidant activity, whereas the lowest total phenolics and flavonoids content and antioxidant activity were measured in commercial flakes and muesli (27). The study might explain the results of our study in which there was a potential positive correlation between bran cereal and the risk of Hand OA.

Conventional views suggest that fresh fruits are beneficial for chronic diseases including osteoarthritis, however, our study did not find a significant association between fresh fruits and OA, but instead unexpectedly found that dried fruit intake was negatively associated with Knee OA and TKR risk. Dried fruits are obtained through a series of drying techniques on fresh fruits and their nutrient content remains similar to the equivalent fresh fruits but more concentrated (28, 29). From a nutritional point of view, a study comparing the nutrient content of dried and fresh fruits found that drying treatments concentrated polyphenol content and consequently antioxidant activity, and it was found that drying techniques might have differential concentration effects on different fruit varieties in dried fruits (30). For the effects of dried fruits on osteoarthritis, Basu et al. investigated the effects of dietary freeze-dried strawberries on obesity-related hormones, inflammatory biomarkers, and lipid peroxidation, and found that freeze-dried strawberries not only lowered serum

TNF-α levels as well as some of the lipid peroxidation products in obese patients with knee OA (31), but also played a role in reducing knee pain. In addition, a large cross-sectional study based on NHANES showed that sultana consumers had higher whole grain intake, lower added sugar intake, and healthier overall diets (32), which may also be a potential factor in the negative association of dried fruit intake with the risk of developing osteoarthritis.

Cheese has long been favored as a healthy diet rich in protein as well as minerals. It has beneficial effects on the musculoskeletal system, and many studies have shown that daily dietary dairy intake significantly increases bone mineral content (33–35) and that cheese intake has a greater effect on increasing bone mass compared to taking calcium supplements (36). In addition to increasing bone mineral content, cheese is rich in whey protein, in which amino acid composition is very similar to that of skeletal muscle, which makes it an effective anabolic supplement for maintaining and building muscle mass (37). More and more studies are beginning to focus on the role of skeletal muscle in osteoarthritis, but it is not clear whether the effect of cheese intake on osteoarthritis is related to skeletal muscle (38, 39). In addition, cheese also produces bioactive compounds with antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties during ripening and leads to changes in the intestinal flora after consumption, which may play a significant role as a preventive measure against osteoarthritis (40).

The effect of alcohol consumption on osteoarthritis in previous observational studies has been less consistent, probably mainly because of the plethora of confounding factors associated with alcohol consumption. It has been shown that excessive alcohol consumption can worsen osteoarthritis by increasing synovial fluid uric acid levels and elevating pro-inflammatory cytokine levels (41). On the contrary, several *in vitro* experiments have demonstrated that resveratrol in wine can have a protective effect on articular cartilage (42, 43) and polyphenols in alcoholic beverages have been shown to modulate the human intestinal flora and increase antioxidant activity (44), which supports the conclusions of our study. The association between alcohol consumption and osteoarthritis may require larger sample sizes and more detailed categorization in future studies. Our study also found a causal relationship between hot drink temperature and osteoarthritis, which was an interesting result. Hot drinking temperature is usually associated with metabolism, but to the best of our knowledge, the relationship between hot drinking temperature and osteoarthritis is not currently reported. A recent study provided experimental evidence of the effect of daily drinking temperature on cognitive function and the development of Alzheimer's disease in mice (45). This study found that drinking water at 0°C reduces pepsin activity, leading to impaired function of the insulin signaling pathway, which may also be a potential metabolic mechanism by which the temperature of hot drinks affects osteoarthritis. In a rat model, it has been demonstrated that caffeine has a detrimental effect on chondrocytes by a mechanism that may be related to endogenous adenosine modulation and a reduction in circulating IGF-1 (46), which may be a potential mechanism by which coffee and tea promote the development of osteoarthritis. Furthermore, it has been shown that higher meat and total protein intake is associated with inflammatory polyarthritis (47). Although red meat is a rich source of protein, it is also rich in saturated fats and other unhealthy substances. It seems to be an accepted fact that excessive intake of red meat and salt may lead to a series of metabolic disorders in the body such as hypertension and hyperlipidaemia thereby increasing the risk of chronic diseases.

## Strength and limitations

To the best of our knowledge, this is the first study to investigate the causal relationship between multiple dietary intakes and osteoarthritis in various parts of the body by means of MR analysis, which can avoid confounding factors as well as reverse causality. The majority of previous studies on diet and osteoarthritis have focused on the impact of obesity on Knee OA due to dietary factors, with a small number of studies exploring the effect of a particular dietary intake on Knee OA. Our study took advantage of MR to include 45 dietary intakes as well as 6 osteoarthritis phenotypes, with exposure and outcome data from the largest sample size dataset available. And after controlling for recognized risk factors such as BMI, we found that certain dietary intakes were still associated with their counterparts in osteoarthritis, implying that there are more other potential mechanisms of dietary influence on osteoarthritis that need to be explored in future studies. In terms of dietary intake, we identified several new factors associated with osteoarthritis, such as dried fruit intake, hot drink temperature, and different drinking habits; and for osteoarthritis, we identified the potential for knee and spine osteoarthritis to be prevented, as well as managed, through diet. Our study provides evidence and references for future studies exploring the in-depth mechanisms of diet and osteoarthritis.

Our study also has some limitations. Firstly, due to lack of the age and gender information from the summary-level GWAS results, this study cannot conduct a stratified analysis of OA based on these factors. Secondly, dietary habits were assessed using a touch-screen questionnaire, which may lead to bias in the assessment as well as in the analyses. Thirdly, Causal associations with pleiotropy or inconsistent directions of the 4 MR analysis methods were not processed further, but rather excluded from the results by directly defining that no association was found, but this does not indicate that these dietary habits are absolutely unrelated to OA. Fourthly, dietary intake habits were used as the exposure phenotype and we did not examine the effect of nutrients on OA. However, a recent study emphasized the matrix effect produced by food (48). Food is complex and not just the sum of certain nutrients. Several studies have found that dairy products have a higher effect on skeletal bone mass than calcium supplements (49, 50), which also emphasizes the overall effect of food on health. Food can also influence health by modulating gut microbes. The gut-bone and gut-muscle axes have been demonstrated, and the gut microbes have an impact on bone beyond calcium and protein intake (51). In the future, we will give more attention to the diet-gut-bone/muscle axis to further explore in depth the potential mechanisms that exist. Fifthly, although our study provides statistical clues to the relationship between diet and OA, and a theoretical basis for subsequent experimental studies and mechanistic explorations, our results should be interpreted carefully. And a combination of evidence from multiple sources such as well-established trials and clinical findings is needed to confirm our findings in the future.

# Conclusion

Our study provides relatively solid evidence for a causal relationship between dietary factors and osteoarthritis. Most

importantly, we identified that cereals, dried fruits, cheese and alcohol usually taken with meals intake remained negatively associated with some OA risk after controlling for BMI, TB-BMD and smoking status. In addition, we found the potential for multiple dietary interventions, management and prevention of knee and spine osteoarthritis. Our study has important implications for the prevention, management, and intervention of osteoarthritis in common sites through rational dietary modification.

## Data availability statement

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

## Author contributions

ZX: Data curation, Investigation, Methodology, Visualization, Writing – original draft. YQ: Supervision, Writing – review & editing.

## Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

## Acknowledgments

We are grateful to the IEU Open GWAS Project and the Genetics of Osteoarthritis (GO) consortium for providing summary statistics.

## 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.2023.1278079/full#supplementary-material

## References

1. Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, et al. Osteoarthritis. *Nat Rev Dis Primers*. (2016) 2:16072. doi: 10.1038/nrdp.2016.72

2. Kraus VB, Jordan JM, Doherty M, Wilson AG, Moskowitz R, Hochberg M, et al. The genetics of generalized osteoarthritis (GOGO) study: study design and evaluation of osteoarthritis phenotypes. *Osteoarthr Cartil*. (2007) 15:120–7. doi: 10.1016/j.joca.2006.10.002

3. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet*. (2019) 393:1745–59. doi: 10.1016/S0140-6736(19)30417-9

4. Dai Z, Niu J, Zhang Y, Jacques P, Felson DT. Dietary intake of fibre and risk of knee osteoarthritis in two US prospective cohorts. *Ann Rheum Dis*. (2017) 76:1411–9. doi: 10.1136/annrheumdis-2016-210810

5. Thomas S, Browne H, Mobasheri A, Rayman MP. What is the evidence for a role for diet and nutrition in osteoarthritis? *Rheumatology (Oxford)*. (2018) 57:iv61–74. doi: 10.1093/rheumatology/key011

6. Liu T, Xu C, Driban JB, Liang GY, Zhang XH, Hu FB, et al. Whole grain consumption and risk of radiographic knee osteoarthritis: a prospective study from the osteoarthritis initiative. *Rheumatology (Oxford)*. (2023) 62:1834–40. doi: 10.1093/rheumatology/keac517

7. Denissen KFM, Boonen A, Nielen JTH, Feitsma AL, van den Heuvel EGHM, Emans PJ, et al. Consumption of dairy products in relation to the presence of clinical knee osteoarthritis: the Maastricht study. *Eur J Nutr*. (2019) 58:2693–704. doi: 10.1007/s00394-018-1818-7

8. Lu B, Driban JB, Duryea J, McAlindon T, Lapane KL, Eaton CB. Milk consumption and progression of medial tibiofemoral knee osteoarthritis: data from the osteoarthritis initiative. *Arthritis Care Res (Hoboken)*. (2014) 66:802–9. doi: 10.1002/acr.22297

9. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol*. (2016) 27:3253–65. doi: 10.1681/ASN.2016010098

10. Lee YH. Investigating the possible causal association of coffee consumption with osteoarthritis risk using a Mendelian randomization analysis. *Clin Rheumatol*. (2018) 37:3133–9. doi: 10.1007/s10067-018-4252-6

11. Li G, Zhang Z, Liu Y. Genetically predicted tea intake increases the risk of osteoarthritis: a Mendelian randomization study. *Front Genet*. (2022) 13:1004392. doi: 10.3389/fgene.2022.1004392

12. Wen MT, Liang XZ, Luo D, Li JC, Yan BZ, Lu BW, et al. Plasma lipids, alcohol intake frequency and risk of osteoarthritis: a Mendelian randomization study. *BMC Public Health*. (2023) 23:1327. doi: 10.1186/s12889-023-16250-1

13. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK biobank resource with deep phenotyping and genomic data. *Nature*. (2018) 562:203–9. doi: 10.1038/s41586-018-0579-z

14. Boer CG, Hatzikotoulas K, Southam L, Stefánsdóttir L, Zhang Y, Coutinho de Almeida R, et al. Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. *Cells*. (2021) 184:4784–4818.e17. doi: 10.1016/j.cell.2021.07.038

15. Hemani G, Zheng J, Elsworth B, Wade KH, 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. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol*. (2011) 40:740–52. doi: 10.1093/ije/dyq151

17. Yuan S, Kim JH, Xu P, Wang Z. Causal association between celiac disease and inflammatory bowel disease: a two-sample bidirectional Mendelian randomization study. *Front Immunol*. (2023) 13:1057253. doi: 10.3389/fimmu.2022.1057253

18. Zheng J, Zhang Y, Rasheed H, Walker V, Sugawara Y, Li J, et al. Trans-ethnic Mendelian-randomization study reveals causal relationships between cardiometabolic factors and chronic kidney disease. *Int J Epidemiol*. (2022) 50:1995–2010. doi: 10.1093/ije/dyab203

19. Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh PR, et al. An atlas of genetic correlations across human diseases and traits. *Nat Genet*. (2015) 47:1236–41. doi: 10.1038/ng.3406. Epub 2015 Sep 28

20. Bulik-Sullivan B, Loh PR, Finucane HK, Ripke S, Yang J, Patterson N, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. (2015) 47:291–5. doi: 10.1038/ng.3211. Epub 2015 Feb 2

21. Wang Y, Simpson JA, Wluka AE, English DR, Giles GG, Graves S, et al. Meat consumption and risk of primary hip and knee joint replacement due to osteoarthritis: a prospective cohort study. *BMC Musculoskelet Disord*. (2011) 12:17. doi: 10.1186/1471-2474-12-17

22. Makarem N, Nicholson JM, Bandera EV, McKeown NM, Parekh N. Consumption of whole grains and cereal fiber in relation to cancer risk: a systematic review of longitudinal studies. *Nutr Rev*. (2016) 74:353–73. doi: 10.1093/nutrit/nuw003

23. Kashino I, Eguchi M, Miki T, Kochi T, Nanri A, Kabe I, et al. Prospective association between whole grain consumption and hypertension: the Furukawa nutrition and health study. *Nutrients*. (2020) 12:902. doi: 10.3390/nu12040902

24. Kissock KR, Neale EP, Beck EJ. Whole grain food definition effects on determining associations of whole grain intake and body weight changes: a systematic review. *Adv Nutr*. (2021) 12:693–707. doi: 10.1093/advances/nmaa122

25. Tosh SM, Bordenave N. Emerging science on benefits of whole grain oat and barley and their soluble dietary fibers for heart health, glycemic response, and gut microbiota. *Nutr Rev*. (2020) 78:13–20. doi: 10.1093/nutrit/nuz085

26. Van Hung P. Phenolic compounds of cereals and their antioxidant capacity. *Crit Rev Food Sci Nutr*. (2016) 56:25–35. doi: 10.1080/10408398.2012.708909

27. Sumczynski D, Bubelova Z, Sneyd J, Erb-Weber S, Mlcek J. Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. *Food Chem*. (2015) 174:319–25. doi: 10.1016/j.foodchem.2014.11.065

28. Mossine VV, Mawhinney TP, Giovannucci EL. Dried fruit intake and Cancer: a systematic review of observational studies. *Adv Nutr*. (2020) 11:237–50. doi: 10.1093/advances/nmz085

29. Alasalvar C, Salvadó JS, Ros E. Bioactives and health benefits of nuts and dried fruits. *Food Chem*. (2020) 314:126192. doi: 10.1016/j.foodchem.2020.126192

30. Sadler MJ, Gibson S, Whelan K, Ha MA, Lovegrove J, Higgs J. Dried fruit and public health - what does the evidence tell us? *Int J Food Sci Nutr*. (2019) 70:675–87. doi: 10.1080/09637486.2019.1568398

31. Basu A, Kurien BT, Tran H, Byrd B, Maher J, Schell J, et al. Strawberries decrease circulating levels of tumor necrosis factor and lipid peroxides in obese adults with knee osteoarthritis. *Food Funct*. (2018) 9:6218–26. doi: 10.1039/c8fo01194j

32. Fulgoni VL 3rd, Painter J, Carughi A. Association of raisin consumption with nutrient intake, diet quality, and health risk factors in US adults: National Health and nutrition examination survey 2001-2012. *Food Nutr Res*. (2017) 61:1378567. doi: 10.1080/16546628.2017.1378567

33. Cheng S, Lyytikäinen A, Kröger H, Lamberg-Allardt C, Alén M, Koistinen A, et al. Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10-12-y-old girls: a 2-y randomized trial. *Am J Clin Nutr*. (2005) 82:1115–26; quiz 1147-8. doi: 10.1093/ajcn/82.5.1115

34. Rizzoli R, Biver E. Effects of fermented Milk products on bone. *Calcif Tissue Int*. (2018) 102:489–500. doi: 10.1007/s00223-017-0317-9

35. Moschonis G, van den Heuvel EG, Mavrogianni C, Manios Y. Effect of vitamin D-enriched gouda-type cheese consumption on biochemical markers of bone metabolism in postmenopausal women in Greece. *Nutrients*. (2021) 13:2985. doi: 10.3390/nu13092985

36. de Lamas C, de Castro MJ, Gil-Campos M, Gil Á, Couce ML, Leis R. Effects of dairy product consumption on height and bone mineral content in children: a systematic review of controlled trials. *Adv Nutr*. (2019) 10:S88–96. doi: 10.1093/advances/nmy096

37. Cuesta-Triana F, Verdejo-Bravo C, Fernández-Pérez C, Martín-Sánchez FJ. Effect of Milk and other dairy products on the risk of frailty, sarcopenia, and cognitive performance decline in the elderly: a systematic review. *Adv Nutr*. (2019) 10:S105–19. doi: 10.1093/advances/nmy105

38. Krishnasamy P, Hall M, Robbins SR. The role of skeletal muscle in the pathophysiology and management of knee osteoarthritis. *Rheumatology (Oxford)*. (2018) 57:iv22–33. doi: 10.1093/rheumatology/kex515

39. Siqueira MS, Souto LR, Martinez AF, Serrão FV, de Noronha M. Muscle activation, strength, and volume in people with patellofemoral osteoarthritis: a systematic review and meta-analysis. *Osteoarthr Cartil*. (2022) 30:935–44. doi: 10.1016/j.joca.2022.01.013

40. Santiago-López L, Aguilar-Toalá JE, Hernández-Mendoza A, Vallejo-Cordoba B, Liceaga AM, González-Córdova AF. Invited review: bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption. *J Dairy Sci*. (2018) 101:3742–57. doi: 10.3168/jds.2017-13465

41. Denoble AE, Huffman KM, Stabler TV, Kelly SJ, Hershfield MS, McDaniel GE, et al. Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation. *Proc Natl Acad Sci U S A*. (2011) 108:2088–93. doi: 10.1073/pnas.1012743108

42. Csaki C, Keshishzadeh N, Fischer K, Shakibaei M. Regulation of inflammation signalling by resveratrol in human chondrocytes *in vitro*. *Biochem Pharmacol*. (2008) 75:677–87. doi: 10.1016/j.bcp.2007.09.014

43. Liu FC, Hung LF, Wu WL, Chang DM, Huang CY, Lai JH, et al. Chondroprotective effects and mechanisms of resveratrol in advanced glycation end products-stimulated chondrocytes. *Arthritis Res Ther*. (2010) 12:R167. doi: 10.1186/ar3127

44. Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, et al. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am J Clin Nutr*. (2012) 95:1323–34. doi: 10.3945/ajcn.111.027847

45. Wei JP, Wen W, Dai Y, Qin LX, Wen YQ, Duan DD, et al. Drinking water temperature affects cognitive function and progression of Alzheimer's disease in a mouse model. *Acta Pharmacol Sin*. (2021) 42:45–54. doi: 10.1038/s41401-020-0407-5

46. Guillán-Fresco M, Franco-Trepat E, Alonso-Pérez A, Jorge-Mora A, López-Fagúndez M, Pazos-Pérez A, et al. Caffeine, a risk factor for osteoarthritis and longitudinal bone growth inhibition. *J Clin Med*. (2020) 9:1163. doi: 10.3390/jcm9041163

47. Choi HK. Dietary risk factors for rheumatic diseases. *Curr Opin Rheumatol*. (2005) 17:141–6. doi: 10.1097/01.cco.0000152664.87204.3c

48. Geiker NRW, Mølgaard C, Iuliano S, Rizzoli R, Manios Y, van Loon LJC, et al. Impact of whole dairy matrix on musculoskeletal health and aging-current knowledge and research gaps. *Osteoporos Int*. (2020) 31:601–15. doi: 10.1007/s00198-019-05229-7

49. Weaver CM, Janle E, Martin B, Browne S, Guiden H, Lachcik P, et al. Dairy versus calcium carbonate in promoting peak bone mass and bone maintenance during subsequent calcium deficiency. *J Bone Miner Res*. (2009) 24:1411–9. doi: 10.1359/jbmr.090303

50. Faghih S, Abadi AR, Hedayati M, Kimiagar SM. Comparison of the effects of cows' milk, fortified soy milk, and calcium supplement on weight and fat loss in premenopausal overweight and obese women. *Nutr Metab Cardiovasc Dis*. (2011) 21:499–503. doi: 10.1016/j.numecd.2009.11.013

51. Rizzoli R. Nutritional influence on bone: role of gut microbiota. *Aging Clin Exp Res*. (2019) 31:743–51. doi: 10.1007/s40520-019-01131-8

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 04 December 2023
DOI 10.3389/fnut.2023.1284497



OPEN ACCESS

EDITED BY
Attilio Parisi,
Foro Italico University of Rome, Italy

REVIEWED BY
Joshua Wooten,
Southern Illinois University Edwardsville, United States
Ciarán Eoin Fealy, United Kingdom

*CORRESPONDENCE
Osamu Ezaki
✉ ezaki1952@yahoo.co.jp

RECEIVED 28 August 2023
ACCEPTED 14 November 2023
PUBLISHED 04 December 2023

CITATION
Ezaki O and Abe S (2023) Medium-chain triglycerides (8:0 and 10:0) increase muscle mass and function in frail older adults: a combined data analysis of clinical trials. *Front. Nutr.* 10:1284497. doi: 10.3389/fnut.2023.1284497



# Medium-chain triglycerides (8:0 and 10:0) increase muscle mass and function in frail older adults: a combined data analysis of clinical trials

Osamu Ezaki[1]* and Sakiko Abe[2]

[1]Institute of Women's Health Science, Showa Women's University, Tokyo, Japan, [2]Department of Food and Nutrition, Faculty of Contemporary Human Life Science, Tezukayama University, Nara, Japan

**Background:** Three clinical trials have examined the chronic effects of medium-chain triglycerides (MCTs) on muscle mass and function in frail older adults (mean age 85 years old). However, significant increases in muscle mass and some muscle function relative to long-chain triglycerides (LCTs) have yet to be shown, possibly due to the small number of participants in each trial.

**Objective:** We re-analyzed these previous clinical trials to clarify whether MCT supplementation can increase muscle mass and function.

**Analysis:** After adding *post hoc* tests to the original report, we compared changes in measurement between the MCT and LCT groups in the first 2 trials and conducted a combined data analysis.

**Methods:** In a combined data analysis, changes from baseline in measurements at the 3 months intervention in the MCTs- and LCTs-containing groups were assessed by analysis of covariance adjusted for baseline values of each measurement, age, sex, BMI, allocation to trial, habitual intakes in energy, protein, leucine, octanoic acid, decanoic acid, and vitamin D during the baseline period. The Mann–Whitney U test was used to analyze data on right and left knee extension times.

**Results:** MCT supplementation for 3 months increased muscle function relative to LCT supplementation with and without an L-leucine (1.2 g) and vitamin D (cholecalciferol, 20 μg)-enriched supplement. In a combined data analysis ($n = 29$ in MCTs, $n = 27$ in LCTs), relative to supplementation with 6 g LCTs/day, supplementation with 6 g MCTs/day at dinner for 3 months significantly increased body weight (adjusted mean change from baseline: MCTs 1.2 vs. LCTs 0.2 kg, $p = 0.023$), right arm muscle area (MCTs 1.4 vs. LCTs-0.7 $cm^2$, $p = 0.002$), left calf circumference ($p = 0.015$), right-hand grip strength (MCTs 1.6 vs. LCTs 0.3 kg, $p = 0.017$), right knee extension time ($p = 0.021$), left knee extension time ($p = 0.034$), walking speed ($p = 0.002$), and number of iterations in leg open and close test ($p < 0.001$) and decreased right triceps skinfold thickness ($p = 0.016$).

**Conclusion:** In frail older adults, supplementation for 3 months with a low dose (6 g/day) of MCTs (C8:0 and C10:0) increased muscle mass and function. These findings indicate the potential for the practical use of MCTs in daily life in treating sarcopenia.

KEYWORDS

**ketone body, ghrelin, frailty, sarcopenia, adipocyte triglyceride lipase, medium chain fatty acid, muscle mass, medium chain triglyceride**

# 1 Introduction

Sarcopenia is characterized by the low skeletal muscle mass, strength, and function observed with aging (1). Exercise training and ingestion of adequate amounts of proteins are considered to be two primary lifestyles to slow the progression of sarcopenia (2). However, exercise training becomes difficult because of frailty, as does dietary intake, because of decreased appetite and digestive activity. Therefore, small amounts of foods or supplements that can be easily eaten and would maintain muscle mass and function are needed.

In an attempt to search for such nutrients, we found through a series of clinical trials that supplementation with a low dose of medium-chain triglycerides (MCTs) (6 g/day) may increase muscle mass and function without altering body weight in frail older adults (3–5). To compare the effects of the MCTs with those of long-chain triglycerides (LCTs), the changes from baseline between groups after interventions were compared in multiple groups (3, 4). In one trial, following 3 months of intervention, the increases in the numbers of iterations in the leg open and close test and the numbers of swallows were significantly greater in the MCT group than in the LCT group. However, the statistical significance of the increases in right arm muscle area (AMA), right-hand grip strength, right and left knee extension time, walking speed, and peak expiratory flow in the MCT group was not examined by a *post hoc* test because the fixed effect of the group by time was nonsignificant in a mixed-effect model, although these measurements in the MCT group showed a tendency to increase compared with those of LCT group (4). We re-analyzed the previous data by allowing the conduction of a *post hoc* test when the fixed effect of the group was significant, and the results were compared between trials to obtain a more accurate conclusion. In addition, combining the data in trials, a new analysis was conducted to compare the groups, including the MCTs and LCTs.

# 2 Materials and methods

## 2.1 Outline of clinical trials

Three clinical trials were conducted in a nursing home (Day Care SKY facility in Yokohama, Japan), and their protocols and methods were described in detail (3–7). We targeted all participants (mean age around 85 years) who resided in this nursing home and required special care from a helper. They were selected by the application of the exclusion criteria before enrollment as described previously (3–5). All trials were randomized, controlled (negative or positive control), single-blinded (or double-blinded for some measurements), and parallel-group trials. Each trial had a different purpose, group, and intervention period (Table 1). Six grams of MCTs (75% C8:0, 25% C10:0) per day were mixed with foods at mealtime in all trials. This article focuses on muscle mass and function in response to MCT supplementation. Therefore, we did not describe the cognition of the subjects in detail here.

The first trial (Trial 1), which started in September 2014 and ended in December 2014 (3, 6), was conducted to find a combination of nutrients that could treat sarcopenia. The participants ($n = 38$) were randomly allocated to three groups: the first group received an L-leucine (1.2 g) and vitamin D (cholecalciferol, 20 μg)-enriched supplement with 6 g of MCTs (LD + MCT); the second group received the same energy-matched supplement with 6 g of LCTs (LD + LCT); and the third group did not receive any supplements (no-supplements). The supplement and oils were taken at dinner. After three months of intervention, the LD + MCT participants had increased right-hand grip strength, walking speed, 10 s leg open and close test performance, and peak expiratory flow from baseline. In contrast, no significant improvements were observed in muscle function of the LD + LCT and no-supplements groups. Therefore, it was concluded that MCTs (6 g/d) played a pivotal role in the increase in muscle function in frail older individuals.

Trial 2 started in September 2016 and ended in February 2017 (4, 7). This trial aimed to clarify whether the favorable effects observed in the LD + MCT group in Trial 1 were due to MCT itself or the interaction between MCT and LD. The participants ($n = 64$) were randomly allocated to three groups: LD + MCT (positive control), MCT only (target), and LCT only (negative control). Each participant's body weight, appendicular muscle mass, and function were assessed at four equally spaced time points: baseline, 1.5 and 3.0 months after initiation of the intervention (intervention), and 1.5 months after termination of the intervention (washout). MCT (6 g/d) supplementation alone increased the muscle function of frail older individuals, suggesting that MCT oil is a responsible nutrient involved in the favorable effects of LD + MCT on muscle function observed in Trial 1.

Trial 3 started in April 2019 and ended in June 2019 (5). In Trials 1 and 2, MCTs were given at dinnertime. This study aimed to determine the suitable timing of MCT supplementation during the day. The participants ($n = 40$) were randomly allocated to two groups, and we compared the effects of MCTs at breakfast or dinnertime for 1.5 months. Irrespective of ingestion at breakfast or dinnertime, supplementation with 6 g MCTs/day for 1.5 months increased muscle mass and function from baseline. However, a negative control group (i.e., the LCT group) was not set because the number of participants was insufficient.

## 2.2 Ethical approval

These clinical trials were approved by the Human Ethics Committee of Showa Women's University (Nos. 14–10, 16–17, and 16–49) and by the Human Ethics Committee of Japan Society of Nutrition and Food Science (Approval No. 87). The procedures were conducted according to either the ethical standards of the institutional committee on human study or the Helsinki Declaration of 2000. Written informed consent was obtained from the participants and/or their family members in all trials.

## 2.3 Study products

The MCTs (75% C8:0 and 25% C10:0 from total fatty acids in oils) and LCTs (64% C18:1, 19% C18:2, and 9% C18:3 from total fatty acids in oils) were provided (Trial 1) by or purchased (Trials 2, 3) from Nisshin OilliO Group, Ltd. (Kanagawa, Japan). Six grams of MCTs (50 kcal; 8.3 kcal/g) or LCTs (54 kcal; 9 kcal/g) per day were mixed with foods such as steamed rice or miso soup at dinnertime (3, 4). The L-leucine (1.2 g) and vitamin D (cholecalciferol, 20 μg) (LD) -enriched supplement (Amino L40) was purchased from Ajinomoto Inc. (Tokyo,

TABLE 1 Outline of the clinical trials.

| Trial | Group | $n^a$ | $n^b$ | Times of measurement | Analysis of the change |
|---|---|---|---|---|---|
| 1 | | 38 | 36 | Baseline, 3.0-mo intervention | ANCOVA, per protocol analysis |
| | LD + MCT | 13 | 13 | | |
| | LD + LCT | 13 | 12 | | |
| | No supplements | 12 | 11 | | |
| 2 | | 64 | 49 | Baseline, 1.5-mo intervention, 3.0-mo intervention, washout (follow-up) | Mixed-effects model, intention-to-treat analysis |
| | LD + MCT | 21 | 18 | | |
| | MCT | 21 | 16 | | |
| | LCT | 22 | 15 | | |
| 3 | | 40 | 0 | Baseline, 1.5-mo intervention | ANCOVA, per protocol analysis |
| | MCT at breakfast | 20 | 0 | | |
| | MCT at dinner | 20 | 0 | | |

[a]The number of participants at the enrollment and assignment to groups.
[b]The number of participants at the 3.0-mo intervention.ANCOVA, analysis of covariance; LCT, long-chain triglyceride; LD, L-leucine + vitamin D; MCT, medium-chain triglyceride; mo, month.

Japan). One tube (100 g, 30 kcal) of Amino L40 was given at the beginning of dinner (3).

## 2.4 Nutrient intake

The nursing care home served breakfast, lunch, and dinner daily. The individual participants' habitual daily energy and nutrient intake during the baseline and intervention periods was calculated from data on food intakes for 7 separate days during each period using the Japanese Standard Tables of Food Composition as described previously (3–5).

## 2.5 Daily physical activity, including rehabilitation

Daily physical activities in this nursing home were as described previously (4). The individual daily activities and rehabilitation/exercise were not changed during the baseline and intervention periods.

## 2.6 Anthropometric measurements, muscle strength, and function measures

The items analyzed in each trial are described in detail in the original reports (3–5), and the measurements used in this article are briefly described below.

Measurement of the right mid-upper AMA was the best approach to assess the change in muscle mass in response to MCT supplementation among the anthropometric measures we have conducted. The AMA was calculated as follows: AMA = [mid-upper-arm circumference (AC) (cm) $-\ \pi \cdot$ triceps skinfold thickness (TSF) (cm)]$^2$ / $(4 \cdot \pi)$ (8).

Maximal calf circumference (CC) was measured with each participant supine, with the left knee raised and the calf at right angles to the thigh. CC was used to assess calf muscle mass (9, 10).

The methods used to evaluate muscle function are restricted by the limited functional capabilities of frail older adults and the effect size of MCT supplementation. The methods that were feasible for frail older adults are described as follows.

For knee extension time, which is measured to examine muscle endurance of the quadriceps, the duration of holding each lower leg in the horizontal position was measured with the participant seated in a straight-backed chair.

For walking speed measurement, participants who could walk unaided were asked to walk for 10 m as fast as they could (3). Participants who had difficulty walking alone were asked to walk for 2.85 m with the support of parallel bars as fast as possible. Participants who could not walk for 2.85 m with the support of parallel bars used a walking aid to measure walking speed. Walking speed was calculated from the time and distance completed by each participant. The method to measure walking speed for individuals remained the same during the baseline and intervention periods.

For the leg open and close test, the number of iterations of opening and closing of the legs over a 10 s period with the participant sitting in a chair was calculated as described previously (11). Among the measurements we conducted, this test was the most sensitive for examining the impact of MCT supplementation on muscle function in frail older adults.

In people without lung disorders, peak expiratory flow was determined as an indicator of the strength of the respiratory muscles (12).

## 2.7 Statistical analysis

### 2.7.1 Differences in statistical analyses between this article and the original reports

To compare results from Trial 1 with Trial 2, a similar statistical analysis of multiple comparisons between the 2 trials is presented in this article. Thus, in both trials, the adjusted mean changes for each baseline value and their relevant statistical analysis are shown in the figures (analysis of covariance [ANCOVA] in Trial 1, mixed-effects model in Trial 2).

In Trial 1, the numerical values of the non-adjusted (actual) mean changes were shown in tables of the original report (3), whereas, in Trial 2, those of the adjusted mean changes for each baseline value were shown in supplemental tables 1, 2 of the original report (4).

In the original report for Trial 2, when the fixed effect of the group was significant but that of the group by time was nonsignificant, *post hoc* tests were not performed for between-group and within-group analyses (4). However, in this article, *post hoc* tests were conducted because when the fixed effect of the group was significant, but that of the group by time interaction was nonsignificant, this indicates that there are statistically significant differences in the overall changes (mean of three time points) between the groups but not in their patterns. However, the third time point differed from the first and second times in that the third was in a follow-up period (washout) and not an intervention period. Thus, the overall changes (mean of three time points) between the groups were not compared because their comparisons were not meaningful. Therefore, in this article, when the fixed effect of the group was significant, *post hoc* tests with Bonferroni correction were performed for the between-group and within-group analyses. Thus, some of the results of the *post hoc* test presented in the figures of this article were not shown in supplemental tables 1, 2 of the original report (4).

### 2.7.2 Combined analysis of clinical trials (Trials 1 and 2)

To interpret the impact of MCTs in Trials 1 and 2 together and increase the statistical power, the MCTs-containing group ($n = 29$, 84.6 + 6.0 years old) was created to combine the LD + MCT group ($n = 13$) in Trial 1 with the MCT group ($n = 16$) in Trial 2; also, the LCTs-containing group ($n = 27$, 86.1 + 5.4 years old) combined the LD + LCT group ($n = 12$) in Trial 1 with the LCT group ($n = 15$) in Trial 2. The number of participants in the group from each trial was that of participants who completed measurements at the 3.0-mo intervention, as described in Table 1. No duplications of participants from each trial were found in the combined data. Outcomes in a combination analysis were common to Trial 1 and Trial 2.

To eliminate the effects of possible confounding factors due to the different trials and genetic and environmental factors, changes in measurements from the baseline values at the 3 months intervention between the 2 groups were compared using one-way ANCOVA considering the following covariates: in model 1, the baseline value of the respective change; in model 2, additional adjustment for age, sex, and body mass index (BMI) at baseline and allocation to trials (Trial 1 or Trial 2); and in model 3, further additional adjustment for habitual intakes in energy (kcal/day), protein (g/day), leucine (g/day), octanoic acid (C8:0, mg/day), decanoic acid (C10:0, mg/day), and vitamin D (μg/day) during the baseline period. Additional covariates in model 3 were energy and nutrients that may relate to sarcopenia. Note that an assumption was made for adjusting the covariates for all outcomes; the group by the covariate interaction was assumed to be nonsignificant in these models: thus, linear regression curves between the covariate and the outcome in the 2 groups were assumed to be parallel in these models.

The reason for conducting ANCOVA to compare the 2 groups instead of an unpaired *t*-test was to adjust for possible confounding factors (13). However, when Levene's test showed the variances of the changes in the 2 groups to be inhomogeneous, the Mann–Whitney U test was used to compare the changes between groups instead of ANCOVA. The Mann–Whitney U test was used to analyze the data on right and left knee extension times, but in this case, no adjustment was made for possible confounding factors. When the difference in changes between the 2 groups was statistically significant, the baseline and 3 months intervention values in each group were compared using the Wilcoxon signed-rank test.

### 2.7.3 Additional details

Statistically significant differences in values between the groups with Bonferroni correction are shown in the figures as superscript letters, e.g., $^{a, b}$. The adjusted mean changes without a common superscript letter indicate a statistically significant difference between the groups at the same time point ($p < 0.05$). For example, $X^a$, $Y^b$, and $Z^a$ indicate that X and Y, and Y and Z are statistically significant because there is no common superscript letter between $X^a$ vs. $Y^b$ and $Y^b$ vs. $Z^a$. In contrast, X and Z are not statistically significant because there is a common superscript letter $^a$ between $X^a$ vs. $Z^a$.

Statistical analyses were performed using the SPSS 20.0 and SPSS 28.0.1.0 (142) software programs (IBM, Chicago, IL). An α level of 0.05 was used to determine statistical significance.

## 3 Results

### 3.1 Comparison of MCT supplementation with (Trial 1) and without (Trial 2) the LD supplement

To examine the impact of MCTs relative to LCTs, the LD + MCT group was compared with the LD + LCT group (Trial 1), and the MCT group was compared with the LCT group (Trial 2). The data from Trial 3 were neither compared nor combined with others because there was no negative control group (LCT) and the intervention period was 1.5 months (the others were 3 months) (Table 1). Representative results are presented here.

#### 3.1.1 MCT supplementation did not alter right AC, decreased right TSF, and increased right AMA compared with LCT supplementation

The mean adjusted changes in the right AC, TSF, and calculated AMA are shown in Figure 1. The differences in changes in the groups in right AC were nonsignificant in Trial 1 (ANCOVA, $p = 0.34$) and in Trial 2 (fixed effect of the group, $p = 0.16$) (Figure 1A). The differences in changes in the groups in right TSF were significant in Trial 1 (ANCOVA, $p = 0.001$) and in Trial 2 (fixed effect of the group, $p = 0.003$) (Figure 1B). The decrease in right TSF was greater in the MCT group than in the LCT group (Trial 2). The differences in changes in the groups in calculated right AMA were significant in Trial 1 (ANCOVA, $p = 0.003$) and in Trial 2 (fixed effect of the group, $p = 0.013$) (Figure 1C). The increase in the calculated right AMA at the 3.0-month intervention was greater in the MCT group than in the LCT group (Trial 2).

The differences in changes in the groups in calculated left AMA and right and left CCs were nonsignificant by ANCOVA and fixed effect of the group in Trials 1 and 2, respectively (data not shown in Figures) but did show a similar tendency to those in calculated right AMA (3, 4).

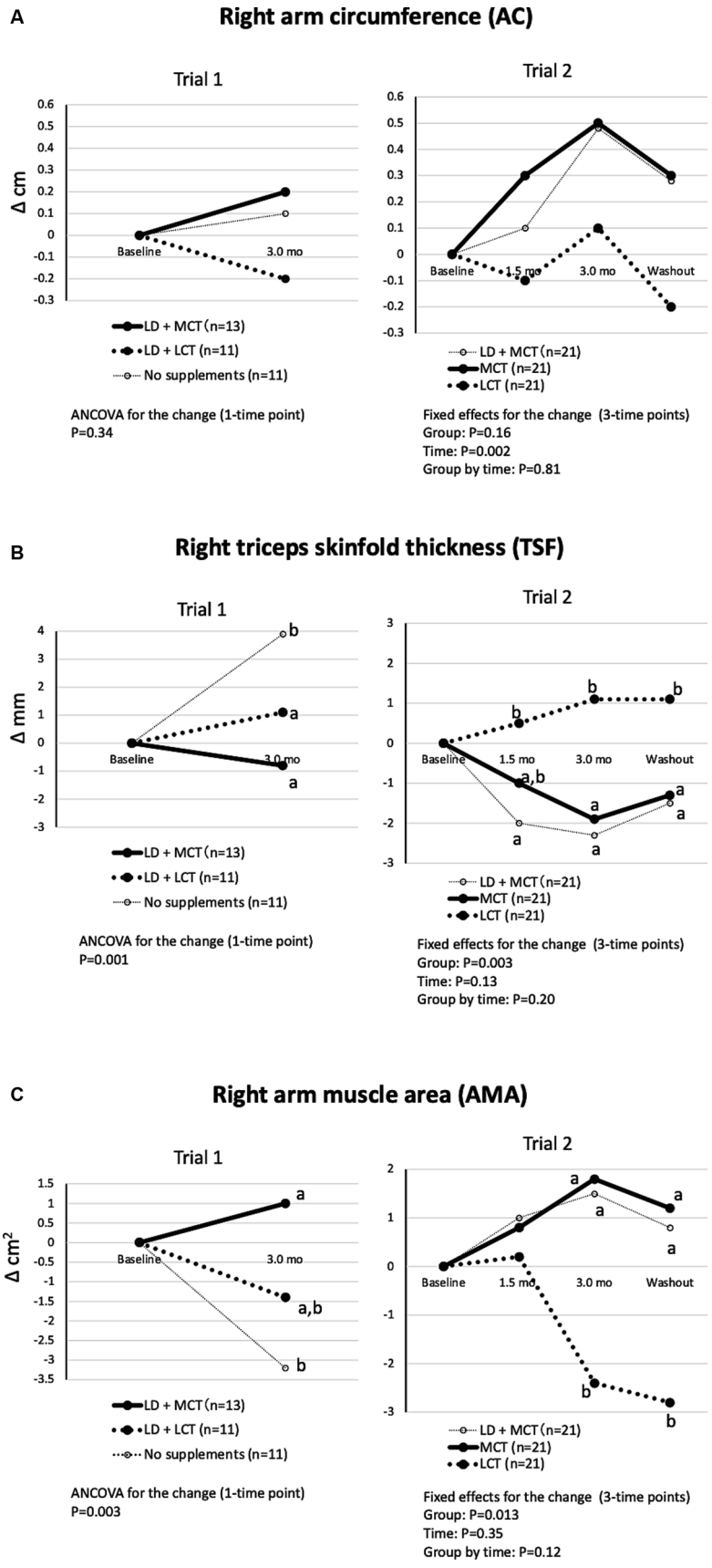



FIGURE 1

*(Continued)*

FIGURE 1 (Continued)
MCT supplementation did not alter right AC **(A)**, decreased right TSF **(B)**, and increased right AMA **(C)** compared with LCT supplementation. **(A)** Changes in right AC from baseline in Trials 1 and 2. Overall mean baseline right ACs were 21.9 cm (Trial 1) and 21.9 cm (Trial 2). **(B)** Changes in right TSF from baseline in Trials 1 and 2. Overall mean baseline right TSFs were 9.5 mm (Trial 1) and 10.6 mm (Trial 2). **(C)** Changes in right AMA from baseline in Trials 1 and 2. Overall mean baseline right AMAs were 28.7 $cm^2$ (Trial 1) and 29.1 $cm^2$ kg (Trial 2). The adjusted mean changes at a time point without a common letter indicate statistically significant differences between the groups, $p < 0.05$.

### 3.1.2 MCT supplementation increased muscle function compared with LCT supplementation

The differences in changes in the groups in right-hand grip strength were significant in Trial 1 (ANCOVA, $p = 0.012$) and Trial 2 (fixed effect of the group, $p = 0.006$) (Figure 2A). In Trial 1, the increase in right-hand grip strength at the 3.0-month intervention was nonsignificant between the LD + MCT group and the LD + LCT group. However, in Trial 2, the increase in right-hand grip strength at the 3.0-month intervention and washout period was greater in the MCT group than in the LCT group. However, the change in left-hand grip strength did not differ between the groups (Figure 2B). This may be due to most participants' left hand being non-dominant.

The differences in changes in the groups in walking speed were significant in Trial 1 (Kruskal-Wallis test, $p = 0.022$) but not in Trial 2 (fixed effect of the group, $p = 0.63$) (Figure 3A). In Trial 1, the increase in walking speed at the 3.0-month intervention was greater in the LD + MCT group than in the LD + LCT group. Similarly, in Trial 2, the increase in walking speed at the 3.0-month intervention appeared to be greater in the MCT group than in the LCT group. However, a *post hoc* test was not conducted because the fixed effect of the group was nonsignificant.

The differences in changes in the groups in the number of iterations in the leg open and close test were significant in Trial 1 (ANCOVA, $p < 0.001$) and in Trial 2 (fixed effect of the group by time, $p = 0.041$) (Figure 3B). In Trial 1, the increase in the number of iterations at the 3.0-month intervention was greater in the LD + MCT group than in the LD + LCT group. Similarly, in Trial 2, the increases in the number of iterations at the 1.5- and 3.0-month interventions and the washout were greater in the MCT group than in the LCT group. At washout (1.5 months after termination of the intervention), the increase in the number of iterations returned to the level measured at the 1.5-month intervention.

As a result, similar effects of MCTs on muscle mass and function were observed in the presence (Trial 1) and absence (Trial 2) of the LD supplement. Then, to interpret the impact of MCTs in Trials 1 and 2 together and increase the statistical power, a combined data analysis was conducted.

## 3.2 Combined data analysis

Changes in measurements from baseline at the 3.0-month intervention between the MCTs- and LCTs-containing groups (combined groups from Trials 1 and 2) and their comparisons are shown in Table 2 (habitual energy and nutrient intakes), Table 3 (anthropometric measures) and Table 4 (muscle strength and functions).

### 3.2.1 Habitual energy and nutrient intakes in a combined data analysis

Note that to examine the effects of the supplements on habitual energy and nutrient intakes, the energy and nutrients in the supplements were not included in Table 2. There was a statistically significant difference between the 2 groups in the energy of fat and carbohydrate intake from total energy; MCT supplementation increased the fat energy % by 1.6%, whereas it decreased carbohydrates by-1.6% compared with LCT supplementation. These differences were also observed after adjusting each baseline value or allocation to trials (Table 2). The increase in fat energy was due to increased fat and decreased carbohydrate intakes in the MCTs-containing group. Similar changes, but nonsignificant changes, were observed after MCT supplementation in each of Trial 1 (3) and Trial 2 (data not shown). The physiological and clinical effects of this slight increase in fat energy % are unclear. It may reflect the increased fat oxidation observed after MCT supplementation (14). No differences in changes in the 2 groups were observed in the intakes of energy and other nutrients.

### 3.2.2 Anthropometric measures, muscle strength, and functions in a combined data analysis

In Tables 3, 4, possible confounding factors were adjusted by 3 stepwise adjustments (models 1, 2, 3) for covariates by ANCOVA (see Materials and methods for details). In Table 3, the increase in right AC between the 2 groups was significant without the adjustment ($p = 0.039$) but nonsignificant after the adjustment of its baseline value (model 1) ($p = 0.06$), whereas that in left CC became significant after the adjustment of energy and nutrient intakes (model 3) ($p = 0.015$). In Table 4, the significance of peak expiratory flow varied; it was significant in models 1 and 2 but not in the non-adjusted model and model 3. The presence of statistical significance in other measurements remained the same after the adjustments.

In the fully adjusted model (model 3), statistically significant increases from baseline in the changes after the 3.0-month intervention in measurements in the MCTs-containing group, relative to the LCTs-containing group, were manifested in body weight (adjusted mean change from baseline: MCTs 1.2 kg vs. LCTs 0.2 kg, $p = 0.023$), BMI (MCTs 0.6 $kg/m^2$ vs. LCTs 0.1 $kg/m^2$, $p = 0.039$), right AMA (MCTs 1.4 $cm^2$ vs. LCTs-0.7 $cm^2$, $p = 0.002$), left CC (MCTs 0.5 cm vs. LCTs-0.4 cm, $p = 0.015$), right-hand grip strength (MCTs 1.6 kg vs. LCTs 0.3 kg, $p = 0.017$), walking speed (MCTs 0.084 m/s vs. LCTs-0.033 m/s, $p = 0.002$), and number of iterations in leg open and close test (MCTs 2.40 n/10 s vs. LCTs-0.92 n/10 s, $p < 0.001$). In contrast, statistically significant decreases were observed in the right TSF (MCTs-1.2 mm vs. LCTs 0.8 mm, $p = 0.016$). Increases in the MCTs-containing group were also observed in right knee extension time (MCTs 31 s vs. LCTs 3 s, $p = 0.021$) and left knee extension time (MCTs 28 s vs. LCTs 2 s, $p = 0.034$) by the Mann–Whitney U test.

# 4 Discussion

A combined data analysis showed that relative to supplementation with 6 g LCTs/day, supplementation with 6 g MCTs/day for 3 months

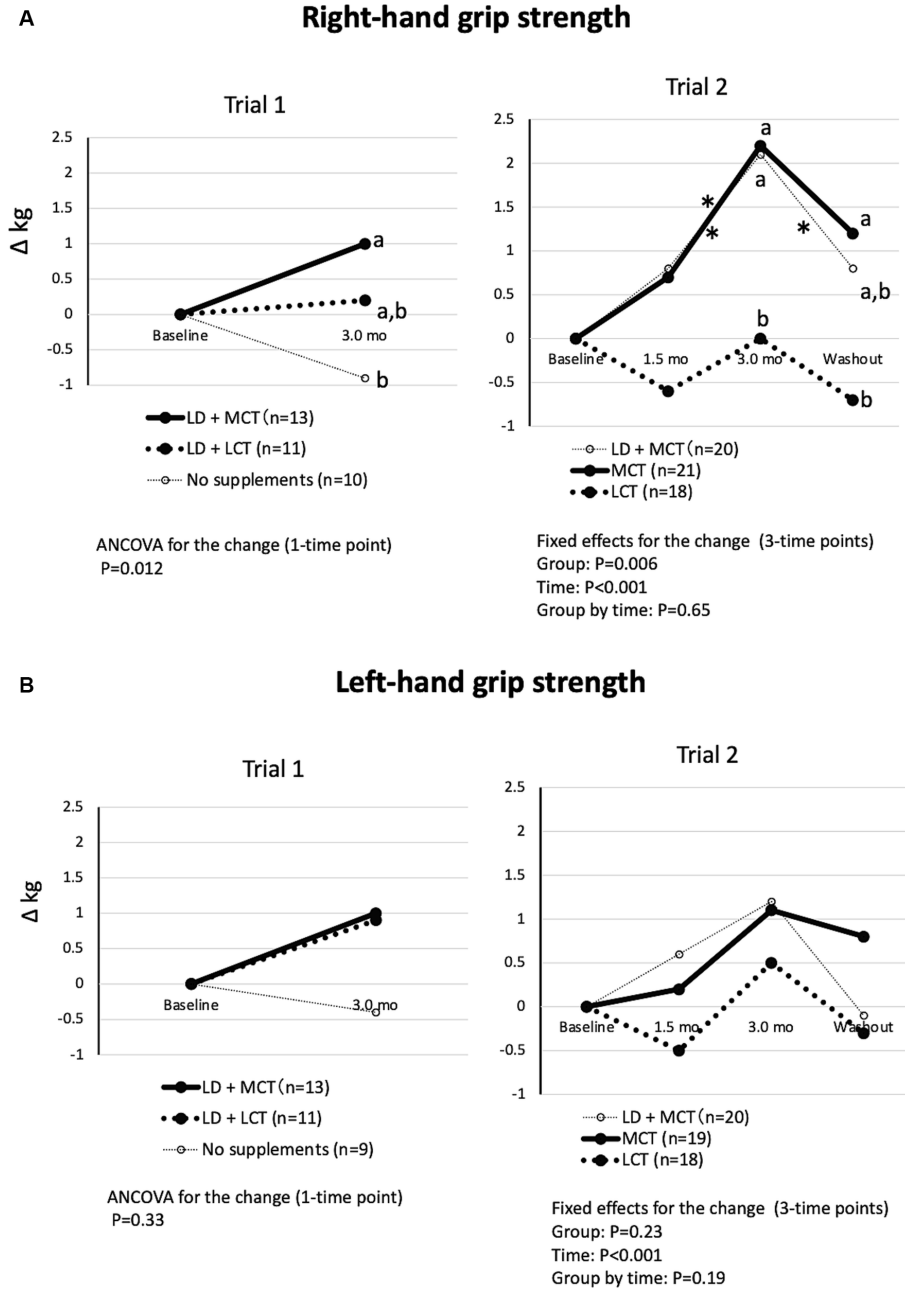



FIGURE 2
MCT supplementation increased right-hand grip strength **(A)** but not left-hand grip strength **(B)** compared with LCT supplementation. **(A)** Changes in right-hand grip strength from baseline in Trials 1 and 2. Overall mean baseline right-hand grip strengths were 10.7 kg (Trial 1) and 10.7 kg (Trial 2). **(B)** Changes in left-hand grip strength from baseline in Trials 1 and 2. Overall mean baseline left-hand grip strengths were 10.0 kg (Trial 1) and 10.6 kg (Trial 2). The adjusted mean changes at a time point without a common letter indicate statistically significant differences between the groups, $p < 0.05$. Asterisks indicate a statistically significant difference vs. at 3.0-mo intervention within the group, $*p < 0.05$.

statistically significantly increased body weight, BMI, muscle mass (right AMA, left CC), and function (right-hand grip strength, right and left knee extension times, walking speed, and number of iterations in leg open and close test) and decreased fat mass (right triceps skinfold thickness) (Tables 3, 4). This is the first report to show a statistically significant increase in muscle mass in response to MCT supplementation relative to LCT supplementation.

Three factors, (1) the number of participants, (2) the selection of confounding factors, and (3) the selection of outcomes, affect the contributions of independent variables to the outcomes. In a combined data analysis, we increased the number of participants ($n = 56$) and considered 11 possible confounding factors. The advantages of covariate-adjusted analysis were reviewed previously (13). Also, we conducted 7 outcome measurements of muscle function in Trials 1 and 2 and performed a combined data analysis for each measurement. Although the precision of the measures for body weight, muscle mass, and function to detect their small changes in frail old adults was unclear, incorporating these 3 appropriate factors

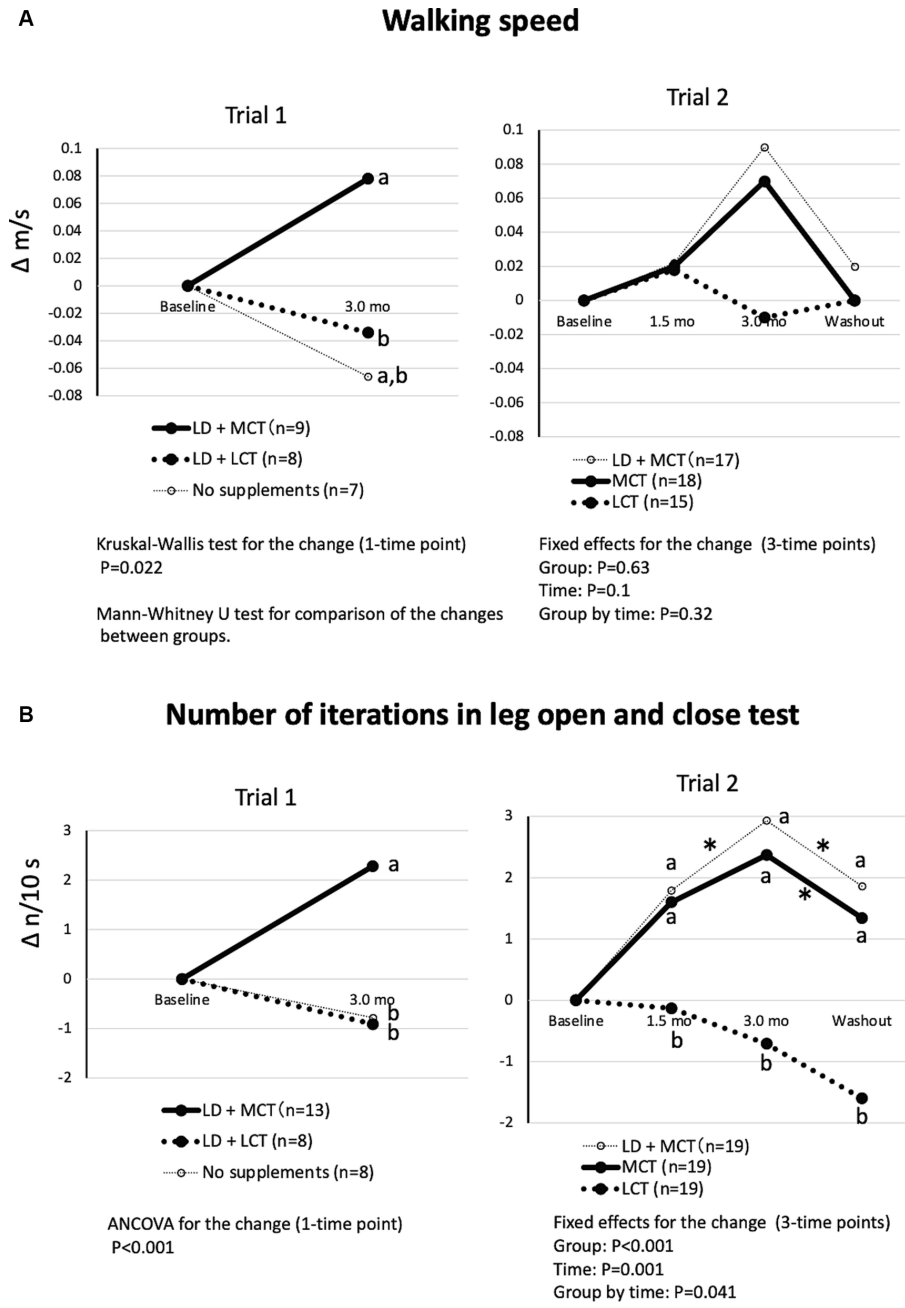



FIGURE 3
MCT supplementation increased walking speed **(A)** and number of iterations in the leg open and close test **(B)** compared with LCT supplementation. **(A)** Changes in walking speed from baseline in Trials 1 and 2. Overall mean baseline walking speeds were 0.50 m/s (Trial 1) and 0.52 m/s (Trial 2). **(B)** Changes in the number of iterations in the leg open and close test from baseline in Trials 1 and 2. Overall mean baseline numbers of iterations in the leg open and close test were 5.52 n/10 s (Trial 1) and 4.41 n/10 s (Trial 2). The adjusted mean changes at a time point without a common letter indicate statistically significant differences between the groups, $p < 0.05$. Asterisks indicate a statistically significant difference vs. at 3.0-mo intervention within the group, $*p < 0.05$.

in a combined analysis may have increased the statistical power to detect the difference in outcomes between the 2 groups.

A dose of 6 g/day is much less than that aimed to increase ketone bodies in the blood (usually 20 ~ 40 g/day of MCTs) (15). A 6 g/day intake of MCTs corresponds to about 4% of habitual energy intake (overall mean habitual energy intake was 1,430 kcal/day), 15% of habitual fat intake (overall mean fat intake was 39 g/day), and 0.14 g/kg body weight (overall mean body weight was 42.6 kg) in this population (overall mean age 85 years old). The significant increase in body weight in the MCTs-containing group is somewhat surprising because MCTs have been considered to reduce body weight and total body fat relative to LCTs (16, 17). The increase in muscle mass may contribute to maintaining or increasing body weight despite a decrease in fat mass in frail older adults.

TABLE 2 Combined data analysis in Trials 1 and 2; habitual energy and nutrient intakes at baseline and after the 3-mo intervention and their changes from baseline in the MCTs-containing (MCT or LD + MCT) and LCTs-containing (LCT or LD + LCT) groups (excluding supplements and added oils) ($n$ = 56).[a]

| Measure | Group | $n$ | Baseline | 3-mo intervention | Non-adjusted change | Adjusted change[c] (model 1) | Adjusted change[d] for trial |
|---|---|---|---|---|---|---|---|
| Energy, kcal/d | MCT, LD + MCT | 29 | 1,379 ± 242 | 1,358 ± 230 | −21 ± 113 | −23 (−63, 17) | −22 (−63, 20) |
| | LCT, LD + LCT | 27 | 1,406 ± 297 | 1,410 ± 294 | 4 ± 109 | 6 (−35, 47) | 4 (−38, 47) |
| $p$ value[b] | | | | | 0.39 | 0.32 | 0.39 |
| Energy, kJ/d | MCT, LD + MCT | 29 | 5,873 ± 1,014 | 5,692 ± 966 | −91 ± 476 | −96 (−266, 72) | −91 (−263, 82) |
| | LCT, LD + LCT | 27 | 5,894 ± 1,243 | 5,912 ± 1,232 | 18 ± 457 | 25 (−151, 200) | 18 (−161, 197) |
| $p$ value | | | | | 0.39 | 0.32 | 0.38 |
| Protein, en% | MCT, LD + MCT | 29 | 16.4 ± 1.9 | 16.5 ± 2.2 | 0.1 ± 1.3 | 0.1 (−0.3, 0.5) | 0.1 (−0.4, 0.5) |
| | LCT, LD + LCT | 27 | 16.2 ± 2.2 | 15.8 ± 1.8 | −0.4 ± 1.0 | −0.4 (−0.9, −0.0) | −0.4 (−0.8, −0.0) |
| $p$ value | | | | | 0.14 | 0.08 | 0.13 |
| Protein, g/d | MCT, LD + MCT | 29 | 55.9 ± 7.4 | 55.2 ± 7.1 | −0.7 ± 5.3 | −0.7 (−2.4, 1.1) | −0.7 (−2.3, 1.0) |
| | LCT, LD + LCT | 27 | 55.7 ± 9.4 | 55.0 ± 10.6 | −0.6 ± 4.0 | −0.7 (−2.5, 1.2) | −0.7 (−2.4, 1.0) |
| $p$ value | | | | | 0.97 | 0.98 | 0.98 |
| Protein, g/(kg BW·d) | MCT, LD + MCT | 29 | 1.35 ± 0.33 | 1.28 ± 0.25 | −0.07 ± 0.15 | −0.06 (−0.11, −0.02) | −0.07 (−0.12, −0.02) |
| | LCT, LD + LCT | 27 | 1.30 ± 0.28 | 1.27 ± 0.29 | −0.03 ± 0.10 | −0.03 (−0.08, 0.01) | −0.03 (−0.08, 0.02) |
| $p$ value | | | | | 0.23 | 0.31 | 0.21 |
| Leucine, g/d | MCT, LD + MCT | 29 | 4.23 ± 0.59 | 4.12 ± 0.61 | −0.11 ± 0.42 | −0.11 (−0.26, 0.03) | −0.11 (−0.22, 0.01) |
| | LCT, LD + LCT | 27 | 4.39 ± 0.66 | 4.20 ± 0.76 | −0.19 ± 0.35 | −0.18 (−0.33, −0.03) | −0.19 (−0.31, −0.07) |
| $p$ value | | | | | 0.43 | 0.53 | 0.32 |
| EAAs, g/d | MCT, LD + MCT | 29 | 21.7 ± 3.1 | 21.0 ± 3.1 | −0.6 ± 2.1 | −0.7 (−1.6, 0.3) | −0.6 (−1.5, 0.2) |
| | LCT, LD + LCT | 27 | 22.0 ± 3.5 | 21.5 ± 4.3 | −0.5 ± 2.9 | −0.5 (−1.4, 0.5) | −0.5 (−1.4, 0.4) |
| $p$ value | | | | | 0.80 | 0.75 | 0.81 |
| Carbohydrate, en% | MCT, LD + MCT | 29 | 62.7 ± 5.7 | 61.1 ± 6.9 | −1.6 ± 3.8 | −1.6 (−2.8, −0.4) | −1.6 (−2.8, −0.4) |
| | LCT, LD + LCT | 27 | 63.1 ± 6.1 | 63.5 ± 5.1 | 0.4 ± 2.5 | 0.4 (−0.8, 1.6) | 0.4 (−0.9, 1.6) |
| $p$ value | | | | | 0.026 | 0.021 | 0.027 |
| Carbohydrate, g/d | MCT, LD + MCT | 29 | 218 ± 51 | 210 ± 52 | −9 ± 23 | −9 (−17, −8) | −9 (−17, −4) |
| | LCT, LD + LCT | 27 | 224 ± 60 | 226 ± 57 | 1 ± 22 | 2 (−7, 10) | 1 (−7, 10) |
| $p$ value | | | | | 0.10 | 0.08 | 0.10 |
| Fat, en% | MCT, LD + MCT | 29 | 20.9 ± 4.2 | 22.4 ± 4.8** | 1.6 ± 2.9 | 1.6 (0.7, 2.5) | 1.6 (0.7, 2.5) |
| | LCT, LD + LCT | 27 | 20.8 ± 4.4 | 20.8 ± 4.0 | 0.0 ± 1.8 | 0.0 (−0.9, 1.0) | 0.0 (−0.9, 1.0) |
| $p$ value | | | | | 0.024 | 0.021 | 0.023 |
| Fat, g/d | MCT, LD + MCT | 29 | 31.3 ± 5.5 | 33.1 ± 5.3 | 1.8 ± 4.2 | 1.7 (0.5, 3.0) | 1.8 (0.4, 3.1) |
| | LCT, LD + LCT | 27 | 31.8 ± 7.5 | 32.0 ± 7.1 | 0.2 ± 2.5 | 0.3 (−1.1, 1.6) | 0.2 (−1.2, 1.6) |
| $p$ value | | | | | 0.11 | 0.11 | 0.11 |
| Octanoic acid, mg/d | MCT, LD + MCT | 29 | 74 ± 157 | 80 ± 158 | 6 ± 34 | 6 (−5, 18) | 6 (−5, 18) |
| | LCT, LD + LCT | 27 | 65 ± 119 | 70 ± 123 | 5 ± 26 | 5 (−6, 17) | 5 (−6, 17) |
| $p$ value | | | | | 0.91 | 0.91 | 0.92 |
| Decanoic acid, mg/d | MCT, LD + MCT | 29 | 68 ± 71 | 78 ± 82 | 9 ± 36 | 9 (−3, 22) | 9 (−4, 22) |
| | LCT, LD + LCT | 27 | 83 ± 71 | 93 ± 82 | 10 ± 32 | 10 (−3, 23) | 10 (−3, 23) |
| $p$ value | | | | | 0.89 | 0.96 | 0.89 |
| Sodium, mg/d | MCT, LD + MCT | 29 | 2,979 ± 725 | 3,074 ± 741 | 95 ± 327 | 103 (−2, 209) | 95 (−11, 201) |
| | LCT, LD + LCT | 27 | 2,657 ± 1,014 | 2,786 ± 1,003 | 129 ± 223 | 121 (11, 230) | 130 (20, 240) |
| $p$ value | | | | | 0.65 | 0.82 | 0.65 |

*(Continued)*

TABLE 2 (Continued)

| Measure | Group | *n* | Baseline | 3-mo intervention | Non-adjusted change | Adjusted change[c] (model 1) | Adjusted change[d] for trial |
|---|---|---|---|---|---|---|---|
| Thiamin, mg/d | MCT, LD + MCT | 29 | 1.1 ± 0.4 | 1.1 ± 0.4 | 0.0 ± 0.1 | 0.0 (−0.0, 0.1) | 0.0 (−0.0, 0.1) |
| | LCT, LD + LCT | 27 | 1.0 ± 0.4 | 1.0 ± 0.4 | 0.0 ± 0.1 | 0.0 (−0.0, 0.1) | 0.0 (−0.0, 0.1) |
| *p* value | | | | | 0.48 | 0.53 | 0.48 |
| Pyridoxine, mg/d | MCT, LD + MCT | 29 | 1.5 ± 0.6 | 1.5 ± 0.6 | −0.0 ± 0.3 | −0.0 (−0.1, 0.1) | −0.0 (−0.1, 0.1) |
| | LCT, LD + LCT | 27 | 1.3 ± 0.6 | 1.3 ± 0.6 | −0.0 ± 0.1 | −0.0 (−0.1, 0.1) | −0.0 (−0.1, 0.1) |
| *p* value | | | | | 0.97 | 0.91 | 0.97 |
| Cyanocobalamin, μg/d | MCT, LD + MCT | 29 | 3.8 ± 1.0 | 3.9 ± 1.2 | 0.1 ± 0.7 | 0.1 (−0.1, 0.3) | 0.1 (−0.1, 0.3) |
| | LCT, LD + LCT | 27 | 3.6 ± 1.1 | 3.7 ± 1.1 | 0.1 ± 0.3 | 0.1 (−0.1, 0.3) | 0.1 (−0.1, 0.3) |
| *p* value | | | | | 0.85 | 0.83 | 0.85 |
| Vitamin D, μg/d | MCT, LD + MCT | 29 | 5.0 ± 2.0 | 4.4 ± 1.2 | -0.6 ± 1.0 | −0.5 (−0.8, −0.3) | −0.6 (−0.8, −0.4) |
| | LCT, LD + LCT | 27 | 4.6 ± 2.2 | 4.1 ± 1.6 | -0.5 ± 0.9 | −0.6 (−0.9, −0.4) | −0.6 (−0.8, −0.3) |
| *p* value | | | | | 0.82 | 0.56 | 0.74 |

[a]Values are means ± SD or adjusted mean (95% CI). Difference from baseline by Wilcoxon signed-rank test, ** $p < 0.01$.
[b]*p* value represents the differences in the changes of variables between the 2 groups assessed by 1-factor ANCOVA.
[c]Adjusted for each baseline value (model 1).
[d]Adjusted for allocation to trial (Trial 1 or Trial 2).En%, energy%; EAAs, essential amino acids; LCT, 6 g/d of long-chain triglycerides; LD + LCT, leucine and cholecalciferol-enriched supplement with 6 g/d of long-chain triglycerides; LD + MCT, leucine and cholecalciferol-enriched supplement with 6 g/d of medium-chain triglycerides; MCT, 6 g/d of medium-chain triglycerides.

## 4.1 Rationale for combining groups from different trials

Because MCT supplementation for 3 months increased muscle function similarly relative to LCT supplementation in Trials 1 and 2, irrespective of the LD supplement given (Figures 1–3), the MCTs-containing group ($n = 29$) was created to combine the LD + MCT group in Trial 1 with the MCT group in Trial 2; also, the LCTs-containing group ($n = 27$) combined the LD + LCT group in Trial 1 with the LCT group in Trial 2, and these 2 groups were compared. Thus, the MCTs- and LCTs-containing groups have a similar number of participants from Trials 1 and 2 (balanced data) and are comparable. However, participants from Trial 1 received an additional supplement of LD. To examine the possible effects of LD and the difference in the trials on the outcomes in the combined data analysis, allocation to trial (Trials 1 or 2) was adjusted for a covariate. There were almost no effects on either the changes in each group or on the difference in changes between the 2 groups for any of the measurements after additional adjustment for "allocation to trial" in the non-adjusted model in energy and nutrient intakes (Table 2) and in the non-adjusted model or model 1 in anthropometric measures and muscle function, respectively (Supplementary Tables S1, S2). However, there was an increase in the statistical power of the MCT effect with this adjustment in the measurement of peak expiratory flow: the *p* value of the difference in the non-adjusted changes between the 2 groups was 0.08, whereas that in the adjusted change for "allocation to trial" was 0.045; that in the adjusted change for baseline value was 0.049, whereas that in the adjusted change for baseline value and "allocation to trial" was 0.028 (the last line in Supplementary Table S2). These results suggest that the LD supplement or "allocation to trial" did not affect most outcomes but somehow affected those of the peak expiratory flow for unknown reasons. This may be one of the reasons that the significance of changes between the 2 groups in the peak expiratory flow varied in the models (Table 4).

## 4.2 Confounding factors

Overall, the confounding factors we examined showed minimal effects on the outcomes (Tables 3, 4). However, an increase in left CC in the MCTs-containing group relative to the LCTs-containing group became significant after the additional adjustment of habitual energy and several nutrient intakes during the baseline period (MCTs 0.5 cm [0.0, 0.9] vs. LCTs-0.4 cm [−0.9, 0.1], $p = 0.015$) (Table 3). A similar tendency was observed in right CC, but this was nonsignificant (MCTs 0.4 cm [−0.1, 0.8] vs. LCTs-0.2 cm [−0.7, 0.3], $p = 0.13$). The following 3 covariates in model 3 contributed significantly to the change in the left CC: the estimated partial regression coefficient B for BMI at baseline was 0.324 (SE 0.092, $p = 0.001$); energy intake during baseline was 0.003 (SE 0.001, $p = 0.039$); and left CC at baseline was-0.306 (SE 0.077, $p < 0.001$) (data not shown). Although these mixed confounding factors may affect the outcome, their clinical significance is unclear.

## 4.3 Is increased muscle mass required for increased muscle strength and function in response to MCT supplementation?

Although a decrease in muscle mass is essential to the definition of sarcopenia (12), physiologically, an increase in muscle mass might not necessarily result in increased muscle strength and function. As observed in exercise training, improved functions of the mitochondria

TABLE 3 Combined data analysis in Trials 1 and 2; anthropometric measures at baseline and after the 3-mo intervention and their changes from baseline in the MCTs-containing (MCT or LD + MCT) and LCTs-containing (LCT or LD + LCT) groups ($n$ = 56).[a]

| Measure | Group | $n$ | Baseline | 3-mo intervention | Non-adjusted change | Adjusted change[c] (model 1) | Adjusted change[d] (model 2) | Adjusted change[e] (model 3) |
|---|---|---|---|---|---|---|---|---|
| Body weight, kg | MCT, LD + MCT | 29 | 43.4 ± 10.6 | 44.6 ± 10.1*** | 1.2 ± 1.7 | 1.2 (0.6, 1.8) | 1.2 (0.6, 1.8) | 1.2 (0.7, 1.8) |
| | LCT, LD + LCT | 27 | 43.6 ± 5.8 | 43.8 ± 5.4 | 0.2 ± 1.6 | 0.2 (−0.4, 0.8) | 0.3 (−0.4, 0.9) | 0.2 (−0.4, 0.8) |
| *p* value[b] | | | | | 0.026 | 0.021 | 0.036 | 0.023 |
| BMI, kg/m$^2$ | MCT, LD + MCT | 29 | 18.5 ± 3.4 | 19.1 ± 3.4*** | 0.6 ± 0.8 | 0.5 (0.3, 0.8) | 0.6 (0.3, 0.8) | 0.6 (0.3, 0.8) |
| | LCT, LD + LCT | 27 | 19.2 ± 1.9 | 19.3 ± 1.7 | 0.1 ± 0.7 | 0.1 (−0.2, 0.4) | 0.1 (−0.2, 0.4) | 0.1 (−0.2, 0.4) |
| *p* value | | | | | 0.027 | 0.042 | 0.031 | 0.039 |
| Right AC, cm | MCT, LD + MCT | 29 | 22.0 ± 3.6 | 22.4 ± 3.7** | 0.5 ± 0.9 | 0.5 (0.1, 0.8) | 0.4 (0.1, 0.8) | 0.5 (0.1, 0.8) |
| | LCT, LD + LCT | 26 | 22.8 ± 2.1 | 22.8 ± 1.8 | −0.0 ± 0.9 | −0.0 (−0.4, 0.3) | 0.0 (−0.4, 0.4) | −0.0 (−0.3, 0.3) |
| *p* value | | | | | 0.039 | 0.06 | 0.10 | 0.06 |
| Left AC, cm | MCT, LD + MCT | 29 | 21.9 ± 3.5 | 22.3 ± 3.5 | 0.4 ± 0.6 | 0.4 (0.2, 0.7) | 0.4 (0.2, 0.7) | 0.4 (0.1, 0.7) |
| | LCT, LD + LCT | 26 | 22.4 ± 2.5 | 22.7 ± 2.2 | 0.3 ± 0.9 | 0.3 (−0.0, 0.6) | 0.3 (−0.0, 0.5) | 0.3 (0.0, 0.6) |
| *p* value | | | | | 0.35 | 0.44 | 0.35 | 0.57 |
| Right TSF, mm | MCT, LD + MCT | 29 | 10.2 ± 5.2 | 8.8 ± 4.7* | −1.4 ± 3.1 | −1.4 (−2.4, −0.4) | −1.2 (−2.1, −0.3) | −1.2 (−2.3, −0.2) |
| | LCT, LD + LCT | 26 | 10.4 ± 4.9 | 11.3 ± 4.7 | 0.9 ± 2.9 | 0.9 (−0.1, 2.0) | 0.7 (−0.3, 1.7) | 0.8 (−0.3, 1.9) |
| *p* value | | | | | 0.007 | 0.003 | 0.007 | 0.016 |
| Left TSF, mm | MCT, LD + MCT | 29 | 8.3 ± 5.3 | 7.6 ± 4.7 | −0.7 ± 2.7 | −0.7 (−1.7, 0.4) | −0.6 (−1.7, −0.4) | −0.4 (−1.6, 0.7) |
| | LCT, LD + LCT | 26 | 8.4 ± 4.3 | 8.8 ± 4.8 | 0.4 ± 3.2 | 0.4 (−0.7, 1.5) | 0.4 (−0.7, 1.5) | 0.2 (−1.1, 1.4) |
| *p* value | | | | | 0.18 | 0.16 | 0.20 | 0.51 |
| Calculated right | MCT, LD + MCT | 29 | 28.6 ± 6.0 | 30.3 ± 5.7** | 1.7 ± 2.5 | 1.4 (0.5, 2.3) | 1.3 (0.4, 2.1) | 1.4 (0.6, 2.3) |
| AMA, cm$^2$ | LCT, LD + LCT | 26 | 30.7 ± 5.3 | 29.8 ± 4.0 | −0.9 ± 2.7 | −0.7 (−1.6, 0.3) | −0.5 (−1.3, 0.4) | −0.7 (−1.6, 0.2) |
| *P* value | | | | | <0.001 | 0.002 | 0.006 | 0.002 |
| Calculated left | MCT, LD + MCT | 29 | 29.1 ± 5.1 | 30.8 ± 5.4 | 1.7 ± 3.4 | 1.6 (0.5, 2.7) | 1.6 (0.5, 2.7) | 1.5 (0.3, 2.6) |
| AMA, cm$^2$ | LCT, LD + LCT | 26 | 30.8 ± 4.7 | 31.1 ± 5.1 | 0.4 ± 2.5 | 0.5 (−0.7, 1.6) | 0.5 (−0.6, 1.7) | 0.7 (−0.6, 1.9) |
| *p* value | | | | | 0.10 | 0.16 | 0.19 | 0.38 |
| Right CC, cm | MCT, LD + MCT | 28 | 28.8 ± 4.3 | 29.0 ± 4.4 | 0.2 ± 1.0 | 0.2 (−0.2, 0.6) | 0.3 (−0.2, 0.7) | 0.4 (−0.1, 0.8) |
| | LCT, LD + LCT | 25 | 28.9 ± 3.1 | 28.9 ± 2.8 | −0.1 ± 1.2 | −0.0 (−0.5, 0.4) | −0.1 (−0.5, 0.4) | −0.2 (−0.7, 0.3) |
| *p* value | | | | | 0.34 | 0.36 | 0.32 | 0.13 |
| Left CC, cm | MCT, LD + MCT | 28 | 28.5 ± 4.5 | 28.8 ± 4.5 | 0.3 ± 0.8 | 0.3 (−0.2, 0.7) | 0.4 (−0.1, 0.8) | 0.5 (0.0, 0.9) |
| | LCT, LD + LCT | 25 | 28.5 ± 2.8 | 28.3 ± 2.4 | −0.2 ± 1.5 | −0.2 (−0.6, 0.3) | −0.3 (−0.7, 0.2) | −0.4 (−0.9, 0.1) |
| *p* value | | | | | 0.16 | 0.16 | 0.06 | 0.015 |

[a]Values are means ± SD or adjusted mean (95% CI). Difference from baseline by Wilcoxon signed-rank test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[b]*p* value represents the differences in the changes of variables between the 2 groups assessed by 1-factor ANCOVA.
[c]Adjusted for each baseline value (model 1).
[d]Additionally adjusted for age, sex, BMI, and allocation to trial (Trials 1 or 2) (model 2).
[e]Further additionally adjusted for intakes in energy (kcal/day), protein (g/day), leucine (g/day), octanoic acid (C8:0, mg/day), decanoic acid (C10:0, mg/day), and vitamin D (μg/day) during the baseline period (model 3).AC, arm circumference; AMA, arm muscle area; BMI, body mass index; CC, calf circumference; LCT, 6 g/d of long-chain triglycerides; LD + LCT, leucine and cholecalciferol-enriched supplement with 6 g/d of long-chain triglycerides; LD + MCT, leucine and cholecalciferol-enriched supplement with 6 g/d of medium-chain triglycerides; MCT, 6 g/d of medium-chain triglycerides; TSF, triceps skinfold thickness.

in skeletal muscles (18), neuromuscular junctions (19), and the brain (20), in addition to increasing muscle mass (i.e., increases in the number or size of type I or type II fibers) (21, 22), may increase muscle strength and function.

Kojima et al. recently reported that in healthy middle-aged and older adults (mean age 68 years old), supplementation with 6 g/day MCTs (C8:0 or C10:0) combined with moderate-intensity aerobic exercise for 3 months significantly increased muscle function (right knee extension strength) but did not alter skeletal muscle mass (overall mean skeletal muscle mass was 23 kg) compared with 6 g/day LCTs combined with aerobic exercise (23). Also, Mutoh et al. reported that supplementation with 18 g MCTs/day in healthy older adults (mean age 70 years old) for 3 months resulted in the participants showing better balance ability (one of the muscle functions) relative to those receiving a placebo (24). These findings suggested that MCTs could increase muscle function without increasing muscle mass in healthy older adults and that an increase in

TABLE 4 Combined data analysis in Trials 1 and 2; muscle strength and function at baseline and after the 3-mo intervention and their changes from baseline in the MCTs-containing (MCT or LD + MCT) and LCTs-containing (LCT or LD + LCT) groups ($n = 56$).[a]

| Measure | Group | *n* | Baseline | 3-mo intervention | Non-adjusted change | Adjusted change[c] (model 1) | Adjusted change[d] (model 2) | Adjusted change[e] (model 3) |
|---|---|---|---|---|---|---|---|---|
| Right-hand grip | MCT, LD + MCT | 29 | 11.3 ± 7.6 | 13.0 ± 7.2*** | 1.7 ± 2.0 | 1.7 (1.1, 2.3) | 1.7 (1.1, 2.3) | 1.6 (0.9, 2.2) |
| strength, kg | LCT, LD + LCT | 25 | 11.7 ± 5.1 | 11.8 ± 4.9 | 0.2 ± 1.3 | 0.2 (−0.5, 0.8) | 0.2 (−0.5, 0.8) | 0.3 (−0.4, 1.0) |
| *p* value[b] | | | | | 0.002 | 0.001 | 0.002 | 0.017 |
| Left-hand grip | MCT, LD + MCT | 27 | 10.2 ± 4.6 | 11.4 ± 4.5 | 1.3 ± 2.6 | 1.2 (0.3, 2.1) | 1.1 (0.1, 2.0) | 0.8 (−0.2, 1.8) |
| strength, kg | LCT, LD + LCT | 25 | 10.8 ± 5.4 | 11.6 ± 5.5 | 0.8 ± 2.3 | 0.8 (−0.1, 1.8) | 1.0 (0.0, 2.0) | 1.3 (0.3, 2.3) |
| *p* value | | | | | 0.51 | 0.57 | 0.96 | 0.54 |
| Right knee | MCT, LD + MCT | 28 | 74 ± 40 | 105 ± 29*** | 31 ± 35 | | | |
| extension time, s | LCT, LD + LCT | 24 | 70 ± 43 | 73 ± 42 | 3 ± 46 | | Not applicable | |
| *p* value[f] | | | | | 0.021 | | | |
| Left knee | MCT, LD + MCT | 27 | 78 ± 42 | 106 ± 32** | 28 ± 36 | | | |
| extension time, s | LCT, LD + LCT | 25 | 69 ± 42 | 71 ± 44 | 2 ± 46 | | Not applicable | |
| *p* value | | | | | 0.034 | | | |
| Walking speed, | MCT, LD + MCT | 23 | 0.637 ± 0.409 | 0.706 ± 0.427** | 0.069 ± 0.124 | 0.068 (0.022, 0.113) | 0.076 (0.032, 0.119) | 0.084 (0.040, 0.128) |
| m/s | LCT, LD + LCT | 20 | 0.436 ± 0.257 | 0.420 ± 0.285 | −0.016 ± 0.073 | −0.014 (−0.062, 0.035) | −0.023 (−0.070, 0.024) | −0.033 (−0.080, 0.015) |
| *p* value | | | | | 0.011 | 0.020 | 0.005 | 0.002 |
| Legs open and | MCT, LD + MCT | 28 | 4.14 ± 3.13 | 6.48 ± 3.90*** | 2.34 ± 1.88 | 2.32 (1.67, 2.97) | 2.38 (1.71, 3.06) | 2.40 (1.70, 3.11) |
| close test, n/10 s | LCT, LD + LCT | 22 | 5.80 ± 2.89 | 4.96 ± 2.59* | −0.84 ± 1.35 | −0.82 (−1.56, −0.08) | −0.89 (−1.66, −0.12) | −0.92 (−1.73, −0.11) |
| *p* value | | | | | < 0.001 | <0.001 | <0.001 | <0.001 |
| Peak expiratory | MCT, LD + MCT | 25 | 189 ± 83 | 220 ± 82** | 31 ± 48 | 32 (13, 52) | 32 (13, 51) | 34 (13, 55) |
| flow, L/min | LCT, LD + LCT | 26 | 173 ± 66 | 179 ± 75 | 6 ± 50 | 5 (−14, 24) | 5 (−13, 23) | 3 (−18, 24) |
| *p* value | | | | | 0.08 | 0.049 | 0.048 | 0.06 |

[a]Values are means ± SD or adjusted mean (95% CI). Difference from baseline by Wilcoxon signed-rank test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[b]*p* value represents the differences in the changes of variables between the 2 groups assessed by 1-factor ANCOVA.
[c]Adjusted for each baseline value (model 1),
[d]Additionally adjusted for age, sex, BMI, and allocation to trial (Trials 1 or 2) (model 2).
[e]Further additionally adjusted for intakes in energy (kcal/day), protein (g/day), leucine (g/day), octanoic acid (C8:0, mg/day), decanoic acid (C10:0, mg/day), and vitamin D (μg/day) during the baseline period (model 3).
[f]The changes in right and left knee extension times were assessed by a nonparametric Mann–Whitney U test.LCT, 6 g/d of long-chain triglycerides; LD + LCT, leucine and cholecalciferol-enriched supplement with 6 g/d of long-chain triglycerides; LD + MCT, leucine and cholecalciferol-enriched supplement with 6 g/d of medium-chain triglycerides; MCT, 6 g/d of medium-chain triglycerides.

muscle mass by MCT supplementation might manifest in subjects whose muscle mass at baseline is very low (overall mean muscle mass was 16 kg), as is observed in frail older adults. It is also conceivable that an increase in muscle function may eventually increase muscle mass in frail older adults. However, precision in the measurement of muscle mass and functions is essential and is required for these studies.

Cognition was also improved after MCT supplementation in all trials (5–7). Therefore, improved cognition might affect muscle function (20). However, in the LD + MCT group in Trial 1, there was no significant correlation between changes in cognition test scores and changes in muscle mass and function (6). In Trial 2, the increases in peak expiratory flow and the number of swallows in 30 s persisted for 1.5 months after termination of the intervention (washout) (4). In contrast, the increases in cognition score returned to the baseline levels (7). These data suggested that the increase in cognition did not directly mediate increases in muscle function. However, it is conceivable that the motor and cognition areas in the brain were activated by a similar mechanism after MCT supplementation.

## 4.4 Acyl-ghrelin (an active form) may mediate increased muscle mass in response to MCT supplementation

After MCT supplementation in frail older adults, slight but significant increases in body weight and muscle mass with a decrease in fat mass were observed without a concomitant increase in energy intake. These findings indicated that the energy in fat tissues might be used for muscle tissues, and an anabolic effect was seen on muscle mass. This anabolic effect is similar to that of growth hormone (GH) (25). Interestingly, there is a close relationship between MCTs and GH via ghrelin.

Activated ghrelin (acyl-ghrelin) formed by proghrelin and octanoic acid (C8:0) via ghrelin-O-acyltransferase in the stomach, enters the blood circulation and stimulates GH release in the brain (26) and GH increases muscle mass (the MCTs/Ghrelin/GH hypothesis). MCT supplementation increases blood acyl-ghrelin concentrations in humans (27–29). Supplementation with 6 g MCTs/day for 2 weeks increased acyl-ghrelin from 20 fmol/mL (baseline) to 40 fmol/mL (2-fold

increase) and decreased desacyl-ghrelin (an inactive form) from 210 fmol/mL (baseline) to 160 fmol/mL in patients with anorexia nervosa (mean age, 26.4 years; BMI, 13.0 kg/m$^2$) (28). A lower dose of MCTs was able to increase acyl-ghrelin: single oral ingestion of 3 g of MCT (100% C8:0) with enteral nutrition formula in cachectic patients increased plasma acyl-ghrelin, and the 2 weeks administration of MCTs increased appetite score, body weight, and serum albumin and insulin-like growth factor-1 (but not GH) concentrations compared with their baseline levels (27). Considering the effects of a low dose of MCTs on acyl-ghrelin concentration, acyl-ghrelin may mediate the increases in muscle mass and function in response to MCT supplementation in frail older adults. Because a decrease in GH secretion was observed with aging, MCT supplementation in older adults might be timely (30). However, it has not been shown that MCT supplementation increases GH secretion.

Many reports show that acute acyl-ghrelin injection (or infusion) increased GH concentration, appetite, and food intake and that chronic acyl-ghrelin injection increased body weight, as listed in a supplemental table of a review (31). Chronic effects of acyl-ghrelin for years are mimicked by growth hormone secretagogues receptor agonists, which require no injections. Indeed, the impact of these oral agonists on body composition has been reported in three clinical trials (32–34), all of which showed increases in body weight and fat-free mass with no change in fat mass. These data suggested that acyl-ghrelin might lead to an increase in muscle mass but not a decrease in fat mass. Because MCT supplementation decreases fat mass, other mechanisms may be involved in reducing fat mass in response to MCT supplementation.

## 4.5 Effects of MCT supplementation on heart and skeletal muscles in patients with triglyceride deposit cardiomyovasculopathy (TGCV) or neutral lipid storage disease with myopathy (NLSDM)

Because in both TGCV and NLSDM (primary TGCV and NLSDM might be the same disease because the responsive gene is identical), lipid accumulation in cardiomyocytes and myocytes may cause heart failure and muscle atrophy, respectively (35, 36), MCTs, which decrease fat accumulation, have been considered a promising candidate for treating patients suffering from these conditions (37).

TGCV is a rare intractable disease in which impaired intracellular lipolysis results in massive triglyceride accumulation in the myocardium and coronary arteries, caused by genetic (primary) or acquired dysfunction of adipose triglyceride lipase (ATGL) (36). This leads to heart failure and ischemic heart disease (38). Long-chain fatty acids (LCFAs) entering cardiomyocytes are re-esterized to LCTs to form an energy pool in cells and are immediately hydrolyzed by intracellular lipases such as ATGL. In TGCV, the impaired intracellular lipolysis of LCTs results in ectopic deposition and loss of LCFA supply to mitochondria, leading to lipotoxicity and energy failure in cardiomyocytes and coronary smooth muscle cells. Because cytoplasmic MCTs were degraded at an average rate, whereas LCTs remained undegraded in fibroblasts from NLSDM patients (39), MCTs might be effective in treating TGCV. Indeed, cardiac imaging tests showed that a tricaprin diet (100% C10:0 with MCTs corresponding to 80% of total fat intake) reduced triglyceride accumulation and improved metabolism of LCFAs and left ventricular function in ATGL knockout (KO) mice compared with the ATGL KO mice fed the control diet (40). Recently, a randomized controlled MCT trial for idiopathic TGCV in humans ($n = 17$) was reported (41). In agreement with the results in ATGL KO mice, compared with the placebo ($n = 8$), supplementation with a 1.5 g/day of CNT-01 (100% C10:0) for 8 weeks ($n = 9$) improved myocardial lipolysis ($p = 0.035$) as estimated by iodine-123-beta-methyl-p-iodophenylpentadecanoic acid scintigraphy. However, decreases in TGCV severity scores (lower score indicates better outcome) between the 2 groups were nonsignificant, probably due to the short term of the intervention period. The adjusted mean changes from baseline (95% CI) were as follows: TGCV severity symptom score [CNT-01-2.78 (−4.61, −0.94) vs. placebo-0.75 points (−3.38, 1.88)] and TGCV severity ADL score [CNT-01-2.67 (−6.47, 1.14) vs. placebo-0.88 points (−3.30, 1.55)] (41).

In contrast, in a 26-year-old female patient with NLSDM caused by a mutation of ATGL without cardiac involvement, no beneficial effects on progressive muscle weakness were observed after MCT supplementation in a low-fat diet (medium-chain fatty acids [MCFAs] not specified; 30 g/day of MCTs plus 15 g/day of natural fat) for several years (42).

The difference in the effects of MCT supplementation between patients with TGCV and NLSDM might be explained as follows. (1) A high dose of MCTs was ineffective in reducing fat deposits in cells because a relatively large amount of MCT supplementation over the long term might lead to lipid accumulation in myocytes. (2) MCTs might be more effective in patients suffering effects on the heart than skeletal muscles because the heart might utilize more energy from fatty acids than the skeletal muscles do. (3) The effects of MCTs might depend on the patient's lifestyle or environment. Therefore, there were large variations in the effects of MCTs between patients, possibly due to confounding factors. These possible effects of MCTs on muscles in patients with TGCV and NLSDM may be applied to treating sarcopenia.

## 4.6 Limitations

This study has several limitations. We estimated muscle size by an anthropometric analysis; however, other modalities such as computed tomography, magnetic resonance imaging, and dual-energy X-ray absorptiometry may be required to confirm the results. The increase in muscle mass might reflect the accumulation of intramuscular lipids rather than an increase in muscle cell number or size; therefore, a muscle biopsy may be necessary. Critical confounding factors might have been missed. For example, ANCOVA might include individual physical activity levels as a covariate. Although a combined data analysis increased the number of participants, even more participants might be needed to observe the significant effects of MCTs in some measures relative to LCTs. Because this study targeted only frail older Japanese individuals, we should have addressed whether similar favorable effects of MCTs would be observed in Western populations with larger body sizes or non-frail subjects. Knowledge of the adverse effects of MCTs is also necessary.

## 5 Conclusion

A combined data analysis of clinical studies concluded that relative to LCTs, chronic supplementation with a low dose (6 g/day) of MCTs (C8:0 and C10:0) in frail older adults increased muscle mass and function. In contrast, it decreased fat mass while maintaining or increasing body weight. These findings indicate the potential for the practical use of MCTs in daily life in treating sarcopenia in older adults. Clinical trials in other groups of frail older adults will be required to verify these favorable effects of MCTs.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data presented in this article may be available on request from the corresponding author in accordance with appropriate data transfer and use agreements. Requests to access these datasets should be directed to ezaki1952@yahoo.co.jp.

## Ethics statement

The studies involving humans were approved by Dr. Teruhisa Yamamoto; The Human Ethics Committee of Showa Women's University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

## Author contributions

OE: Conceptualization, Formal analysis, Investigation, Writing – original draft. SA: Data curation, Methodology, Writing – review & editing.

## Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

## Acknowledgments

The authors thank all of our study participants and all personnel at the Day Care SKY nursing home for their collaboration.

## 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.2023.1284497/full#supplementary-material

## References

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *J Nutr*. (1997) 127:990S–1S. doi: 10.1093/jn/127.5.990S

2. Rogeri PS, Zanella R Jr, Martins GL, Garcia MDA, Leite G, Lugaresi R, et al. Strategies to prevent sarcopenia in the aging process: role of protein intake and exercise. *Nutrients*. (2021) 14:14. doi: 10.3390/nu14010052

3. Abe S, Ezaki O, Suzuki M. Medium-chain triglycerides in combination with leucine and vitamin D increase muscle strength and function in frail elderly adults in a randomized controlled trial. *J Nutr*. (2016) 146:1017–26. doi: 10.3945/jn.115.228965

4. Abe S, Ezaki O, Suzuki M. Medium-chain triglycerides (8:0 and 10:0) are promising nutrients for sarcopenia: a randomized controlled trial. *Am J Clin Nutr*. (2019) 110:652–65. doi: 10.1093/ajcn/nqz138

5. Abe S, Ezaki O, Suzuki M. Effects of timing of medium-chain triglycerides (8:0 and 10:0) supplementation during the day on muscle mass, function and cognition in frail elderly adults. *J Frailty Aging*. (2022) 11:1–9. doi: 10.14283/jfa.2021.33

6. Abe S, Ezaki O, Suzuki M. Medium-chain triglycerides in combination with leucine and vitamin D benefit cognition in frail elderly adults: a randomized controlled trial. *J Nutr Sci Vitaminol (Tokyo)*. (2017) 63:133–40. doi: 10.3177/jnsv.63.133

7. Abe S, Ezaki O, Suzuki M. Medium-chain triglycerides (8:0 and 10:0) increase Mini-mental state examination (MMSE) score in frail elderly adults in a randomized controlled trial. *J Nutr*. (2020) 150:2383–90. doi: 10.1093/jn/nxaa186

8. Gurney JM, Jelliffe DB. Arm anthropometry in nutritional assessment: nomogram for rapid calculation of muscle circumference and cross-sectional muscle and fat areas. *Am J Clin Nutr*. (1973) 26:912–5. doi: 10.1093/ajcn/26.9.912

9. Chumlea WC, Guo SS, Vellas B, Guigoz Y. Techniques of assessing muscle mass and function (sarcopenia) for epidemiological studies of the elderly. *J Gerontol A Biol Sci Med Sci*. (1995) 50A:45–51. doi: 10.1093/gerona/50A.Special_Issue.45

10. Asai C, Akao K, Adachi T, Iwatsu K, Fukuyama A, Ikeda M, et al. Maximal calf circumference reflects calf muscle mass measured using magnetic resonance imaging. *Arch Gerontol Geriatr*. (2019) 83:175–8. doi: 10.1016/j.archger.2019.04.012

11. Kobayashi K, Hiiragi Y, Maruyama H. Development of the "10-second open-close stepping test" (OCS-10) and fundamental study of its measurement values through a comparison of healthy young people and COmmunity-dwelling elderly. *J Phys Ther Sci*. (2012) 24:747–9. doi: 10.1589/jpts.24.747

12. 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

13. Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. *Stat Med*. (2002) 21:2917–30. doi: 10.1002/sim.1296

14. Nosaka N, Suzuki Y, Suemitsu H, Kasai M, Kato K, Taguchi M. Medium-chain triglycerides with maltodextrin increase fat oxidation during moderate-intensity exercise and extend the duration of subsequent high-intensity exercise. *J Oleo Sci*. (2018) 67:1455–62. doi: 10.5650/jos.ess18112

15. Vandenberghe C, St-Pierre V, Fortier M, Castellano CA, Cuenoud B, Cunnane SC. Medium chain triglycerides modulate the ketogenic effect of a metabolic switch. *Front Nutr*. (2020) 7:3. doi: 10.3389/fnut.2020.00003

16. Mumme K, Stonehouse W. Effects of medium-chain triglycerides on weight loss and body composition: a meta-analysis of randomized controlled trials. *J Acad Nutr Diet*. (2015) 115:249–63. doi: 10.1016/j.jand.2014.10.022

17. Bueno NB, de Melo IV, Florencio TT, Sawaya AL. Dietary medium-chain Triacylglycerols versus long-chain Triacylglycerols for body composition in adults: systematic review and Meta-analysis of randomized controlled trials. *J Am Coll Nutr*. (2015) 34:175–83. doi: 10.1080/07315724.2013.879844

18. Joseph AM, Adhihetty PJ, Leeuwenburgh C. Beneficial effects of exercise on age-related mitochondrial dysfunction and oxidative stress in skeletal muscle. *J Physiol*. (2016) 594:5105–23. doi: 10.1113/JP270659

19. Nishimune H, Stanford JA, Mori Y. Role of exercise in maintaining the integrity of the neuromuscular junction. *Muscle Nerve*. (2014) 49:315–24. doi: 10.1002/mus.24095

20. Amboni M, Barone P, Hausdorff JM. Cognitive contributions to gait and falls: evidence and implications. *Mov Disord*. (2013) 28:1520–33. doi: 10.1002/mds.25674

21. Harber MP, Konopka AR, Douglass MD, Minchev K, Kaminsky LA, Trappe TA, et al. Aerobic exercise training improves whole muscle and single myofiber size and function in older women. *Am J Physiol Regul Integr Comp Physiol*. (2009) 297:R1452–9. doi: 10.1152/ajpregu.00354.2009

22. Charette SL, McEvoy L, Pyka G, Snow-Harter C, Guido D, Wiswell RA, et al. Muscle hypertrophy response to resistance training in older women. *J Appl Physiol (1985)*. (1991) 70:1912–6. doi: 10.1152/jappl.1991.70.5.1912

23. Kojima K, Ishikawa H, Watanabe S, Nosaka N, Mutoh T. A randomized, double-blind, controlled trial assessing if medium-chain triglycerides in combination with moderate-intensity exercise increase muscle strength in healthy middle-aged and older adults. *Nutrients*. (2023) 15:3275. doi: 10.3390/nu15143275

24. Mutoh T, Kunitoki K, Tatewaki Y, Yamamoto S, Thyreau B, Matsudaira I, et al. Impact of medium-chain triglycerides on gait performance and brain metabolic network in healthy older adults: a double-blind, randomized controlled study. *Geroscience*. (2022) 44:1325–38. doi: 10.1007/s11357-022-00553-z

25. Svensson J, Lonn L, Jansson JO, Murphy G, Wyss D, Krupa D, et al. Two-month treatment of obese subjects with the oral growth hormone (GH) secretagogue MK-677 increases GH secretion, fat-free mass, and energy expenditure. *J Clin Endocrinol Metab*. (1998) 83:362–9. doi: 10.1210/jcem.83.2.4539

26. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. (1999) 402:656–60. doi: 10.1038/45230

27. Ashitani J, Matsumoto N, Nakazato M. Effect of octanoic acid-rich formula on plasma ghrelin levels in cachectic patients with chronic respiratory disease. *Nutr J*. (2009) 8:25. doi: 10.1186/1475-2891-8-25

28. Kawai K, Nakashima M, Kojima M, Yamashita S, Takakura S, Shimizu M, et al. Ghrelin activation and neuropeptide Y elevation in response to medium chain triglyceride administration in anorexia nervosa patients. *Clin Nutr ESPEN*. (2017) 17:100–4. doi: 10.1016/j.clnesp.2016.10.001

29. Yoshimura Y, Shimazu S, Shiraishi A, Nagano F, Tominaga S, Hamada T, et al. Ghrelin activation by ingestion of medium-chain triglycerides in healthy adults: a pilot trial. *J Aging Res Clin Pract*. (2018) 7:42–6. doi: 10.14283/jarcp.2018.9

30. Finkelstein JW, Roffwarg HP, Boyar RM, Kream J, Hellman L. Age-Related Change in the Twenty-Four Hour Spontaneous Secretion of Growth Hormone. *J Clin Endocrinol Metab*. (1972) 35:665–70. doi: 10.1210/jcem-35-5-665

31. Yanagi S, Sato T, Kangawa K, Nakazato M. The homeostatic force of ghrelin. *Cell Metab*. (2018) 27:786–804. doi: 10.1016/j.cmet.2018.02.008

32. Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell FE Jr, Clasey JL, et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. *Ann Intern Med*. (2008) 149:601–11. doi: 10.7326/0003-4819-149-9-200811040-00003

33. Katakami N, Uchino J, Yokoyama T, Naito T, Kondo M, Yamada K, et al. Anamorelin (ONO-7643) for the treatment of patients with non-small cell lung cancer and cachexia: results from a randomized, double-blind, placebo-controlled, multicenter study of Japanese patients (ONO-7643-04). *Cancer*. (2018) 124:606–16. doi: 10.1002/cncr.31128

34. White HK, Petrie CD, Landschulz W, MacLean D, Taylor A, Lyles K, et al. Effects of an oral growth hormone secretagogue in older adults. *J Clin Endocrinol Metab*. (2009) 94:1198–206. doi: 10.1210/jc.2008-0632

35. Fischer J, Lefevre C, Morava E, Mussini JM, Laforet P, Negre-Salvayre A, et al. The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. *Nat Genet*. (2007) 39:28–30. doi: 10.1038/ng1951

36. Hirano K, Ikeda Y, Zaima N, Sakata Y, Matsumiya G. Triglyceride deposit cardiomyovasculopathy. *N Engl J Med*. (2008) 359:2396–8. doi: 10.1056/NEJMc0805305

37. Kobayashi K, Sakata Y, Miyauchi H, Ikeda Y, Nagasawa Y, Nakajima K, et al. The diagnostic criteria 2020 for triglyceride deposit Cardiomyovasculopathy. *Ann Nucl Cardiol*. (2020) 6:99–104. doi: 10.17996/anc.20-00131

38. Hirano K. A novel clinical entity: triglyceride deposit cardiomyovasculopathy. *J Atheroscler Thromb*. (2009) 16:702–5. doi: 10.5551/jat.1669

39. Hilaire N, Salvayre R, Thiers JC, Bonnafé MJ, Nègre-Salvayre A. The turnover of cytoplasmic triacylglycerols in human fibroblasts involves two separate acyl chain length-dependent degradation pathways. *J Biol Chem*. (1995) 270:27027–34. doi: 10.1074/jbc.270.45.27027

40. Suzuki A, Yamaguchi S, Li M, Hara Y, Miyauchi H, Ikeda Y, et al. Tricaprin rescues myocardial abnormality in a mouse model of triglyceride deposit Cardiomyovasculopathy. *J Oleo Sci*. (2018) 67:983–9. doi: 10.5650/jos.ess18037

41. Miyauchi H, Hirano KI, Nakano Y, Shimada K, Nishikawa M, Yamamoto H, et al. (123)I-BMIPP scintigraphy shows that CNT-01 (Tricaprin) improves myocardial lipolysis in patients with idiopathic triglyceride deposit Cardiomyovasculopathy: first randomized controlled, exploratory trial for TGCV. *Ann Nucl Cardiol*. (2022) 8:67–75. doi: 10.17996/anc.22-00167

42. Missaglia S, Tavian D, Angelini C. Neutral lipid storage disease with myopathy: a 10-year follow-up case report. *Eur J Transl Myol*. (2022) 32:32. doi: 10.4081/ejtm.2022.10645

## Glossary

| | |
|---|---|
| AC | mid-upper-arm circumference |
| ADL | activities of daily living |
| AMA | mid-upper-arm muscle area |
| ANCOVA | analysis of covariance |
| ATGL | adipose triglyceride lipase |
| BMI | body mass index |
| CC | calf circumference |
| GH | growth hormone |
| KO | knockout |
| LCFAs | long-chain fatty acids |
| LCTs | long-chain triglycerides |
| LCT group | participants receiving 6 g LCTs/day only |
| LD + LCT | leucine and cholecalciferol-enriched supplement with 6 g LCTs/day |
| LD + LCT group | participants receiving a leucine and cholecalciferol-enriched supplement with 6 g LCTs/day |
| LD + MCT | leucine and cholecalciferol-enriched supplement with 6 g MCTs/day |
| LD + MCT group | participants receiving a leucine and cholecalciferol-enriched supplement with 6 g MCTs/day |
| MCFAs | medium-chain fatty acids |
| MCTs | medium-chain triglycerides |
| MCT group | participants receiving 6 g MCTs/day only |
| NLSDM | neutral lipid storage disease with myopathy |
| TGCV | triglyceride deposit cardiomyovasculopathy |
| TSF | triceps skinfold thickness |

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 17 January 2024
DOI 10.3389/fnut.2024.1310155



OPEN ACCESS

EDITED BY
Giulia Marrone,
University of Rome Tor Vergata, Italy

REVIEWED BY
Maha H. Alhussain,
King Saud University, Saudi Arabia
Ghadeer S. Aljuraiban,
King Saud University, Saudi Arabia

*CORRESPONDENCE
Han Zhou
✉ 1286547530@qq.com
Lianlong Yu
✉ lianlong00a@163.com

†These authors have contributed equally to this work

RECEIVED 09 October 2023
ACCEPTED 03 January 2024
PUBLISHED 17 January 2024

CITATION
Yang W, Zhuang Z, Huang P, Zhang M, Wang K, Jiang Y, Zhou H and Yu L (2024) Short sleep time may be the main reason for irregular breakfast to cause overweight—a cross-sectional study.
*Front. Nutr.* 11:1310155.
doi: 10.3389/fnut.2024.1310155



# Short sleep time may be the main reason for irregular breakfast to cause overweight—a cross-sectional study

Wei Yang[1], Zhao Zhuang[2], Pengxiang Huang[3], Man Zhang[3], Kebo Wang[3], Ying Jiang[3], Han Zhou[3,*†] and Lianlong Yu[3,*†]

[1]Shandong Provincial Third Hospital, Jinan, Shandong, China, [2]Qingdao Central Hospital, Qingdao, Shandong, China, [3]Shandong Center for Disease Control and Prevention, Jinan, Shandong, China

**Introduction:** In recent years, the relationship between circadian rhythm and overweight and obesity has attracted the attention of many scholars.

**Methods:** To evaluate association between the duration of sleep and the regularity of breakfast and overweight. A total of 1,178 students from Qingdao University were selected by stratified cluster sampling. There were 601 males (24.69 ± 0.80 years old) and 569 females (24.54 ± 0.70 years old). We used body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR) to define overweight levels. Chi-square test, Pearson correlation test, and logistic regression were applied to test association among overweight, sleep duration, sleep onset time, and breakfast regularity. Pittsburgh sleep quality index was used to assess the overall sleep quality of the study subjects. Mediation effect and Sobel test were used to analyze the effect of sleep duration on breakfast regularity and overweight.

**Results:** Only 34.1% of the population ate breakfast every day, and eating breakfast 1–3 times per week was associated with a higher risk of overweight (BMI: OR = 2.183, 95%CI: 1.369,3,481; WC: OR = 2.101, 95%CI: 1.232,3,583; WHR: OR = 2.108, 95%CI: 1.331,3,337). The effects of all types of Usual Breakfast Consumption Frequency on overweight were fully mediated by sleep duration ($p < 0.05$). In particular, the subjects exercised outdoors more than five times per week slept longer ($p < 0.05$).

**Conclusion:** Short sleep duration may be the main reason for irregular breakfast leading to overweight. Adequate outdoor exercise is essential for weight maintenance.

KEYWORDS

**students, overweight, sleep time, breakfast, cross-sectional study**

## 1 Introduction

Debate continues over the optimal number and timing of eating episodes for overweight individuals. The results of a meta-analysis showed that (1) skipping breakfast increases the risk of overweight, and these results are consistent across age, sex, region, and economic conditions. Further research has also found that (2) eating breakfast irregularly may be detrimental to weight status. This may be related to changes in energy intake due to dietary intake. Studies have shown that the presence or absence of energy intake and the duration and regularity of energy intake may change energy balance to varying degrees (3), including by affecting fat storage and insulin sensitivity. And the change of energy balance is one of the causes of

overweight (4). At the same time, different forms of energy expenditure such as exercise may also affect the energy balance through energy expenditure.

The relationship between dietary intake and energy balance may be related to circadian rhythms (5). The circadian system represents all physiological processes involved in the 24-h cycle, such as sleep/wake cycles, blood pressure, heart rate, hormone secretion, cognitive performance, and emotion regulation (6). Research shows that (7) SIRT1 gene is associated with circadian rhythm, and the activity of protein can be regulated through BMAL1 dysplasia process to influence the lipid metabolism and sugar. At the same time, circadian dysrhythmization increases postprandial ghrelin levels and decreases leptin levels, leading to the risk of weight gain (8). The duration of feeding is closely related to the expression of genes involved in circadian rhythm, and irregular eating can also affect the stability of diet-related clock oscillators located in peripheral tissues of the central nervous system (9). Sleep is an important component of the circadian rhythm, and different scholars have reached different conclusions in overweight/obesity-related studies. Spruyt et al. (10) showed a positive association between irregular sleep and overweight irrespective of sleep duration. Short sleep duration is a risk factor for overweight in a cohort study of type 2 diabetes mellitus (11).

It can be concluded that both dietary intake and sleep affect circadian rhythm adjustment. And overweight is one possible consequence of the altered circadian rhythms. Most previous studies have focused on the single effects of dietary intake and sleep on overweight, and few have included physical activity factors, which may also affect circadian rhythms. Considering that breakfast is the first meal after a long fast compared to other meal times, it may be particularly important in influencing circadian rhythms (2). Compared with students at other stages, graduate students have more academic pressure and mental pressure, so the overwork, irregular work and rest, obesity, sleep disorders, psychological problems of graduate students are more prominent. Therefore, this study aimed to explore the mediating role of sleep duration in the association between irregular breakfast intake and overweight and obesity in postgraduates. We used different methods to define overweight, and the results were stratified according to frequency of outdoor activity.

# 2 Materials and methods

## 2.1 Study population

A stratified cluster sampling method was used in this study. Specifically, all graduate students in Qingdao University were stratified according to grade, and 8 classes were randomly selected from each class, and all students in the whole class were selected as the research objects. During the research process, we sought the opinions of the school, and after obtaining the consent, we contacted the class teachers and student representatives. After obtaining their cooperation, we summoned all the students in the sampled classes to publicize our research project to the students. Finally, according to the arrangement of the head teacher and the student representative, the questionnaire collection was completed in batches. A total of 1,178 students including 571 females and 602 males were enrolled in this study. After obtaining informed consent, a face-to-face questionnaire was completed to collect anthropometric indicators (It is filled in by the students themselves) and basic information forms, including a lifestyle and physical activity questionnaire (This study was approved by the Ethics Committee of the Third Hospital of Shandong Province and the Affiliated Hospital of Qingdao University with the approval numbers of KYLL-2023085 and QYFYWZLL25548, respectively). In the present analysis, participants were excluded if they had an extreme body mass index (BMI, BMI = weight (kg)/height$^2$ (m); below 18.5 kg/m$^2$, and above 28.0 kg/m$^2$; $n = 2$). Or if they had an extreme caloric intake (<500 or > 5,000 kcal/day; $n = 5$) (12). The effective sample size was 1,170, consisting of 601 boys and 569 girls (Figure 1).

## 2.2 Diet, sleep, and physical activity

An adjusted version of the standard food frequency questionnaire (FFQ) was administered to evaluate dietary intakes. The questionnaire included eight main food groups and food varieties adapted to the Chinese diet. Participants were asked, "During the past year, on average, daily, weekly, or monthly use condition, and average consumption per serving (g)" A similar question asked everyone. Food models and food maps were used to assist in the completion of dietary questionnaires. Average daily energy intake levels for each individual were obtained from the FFQ. The eating habits questionnaire included the eating time of three meals a day, the duration of each meal, whether to eat midnight snacks after dinner, and whether to watch videos during meals. For breakfast, we designed the type of breakfast, how much money to buy breakfast, where to eat breakfast and whether parents value breakfast. Of these, breakfast was defined as eating before 9 a.m. Late-night snacks were defined as meals eaten after dinner in the evening, and any food consumed after dinner was considered a late-night snack.

The Pittsburgh sleep quality index (PSQI) (13) was used to assess the overall sleep quality of the study subjects. It was developed in 1989 by Buysse et al. (14), a psychiatrist at the University of Pittsburgh, USA, to evaluate the sleep quality of the subjects in the last month. It is by far the most widely used sleep quality assessment tool (15). It consists of 18 items consisting of 7 components, each of which is scored on a scale of 0–3, and the cumulative score of each

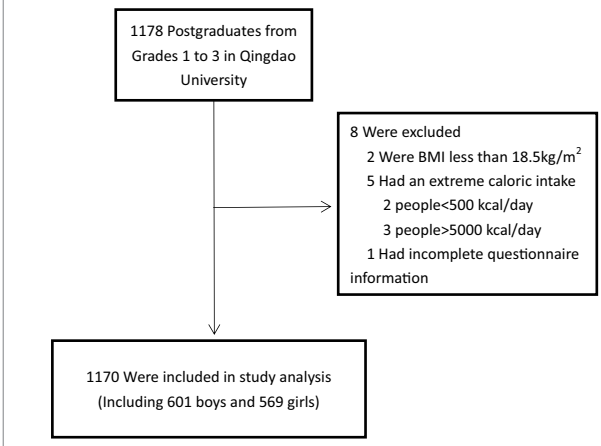



FIGURE 1
Study population.

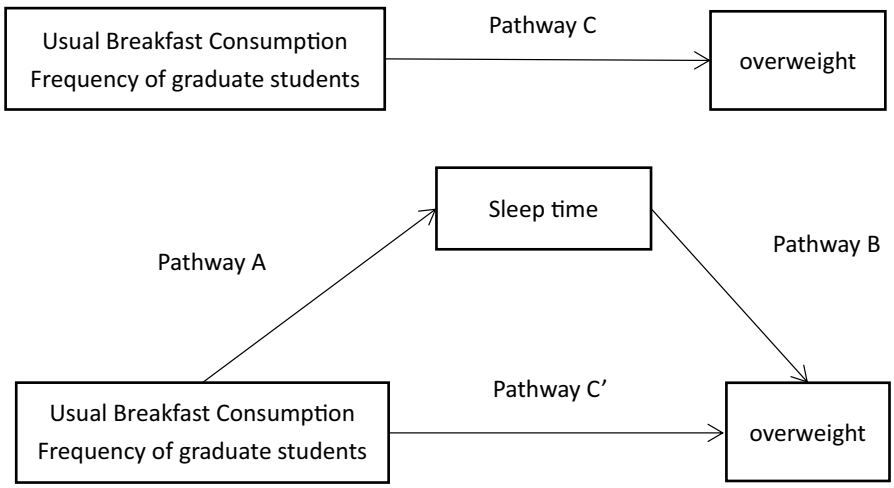



FIGURE 2
Model of the potential mediating effect of sleep duration on the relationship between usual breakfast consumption frequency of college students and overweight.

component is the total PSQI score. The total score ranged from 0 to 21, with higher scores indicating poorer sleep quality. A score of 0–5 is defined as very good sleep quality, 6–10 as good sleep quality, 11–15 as fair sleep quality and 16–21 as very poor sleep quality. The questionnaire recorded the time of getting up in the morning and the time of going to bed the previous day, and we used this data to calculate the time in bed each day, subtracting the "time needed to fall asleep" to obtain the actual amount of sleep each day. Participants were told that the duration of sleep did not include periods of lying awake in bed.

The physical activity questionnaire included the number of outdoor activities per week, the time of each activity, etc. Among them, effective outdoor activity was defined as spontaneous physical activity of moderate to vigorous intensity that required moderate to substantial effort and resulted in significantly increased heart rate or shortness of breath. Such as fast walking, dancing, running, climbing, swimming, skipping rope, football, volleyball, basketball and so on.

## 2.3 Adiposity outcomes

Overweight or Central obesity status was defined in cross-sectional analyses using BMI, waist circumference (WC) and waist-to-hip ratio (WHR, WHR = waist circumference/hip circumference). For data collection, height and weight were self-reported by students. Waist and hip circumference were obtained at the physical examination center with the consent of schools, teachers and students themselves. BMI was used to assess both normal (18.5–24.0 kg/m$^2$) and obesity (≥24.0 kg/m$^2$) as binary outcomes. The definitions of overweight by waist circumference and waist-to-hip ratio refer to previous literature and are adjusted according to the actual situation in China (16, 17). In the separate models, people were considered obese if they had a WC greater than 85 cm for men or 90 cm for women. For WHR, obesity was defined as greater than 0.9 in men and 0.8 in women. We mentioned in the study of obesity are judged to be in the center of the "overweight" in the crowd, the proportion of central obesity.

## 2.4 Statistical analysis

The enumerated data is represented by frequencies and the differences between groups are compared using a chi-square test. We used Pearson correlation and binary logistic regression to analyze the relationship between breakfast consumption regularity and excess weight. In logistics regression, overweight was the dependent variable, breakfast frequency was the independent variable, and the variable was selected as "input." All the results were adjusted for sex and age. The Chi-square test was used to compare the differences in the proportion of obese people in different breakfast frequency groups. Based on the theory of mediating effects proposed by Baron and Kenny (Causal stepwise regression test) (18–20), sleep duration was considered as a mediator factor (M), usual breakfast consumption frequency (X) was considered as an independent variable, and overweight were considered as a dependent variable (Y) for mediating the effect analysis. The total effect (c) of factor X on factor Y can be decomposed into direct effect (c') and mediating effect (ab). 'a' was the effect of factor X on factor M, and 'b' was the effect of factor M on factor Y after adjusting for factor X (Figure 2). The specific steps are as follows: first, analyze the regression of X to Y and test the significance of regression coefficient c; Second, the regression from X to M is analyzed and the significance of the regression coefficient a is tested. Third, we analyze the regression from X to Y with the addition of the mediating variable M, and test the significance of the regression coefficients b and c′. Among them, when both a and b are significant, the significance of c′ is tested to judge the mediation effect; When at least one of a and b is not significant, the Sobel test is used to determine whether a mediation effect is present. Linear regression models were used to analyze the mediation effects, which were subsequently evaluated using the Sobel method. All statistical tests were two-sided at $\alpha = 0.05$ and all analyses were conducted using SPSS 25.0.

# 3 Results

The characteristics of the study population, stratified by breakfast consumption frequency, are shown in Table 1. In short, 34.1% of the

10.3389/fnut.2024.1310155

TABLE 1 Characteristics of study population stratified by breakfast consumption frequency.

| | Usual breakfast consumption frequency (days/week) | | | |
|---|---|---|---|---|
| | 0 | 1–3 | 4–6 | 7 |
| *N* (%) | 50 (4.3) | 167 (14.3) | 553 (47.3) | 400 (34.1) |
| Age | 24.68 ± 1.05 | 24.64 ± 0.65 | 24.63 ± 0.78 | 24.58 ± 0.74 |
| Gender *n* (%) | | | | |
| Male | 32 (64.0) | 93 (55.7) | 297 (53.7) | 179 (44.7) |
| Female | 18 (36.0) | 74 (44.3) | 256 (46.3) | 221 (55.3) |
| Energy intake (kcal/day) | 1406.73 ± 458.37 | 1540.70 ± 675.35 | 1577.29 ± 586.54 | 1574.58 ± 680.17 |
| WC | 76.06 ± 8.5 | 76.15 ± 10.60 | 74.87 ± 10.10 | 74.45 ± 9.02 |
| WHR | 0.80 ± 0.07 | 0.81 ± 0.07 | 0.79 ± 0.07 | 0.79 ± 0.06 |
| BMI (kg/m$^2$) | 21.61 ± 2.76 | 21.95 ± 2.84 | 21.60 ± 2.56 | 21.61 ± 2.44 |
| Average sleep (hours) | 6.84 ± 0.90 | 6.85 ± 0.93 | 7.00 ± 0.75 | 7.00 ± 0.82 |
| PSQI score | 4.76 ± 2.92 | 4.93 ± 2.38 | 4.43 ± 2.34 | 3.96 ± 2.30 |
| Stay up late (usual sleep after 12 o 'clock frequency) *n* (%) | | | | |
| Everyday | 13 (26.0) | 33 (19.8) | 84 (15.2) | 42 (10.5) |
| 4–6 times a week | 8 (16.0) | 37 (22.2) | 115 (20.8) | 42 (10.5) |
| 1–3 times a week | 21 (42.0) | 81 (48.5) | 286 (51.7) | 192 (48.0) |
| Never | 8 (16.0) | 16 (9.6) | 68 (12.3) | 124 (31.0) |
| Usual outdoor exercise frequency | | | | |
| ≥5 times a week | 7 (14.0) | 27 (16.2) | 86 (15.5) | 84 (21.0) |
| 3–4 times a week | 16 (32.0) | 43 (25.7) | 186 (33.7) | 140 (35.0) |
| 1–2 times a week | 24 (48.0) | 92 (55.1) | 261 (47.2) | 157 (39.3) |
| Never | 3 (6.0) | 5 (3.0) | 20 (3.6) | 19 (4.8) |
| Late-night snacks frequency | | | | |
| Everyday | 2(4.0) | 0 | 5 (0.9) | 2 (0.5) |
| 4–6 times a week | 5 (10.0) | 19 (11.4) | 55 (9.9) | 29 (7.2) |
| 1–3 times a month | 4 (8.0) | 24 (14.4) | 63 (11.4) | 27 (6.8) |
| Seldom | 23 (46.0) | 86 (51.5) | 279 (50.5) | 195 (48.8) |
| Never | 16 (32.0) | 38 (22.8) | 151 (27.3) | 147 (36.8) |

participants reported eating breakfast daily, 47.3% reported eating breakfast 4–6 days a week and 14.3% reported eating breakfast 1–3 days a week, while only 4.3% of the participants reported not eating breakfast. On average, those who reported eating breakfast daily and 4–6 days a week consumed the most calories, had lower waistlines and WHR, and slept longer. In addition, both those who never ate breakfast and those who ate breakfast daily reported lower BMI than those who ate breakfast 1–3 times a week. Then, those who ate breakfast daily had more weekly outdoor exercise and fewer late-night snacks than the other groups. Shown in Figure 3.

According to the PSQI scoring rules (Figure 4), 70.5 percent had very good sleep quality, 28.2 percent had good sleep quality and only 1.3 percent had fair sleep quality. The overall PSQI sleep score was 4.35 ± 2.30. The study showed that those who ate breakfast daily had significantly lower PSQI scores than the other groups, with those who ate breakfast 1–3 times a week having the highest mean PSQI scores. We found an association between the duration of sleep and BMI, but not statistically significant between the time of sleep onset and BMI. Shown in Figures 5, 6.

Using BMI to define overweight resulted in the highest prevalence (15.3%), while WHR resulted in the lowest prevalence (11.2%) of obesity, among them, the prevalence of obesity using WC definition was 18.5%. People with different frequency of breakfast intake had different prevalence of overweight, and Pearson's correlation analysis showed that there was a correlation between overweight and breakfast intake frequency. This association persisted after adjusting for sex and age. In addition, all three overweight determination methods showed the same results (shown in Table 2).

Binary logistic regression analysis was performed with overweight as the dependent variable, breakfast frequency as the independent variable, and daily breakfast frequency as the reference level. When BMI was used to define overweight, the results showed that the likelihood of overweight was 218.3% (OR = 2.183, 95%CI: 1.369, 3.481) for those who ate breakfast 1–3 days per week compared with those who ate breakfast every day. This is similar to the results when the WHR defines overweight (OR = 2.101, 95%CI: 1.232, 3.583). Most associations were observed when using WC to define Central obesity, with those who ate breakfast 1–3 days per week or 4–6 days per week

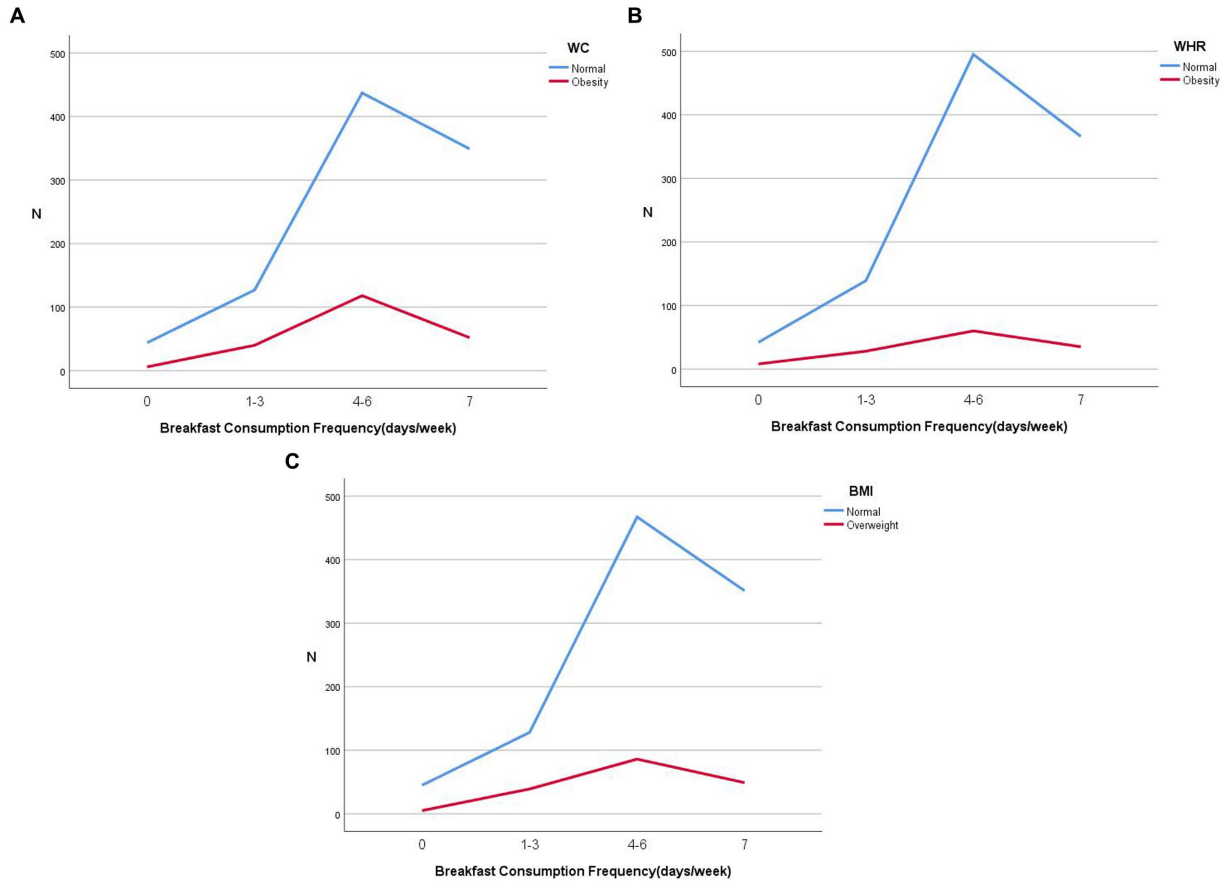



FIGURE 3
Prevalence of overweight and obesity in different breakfast frequency groups, with overweight defined by waist circumference **(A)**, waist-hip ratio **(B)** and BMI **(C)**, respectively.

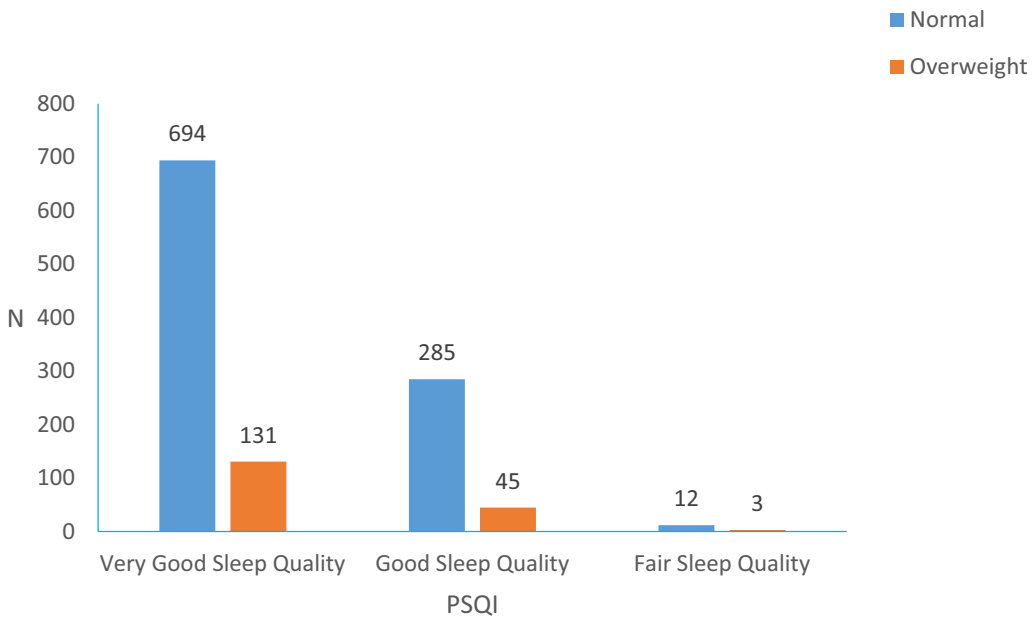



FIGURE 4
Distribution of overweight and obesity in different PSQI scores (overweight assessed by BMI).

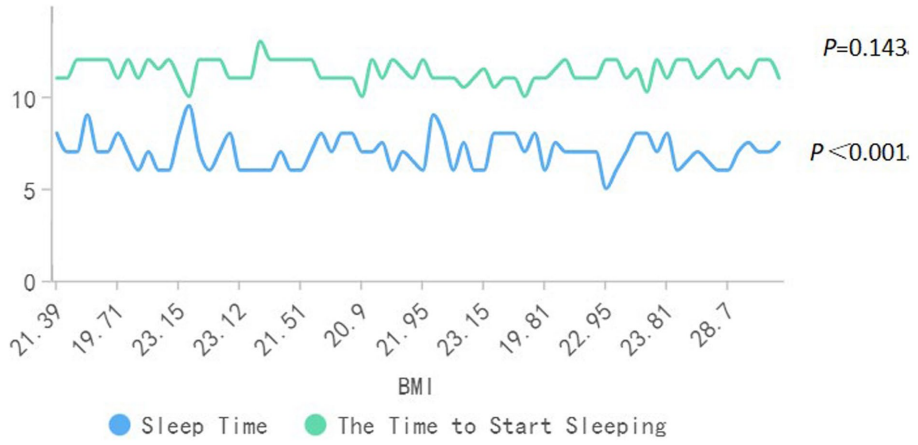



FIGURE 5
Association between sleep and BMI (In order to let two time be displayed on a diagram, it is expressed in 12 h when I start to sleep. I just started to sleep after the early morning, and it is recorded as the time to start sleeping (12 h) +12).

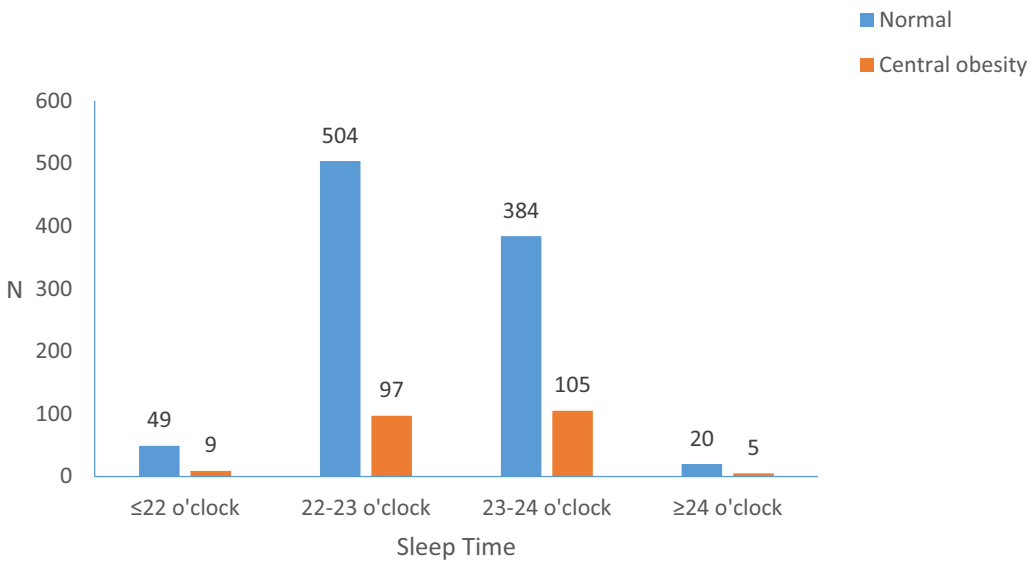



FIGURE 6
The relationship between the time to sleep and the central obesity.

being 110.8% (OR = 2.108, 95%CI: 1.331, 3.337) and 81.5% (OR = 1.815, 95%CI: 1.272, 2.590) more likely to be Central obesity, respectively, compared to people who reported eating breakfast every day. This effect persisted after adjusting for sex and age. The results are shown in Table 3.

We used a causal stepwise regression test to model the mediating effect of sleep duration on the association between frequency of regular breakfast consumption and overweight. We found that sleep duration was associated with the usual breakfast consumption frequency ($p$ = 0.015) and overweight ($p$ = 0.001). The next test coefficient, $c'$, was not significant, indicating that sleep duration was a sufficient mediator of the effect of regular breakfast consumption frequency on overweight. We further adjusted for age and gender (model 2) and yielded the same results. The relevant results are shown in Figure 1 and Table 4.

The mediating effect of sleep duration on the association between frequency of regular breakfast consumption and overweight was stratified by frequency of outdoor activity. Combined with the results of Sobel's test, when the frequency of outdoor activities was 5 or more times per week, the mediating effect of sleep duration on the association between usual breakfast consumption frequency and overweight existed ($p$ = 0.029). The same results were obtained after adjusting for gender and age ($p$ = 0.031). The results are shown in Table 5.

## 4 Discussion

In previous studies (21–23), the prevalence of unhealthy eating habits and overweight among college students has been increasing

TABLE 2 Relationship between regularity in breakfast consumption and weight status.

| Outdoor exercise frequency | | Breakfast consumption frequency (days/week) | | | | $\chi^2$/p | $p^a/p^b$ |
|---|---|---|---|---|---|---|---|
| | | 0 | 1–3 | 4–6 | 7 | | |
| *BMI (kg/m²)* | | | | | | 12.340/0.006 | 0.039/<0.05 |
| 18.5–24 | ≥5 times a week | 4 (2.3) | 22 (12.4) | 73 (41.2) | 78 (44.1) | | |
| | 3–4 times a week | 15 (4.6) | 36 (10.9) | 156 (47.4) | 122 (37.1) | | |
| | 1–2 times a week | 23 (5.0) | 69 (15.1) | 227 (49.7) | 138 (30.2) | | |
| | Never | 3 (10.7) | 1 (3.6) | 11 (39.3) | 13 (46.4) | | |
| ≥24 | ≥5 times a week | 5 (10.0) | 39 (23.4) | 86 (15.6) | 49 (12.3) | | |
| | 3–4 times a week | 1 (1.8) | 7 (12.5) | 30 (53.6) | 18 (32.1) | | |
| | 1–2 times a week | 1 (1.3) | 23 (29.9) | 34 (44.2) | 19 (24.7) | | |
| | Never | 0 | 4(21.1) | 9(47.4) | 6(31.6) | | |
| *WC (Male>85 cm/female>90 cm)* | | | | | | 15.691/0.001 | 0.020 /<0.05 |
| | ≥5 times a week | 2 (5.0) | 11 (27.5) | 19 (47.5) | 8 (20.0) | | |
| | 3–4 times a week | 3 (4.2) | 7(9.9) | 41 (57.7) | 20 (28.2) | | |
| | 1–2 times a week | 1 (1.1) | 18 (20.5) | 48 (54.5) | 21 (23.9) | | |
| | Never | 0 | 4 (23.5) | 10 (58.8) | 3 (17.6) | | |
| *WHR(Male>0.9/female>0.8)* | | | | | | 8.930/0.030 | 0.006/<0.05 |
| | ≥5 times a week | 8 (16.0) | 28 (16.8) | 60 (10.8) | 35 (8.8) | | |
| | 3–4 times a week | 3 (7.9) | 2 (5.3) | 21 (55.3) | 12 (31.6) | | |
| | 1–2 times a week | 3 (4.8) | 20 (32.3) | 24 (38.7) | 15 (24.2) | | |
| | Never | 0 | 1 (14.2) | 3 (42.9) | 3 (42.9) | | |

[a]Pearson correlation.
[b]Pearson correlation (adjusted for age and sex).

TABLE 3 Binary logistics regression analysis of breakfast consumption regularity and overweight in college students under different definitions of overweight.

| | Model 1 | | | | | | Model 2 | |
|---|---|---|---|---|---|---|---|---|
| | Frequency[a] | B | S.E. | Wald | *p* | OR (95%CI) | *p* | OR (95%CI) |
| BMI | Never | −0.228 | 0.495 | 0.212 | 0.645 | 0.796 (0.301, 2.102) | 0.461 | 0.692 (0.260, 1.843) |
| | 1–3 times a week | 0.780 | 0.238 | 10.742 | 0.001 | 2.183 (1.369, 3.481) | 0.003 | 2.059 (1.282, 3.308) |
| | 4–6 times a week | 0.277 | 0.192 | 2.072 | 0.150 | 1.319 (0.905, 1.923) | 0.254 | 1.249 (0.852, 1.831) |
| WC | Never | −0.091 | 0.460 | 0.040 | 0.842 | 0.913 (0.371, 2.248) | 0.636 | 0.803 (0.324, 1.992) |
| | 1–3 times a week | 0.746 | 0.234 | 10.113 | 0.001 | 2.108 (1.331, 3.337) | 0.004 | 1.986 (1.248, 3.161) |
| | 4–6 times a week | 0.596 | 0.181 | 10.815 | 0.001 | 1.815 (1.272, 2.590) | 0.003 | 1.723 (1.203, 2.467) |
| WHR | Never | 0.686 | 0.424 | 2.615 | 0.106 | 1.986 (0.865, 4.564) | 1.150 | 1.850 (0.801, 4.270) |
| | 1–3 times a week | 0.742 | 0.272 | 7.424 | 0.006 | 2.101 (1.232, 3.583) | 0.010 | 2.023 (1.183, 3.458) |
| | 4–6 times a week | 0.238 | 0.224 | 1.136 | 0.286 | 1.269 (0.819, 1.967) | 0.371 | 1.223 (0.787, 1.899) |

[a]usual breakfast consumption frequency. And eating breakfast every day was used as a reference.
Model 2: adjusted for age and sex.

worldwide. In Canada, the prevalence of overweight was 31.6% in men and 26.6% in women, whereas in a survey of 10,810 people in 12 European countries, almost half of the participants reported being overweight (54.1% in men and 42.5% in women). These are, respectively, higher than the results of the present study. In a study on Shandong Province (China) (24), the prevalence of overweight was higher (22.74% in men and 8.42% in women) and the prevalence of central obesity was lower (7.85% in men and 3.02% in women) than in our study. A study of U.S. found that more than 70% of American adults are overweight or obese (25), which may be related to a dramatic shift in food choices and lifestyles among college students (26). This suggests that overweight among college students remains a social problem that needs to be urgently addressed.

The present study showed that people with regular breakfast consumption habits, including never eating breakfast and eating breakfast every day, had lower mean BMI than those who ate breakfast 1–3 times per week, which is consistent with the results of previous studies (2, 27, 28). In theory, people who eat breakfast every day

TABLE 4 The mediating effect of sleep duration on the Relationship between usual breakfast consumption frequency of college students and overweight.

| | Effect | β | S.E. | *t* | *p* | OR (95%CI) |
|---|---|---|---|---|---|---|
| Model 1 | a | 0.071 | 0.029 | 2.448 | 0.015 | 0.014, 0.129 |
| | b | −0.046 | 0.013 | −3.515 | 0.001 | −0.071, −0.020 |
| | C′ | −0.053 | 0.013 | −1.826 | 0.068 | −0.050, 0.002 |
| Model 2 | a | 0.066 | 0.029 | 2.265 | 0.024 | 0.009, 0.124 |
| | b | −0.092 | 0.013 | −3.173 | 0.002 | −0.067, −0.016 |
| | C′ | −0.042 | 0.013 | −1.448 | 0.148 | −0.044, 0.007 |

All analysis adjusted for gender and ages. a, the effect of usual breakfast consumption frequency of undergraduate and sleep duration; b, the effect of sleep duration and overweight; C′ the direct effect of usual breakfast consumption frequency of undergraduate and overweight.

TABLE 5 Coefficients for mediation analysis in different outdoor exercise frequency.

| Frequency[a] | Direct effect Path C′ β/p | Indirect effect | | Sobel test p |
|---|---|---|---|---|
| | | Path A β/p | Path B β/p | |
| ≥5 times a week | −0.082/0.005 | 0.187/0.008 | −0.115/0.101 | 0.029 |
| 3–4 times a week | −0.002/0.933 | −0.007/0.890 | −0.070/0.522 | – |
| 1–2 times a week | −0.025/0.196 | 0.093/0.053 | −0.326/0.001 | – |
| Never | −0.036/0.677 | −0.098/0.531 | 0.011/0.977 | – |
| ≥5 times a week* | −0.076/0.012 | 0.187/0.009 | −0.117/0.096 | 0.031 |
| 3–4 times a week* | 0.003/0.908 | −0.002/0.960 | −0.014/0.548 | – |
| 1–2 times a week* | −0.021/0.268 | 0.083/0.060 | −0.055/0.003 | - |
| Never* | −0.011/0.902 | −0.072/0.646 | 0.011/0.896 | – |

[a]Different outdoor exercise frequency stratified analyses suggest differences according to outdoor exercise ≥ 5 times a week.
Adjusted for age and sex.

should have the highest energy intake and correspondingly the highest weight, but in reality they weigh smaller. At the same time, those who ate breakfast daily spent more time outdoors and ate fewer late night snacks per week, suggesting that normal lifestyle habits are beneficial for weight loss (29). Breakfast consumption was sub-divided into four different frequencies in our study, but the average BMI levels associated with eating breakfast daily and eating breakfast 4–6 days per week were similar. We believe that this may be related to the fact that breakfast consumption habits on weekends are different from those on weekdays (30, 31), and whether there is a difference between eating breakfast 6 days a week and eating breakfast every day still needs additional research, and of course it may be related to sampling error.

In this study, 29.5% of the students had poor sleep quality, which was lower than that of Stanford University (42.4%) (32). The overall PSQI score was 4.35, which was similar to the results of South Korea (4.21) (33), lower than the results of Japanese nursing students (6.86) (34), lower than the results of Anhui province (5.37) (35) and Beijing (5.38) (13). In previous studies (36), short sleep duration and unhealthy eating habits were related to overweight, respectively. This study explored the mediating effect of sleep and showed that the duration of sleep is a sufficient mediator of the effect of breakfast consumption on obesity. Sleep duration, as an essential component of the circadian rhythm, may affect fatty metabolism, energy expenditure, and may also have an effect on eating behavior through the circadian rhythm itself. However, the results from a Japanese cohort study showed that (37) skipping breakfast and short sleep time have nothing to do with the prevalence of male obesity, but are related to female obesity. For this result, we still think that there may be differences in sleep habits between men and women. There are also differences in circadian rhythm and energy balance caused by different sleep habits. So whether different sleep habits can change the circadian rhythm of the body is still a problem worth studying.

It has been suggested that (38) staying up late with insomnia may cause adolescents to consume additional carbohydrates and fats, most likely to gain weight. However, our study did not show a statistically significant association between the time of sleep onset and BMI. It could be that the relatively fixed work-and-rest habits of college masked the effects of late nights on weight, or it could be that the effects of staying up for short periods of time on the body's metabolism have not yet reached the point where they lead to visceral obesity (39). The specific underlying mechanisms require additional investigation.

When the frequency of outdoor activities was ≥5 times per week, the mediating effect of sleep remained significant. In previous studies (40), exercise significantly improved sleep efficiency. Our study may suggest that adequate amounts of exercise are required to have an effective effect on sleep. In fact, numerous scholars also emphasize the importance of regular exercise (41, 42). Regular exercise may improve sleep quality by increasing the production of brain-derived neurotrophic factors (43) and increase skeletal muscle fatty oxidation (44). This may be related to the fact that the effects of exercise on the body's health are reflected in molecular and cellular changes over a

longer period of time (45). This also shows the importance of sticking to exercise and regular lifestyle habits.

There are some limitations in our analysis that should be noted, such as the possible sampling error due to the selection of subjects from only one school. In addition, the strength of our study cancels out the possibility of error due to the small number of people classified as obese. The inclusion of subjects with good homogeneity, similar ages, and consistent living environments also excluded some potential confounding factors for our study.

## 5 Conclusion

In conclusion, this study suggests that short evening sleep duration may be the main cause of overweight caused by irregular breakfast in graduate students. And the mediating effect is still significant when outdoor activities are ≥5 times per week. The differences in the effects of skipping breakfast versus eating breakfast irregularly on obesity are still under debate, and we believe that the effects of breakfast consumption during the week and breakfast consumption during the weekend on obesity remain worthy of study. Sleeping late and sleeping short are two concepts, and the effect of these two different concepts on the body's metabolism is also a point of concern. Most importantly, this study shows that regular breakfast consumption, adequate sleep duration, and adequate outdoor physical activity are all essential for weight maintenance.

## Data availability statement

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

## Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/ participants or patients/participants legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## Author contributions

WY: Formal analysis, Investigation, Methodology, Validation, Writing – original draft. ZZ: Investigation, Validation, Writing – review & editing. PH: Investigation, Methodology, Validation, Writing – review & editing. MZ: Investigation, Methodology, Writing – review & editing. KW: Methodology, Writing – review & editing. YJ: Methodology, Writing – review & editing. HZ: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. LY: Conceptualization, Methodology, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was financed by Shandong Medical and Health Science and Technology Development Project (SMHSTDP) 2019WS436.

## Acknowledgments

We thank all the team members and all the participants. Special thanks to Qingdao university graduate student Wang Liang gives selfless help in the process of investigation.

## 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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

1. Ma X, Chen Q, Pu Y, Guo M, Jiang Z, Huang W, et al. Skipping breakfast is associated with overweight and obesity: a systematic review and meta-analysis. *Obes Res Clin Pract*. (2020) 14:1–8. doi: 10.1016/j.orcp.2019.12.002

2. Guinter MA, Park YM, Steck SE, Sandler DP. Day-to-day regularity in breakfast consumption is associated with weight status in a prospective cohort of women. *Int J Obes*. (2020) 44:186–94. doi: 10.1038/s41366-019-0356-6

3. Chowdhury EA, Richardson JD, Holman GD, Tsintzas K, Thompson D, Betts JA. The causal role of breakfast in energy balance and health: a randomized controlled trial in obese adults. *Am J Clin Nutr*. (2016) 103:747–56. doi: 10.3945/ajcn.115.122044

4. Templeman I, Gonzalez JT, Thompson D, Betts JA. The role of intermittent fasting and meal timing in weight management and metabolic health. *Proc Nutr Soc*. (2020) 79:76–87. doi: 10.1017/S0029665119000636

5. Johnston JD. Physiological responses to food intake throughout the day. *Nutr Res Rev*. (2014) 27:107–18. doi: 10.1017/S0954422414000055

6. Longo VD, Panda S. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metab*. (2016) 23:1048–59. doi: 10.1016/j.cmet.2016.06.001

7. Serin Y, Acar TN. Effect of circadian rhythm on metabolic processes and the regulation of energy balance. *Ann Nutr Metab*. (2019) 74:322–30. doi: 10.1159/000500071

8. Chaput JP, McHill AW, Cox RC, Broussard JL, Dutil C, da Costa BGG, et al. The role of insufficient sleep and circadian misalignment in obesity. *Nat Rev Endocrinol*. (2023) 19:82–97. doi: 10.1038/s41574-022-00747-7

9. Adafer R, Messaadi W, Meddahi M, Patey A, Haderbache A, Bayen S, et al. Food timing, circadian rhythm and Chrononutrition: a systematic review of time-restricted Eating's effects on human health. *Nutrients*. (2020) 12:3770. doi: 10.3390/nu12123770

10. Spruyt K, Molfese DL, Gozal D. Sleep duration, sleep regularity, body weight, and metabolic homeostasis in school-aged children. *Pediatrics*. (2011) 127:e345–52. doi: 10.1542/peds.2010-0497

11. Antza C, Kostopoulos G, Mostafa S, Nirantharakumar K, Tahrani A. The links between sleep duration, obesity and type 2 diabetes mellitus. *J Endocrinol*. (2021) 252:125–41. doi: 10.1530/JOE-21-0155

12. Ding K, Zhou H, Gao T, Xu R, Chen L, Cai J, et al. Dietary patterns and cognitive function in older adults residing in rural China. *Asia Pac J Clin Nutr*. (2021) 30:253–62. doi: 10.6133/apjcn.202106_30(2).0010

13. Zheng B, Li M, Wang KL, Lv J. Analysis of the reliability and validity of the Chinese version of Pittsburgh sleep quality index among medical college students. *Beijing Da Xue Xue Bao*. (2016) 48:424–8. doi: 10.3969/j.issn.1671-167x.2016.03.009

14. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res*. (1989) 28:193–213. doi: 10.1016/0165-1781(89)90047-4

15. Liu D, Kahathuduwa C, Vazsonyi AT. The Pittsburgh sleep quality index (PSQI): psychometric and clinical risk score applications among college students. *Psychol Assess*. (2021) 33:816–26. doi: 10.1037/pas0001027

16. Molla MD, Wolde HF, Atnafu A. Magnitude of central obesity and its associated factors among adults in urban areas of Northwest Ethiopia. *Diabetes Metab Syndr Obes*. (2020) 13:4169–78. doi: 10.2147/DMSO.S279837

17. Chen IJ, Lin WC, Liu CY, Song YL, Chiu JP, Hsu CH. Impact of hypoalphalipoproteinemia on quality of life in Taiwanese women with central obesity. *Qual Life Res*. (2014) 23:1619–27. doi: 10.1007/s11136-013-0601-8

18. Fowler-Brown A, Wee CC, Marcantonio E, Ngo L, Leveille S. The mediating effect of chronic pain on the relationship between obesity and physical function and disability in older adults. *J Am Geriatr Soc*. (2013) 61:2079–86. doi: 10.1111/jgs.12512

19. Gao HQ, Wang BX, Sun LL, Li T, Wu L, Fu LG, et al. The mediating effect of body dissatisfaction in association between obesity and dietary behavior changes for weight loss in Chinese children. *Biomed Environ Sci*. (2019) 32:639–46. doi: 10.3967/bes2019.083. PMID: 31635680

20. Rijnhart JJM, Lamp SJ, Valente MJ, MacKinnon DP, Twisk JWR, Heymans MW. Mediation analysis methods used in observational research: a scoping review and recommendations. *BMC Med Res Methodol*. (2021) 21:226. doi: 10.1186/s12874-021-01426-3

21. Alhashemi M, Mayo W, Alshaghel MM, Brimo Alsaman MZ, Haj KL. Prevalence of obesity and its association with fast-food consumption and physical activity: a cross-sectional study and review of medical students' obesity rate. *Ann Med Surg*. (2022) 79:104007. doi: 10.1016/j.amsu.2022.104007

22. Kolahdooz F, Sadeghirad B, Corriveau A, Sharma S. Prevalence of overweight and obesity among indigenous populations in Canada: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr*. (2017) 57:1316–27. doi: 10.1080/10408398.2014.913003

23. Stival C, Lugo A, Odone A, van den Brandt PA, Fernandez E, Tigova O, et al. Gallus S; TackSHS project investigators. Prevalence and correlates of overweight and obesity in 12 European countries in 2017-2018. *Obes Facts*. (2022) 15:655–65. doi: 10.1159/000525792

24. Zhang YX, Wang SR, Zhao JS, Chu ZH. Prevalence of overweight and central obesity and their relationship with blood pressure among college students in Shandong, China. *Blood Press Monit*. (2016) 21:251–4. doi: 10.1097/MBP.0000000000000189

25. Sa J, Cho BY, Chaput JP, Chung J, Choe S, Gazmararian JA, et al. Sex and racial/ethnic differences in the prevalence of overweight and obesity among U.S. college students, 2011-2015. *J Am Coll Heal*. (2021) 69:413–21. doi: 10.1080/07448481.2019.1679814

26. Stok FM, Renner B, Clarys P, Lien N, Lakerveld J, Deliens T. Understanding eating behavior during the transition from adolescence to young adulthood: a literature review and perspective on future research directions. *Nutrients*. (2018) 10:667. doi: 10.3390/nu10060667

27. Tamez M, Rodriguez-Orengo JF, Mattei J. Higher eating frequency, but not skipping breakfast, is associated with higher odds of abdominal obesity in adults living in Puerto Rico. *Nutr Res*. (2020) 73:75–82. doi: 10.1016/j.nutres.2019.11.005

28. Tee ES, Nurliyana AR, Norimah AK, Mohamed HJBJ, Tan SY, Appukutty M, et al. Breakfast consumption among Malaysian primary and secondary school children and relationship with body weight status - findings from the MyBreakfast study. *Asia Pac J Clin Nutr*. (2018) 27:421–32. doi: 10.6133/apjcn.062017.12

29. Swift DL, McGee JE, Earnest CP, Carlisle E, Nygard M, Johannsen NM. The effects of exercise and physical activity on weight loss and maintenance. *Prog Cardiovasc Dis*. (2018) 61:206–13. doi: 10.1016/j.pcad.2018.07.014

30. Corder K, van Sluijs EM, Ridgway CL, Steele RM, Prynne CJ, Stephen AM, et al. Breakfast consumption and physical activity in adolescents: daily associations and hourly patterns. *Am J Clin Nutr*. (2014) 99:361–8. doi: 10.3945/ajcn.111.027607

31. Verzeletti C, Maes L, Santinello M, Vereecken CA. Soft drink consumption in adolescence: associations with food-related lifestyles and family rules in Belgium Flanders and the Veneto region of Italy. *Eur J Pub Health*. (2010) 20:312–7. doi: 10.1093/eurpub/ckp150

32. Zhang W, Xu R. Effect of exercise intervention on internet addiction and autonomic nervous function in college students. *Biomed Res Int*. (2022) 2022:5935353:1–7. doi: 10.1155/2022/5935353

33. Park BK. The Pittsburg sleep quality index (PSQI) and associated factors in middle-school students: a cross-sectional study. *Child Health Nurs Res*. (2020) 26:55–63. doi: 10.4094/chnr.2020.26.1.55

34. Ota T, Tan C, Ishii A, Shiotani H. Do differences in chronotypes affect sleep and health-related quality of life of nursing students? A cross-sectional study. *Chronobiol Int*. (2022) 39:1435–43. doi: 10.1080/07420528.2022.2117049

35. Yu QC, Ma WJ, Chen GM, Ye DQ. Study on the relationship between college students' physical quality and the quality of sleep. *Zhonghua Liu Xing Bing Xue Za Zhi*. (2013) 34:471–4. doi: 10.3760/cma.j.issn.0254-6450.2013.05.013

36. Tambalis KD, Panagiotakos DB, Psarra G, Sidossis LS. Insufficient sleep duration is associated with dietary habits, screen time, and obesity in children. *J Clin Sleep Med*. (2018) 14:1689–96. doi: 10.5664/jcsm.7374

37. Katsuura-Kamano S, Arisawa K, Uemura H, van Nguyen T, Takezaki T, Ibusuki R, et al. Association of skipping breakfast and short sleep duration with the prevalence of metabolic syndrome in the general Japanese population: baseline data from the Japan multi-institutional collaborative cohort study. *Prev Med Rep*. (2021) 24:101613. doi: 10.1016/j.pmedr.2021.101613

38. Duraccio KM, Whitacre C, Krietsch KN, Zhang N, Summer S, Price M, et al. Losing sleep by staying up late leads adolescents to consume more carbohydrates and a higher glycemic load. *Sleep*. (2022) 45:zsab269. doi: 10.1093/sleep/zsab269

39. Tucker P, Marquié JC, Folkard S, Ansiau D, Esquirol Y. Shiftwork and metabolic dysfunction. *Chronobiol Int*. (2012) 29:549–55. doi: 10.3109/07420528.2012.675259

40. Amara AW, Wood KH, Joop A, Memon RA, Pilkington J, Tuggle SC, et al. Randomized, controlled trial of exercise on objective and subjective sleep in Parkinson's disease. *Mov Disord*. (2020) 35:947–58. doi: 10.1002/mds.28009

41. Min S, Masanovic B, Bu T, Matic RM, Vasiljevic I, Vukotic M, et al. The association between regular physical exercise, sleep patterns, fasting, and autophagy for healthy longevity and well-being: a narrative review. *Front Psychol*. (2021) 12:803421. doi: 10.3389/fpsyg.2021.803421

42. Glavin EE, Ceneus M, Chanowitz M, Kantilierakis J, Mendelow E, Mosquera J, et al. Relationships between sleep, exercise timing, and chronotype in young adults. *J Health Psychol*. (2021) 26:2636–47. doi: 10.1177/1359105320926530

43. Mancini A, Vitucci D, Randers MB, Schmidt JF, Hagman M, Andersen TR, et al. Lifelong football training: effects on autophagy and healthy longevity promotion. *Front Physiol*. (2019) 10:132. doi: 10.3389/fphys.2019.00132

44. Bajer B, Vlcek M, Galusova A, Imrich R, Penesova A. Exercise associated hormonal signals as powerful determinants of an effective fat mass loss. *Endocr Regul*. (2015) 49:151–63. doi: 10.4149/endo_2015_03_151

45. Andreotti DZ, Silva J, Matumoto AM, Orellana AM, de Mello PS, Kawamoto EM. Effects of physical exercise on autophagy and apoptosis in aged brain: human and animal studies. *Front Nutr*. (2020) 7:94. doi: 10.3389/fnut.2020.00094

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 18 June 2024
DOI 10.3389/fnut.2024.1265920



OPEN ACCESS

EDITED BY
Annalisa Noce,
University of Rome Tor Vergata, Italy

REVIEWED BY
Juan Manuel Suárez-Grau,
Virgen del Rocío University Hospital, Spain
Isabela Gobbo Ferreira,
University of São Paulo,
Ribeirão Preto, Brazil
Heba Taher,
Cairo University, Egypt

*CORRESPONDENCE
Wenwen Yang
1243012256@qq.com
Biao Han
hanbiao66@163.com

RECEIVED 24 July 2023
ACCEPTED 28 May 2024
PUBLISHED 18 June 2024

CITATION
Yang Y, Han B and Yang W (2024) Diet and risk for hernia: a Mendelian randomization analysis.
*Front. Nutr.* 11:1265920.
doi: 10.3389/fnut.2024.1265920



# Diet and risk for hernia: a Mendelian randomization analysis

Yanjiang Yang[1], Biao Han[2,3]* and Wenwen Yang[4]*

[1]The People's Hospital of Qiandongnan Autonomous Prefecture, Kaili, Guizhou, China, [2]Department of Thoracic Surgery, The First Hospital of Lanzhou University, Lanzhou, Gansu, China, [3]Gansu Province International Cooperation Base for Research and Application of Key Technology of Thoracic Surgery, The First Hospital of Lanzhou University, Lanzhou, Gansu, China, [4]The First Clinical Medical College, Lanzhou University, Lanzhou, Gansu, China

**Background:** The relationship between dietary factors and hernias is currently unclear.

**Methods:** The UK Biobank was used to extract dietary factors that were used as exposures, including intake of alcohol, non-oily fish, beef, fresh fruit, oily fish, salad/raw vegetables, dried fruit, coffee, cereal, salt, tea, water, cooked vegetables, cheese, Lamb/mutton, pork, poultry, processed meat, and bread. The FinnGen biobank was used to obtain GWAS data on hernias as outcomes. The main analysis of this study was performed using the weighted median, MR-Egger, and IVW methods. Cochran's Q test was utilized to assess heterogeneity. To find potential outliers, the MR-PRESSO method was used. Leave-one-out analysis was employed to assess the IVW method's robustness.

**Results:** Alcoholic consumption per week (OR: 0.614; $p = 0.00614$) reduced the risk of inguinal hernia. Alcohol intake frequency (OR: 1.309; $p = 0.0477$) increased the risk of ventral hernia (mainly including incisional hernia and parastomal hernia). The intake of non-oily fish (OR: 2.945; $p = 0.0214$) increased the risk of inguinal hernia. Salt added to food (OR: 1.841; $p = 0.00267$) increased the risk of umbilical hernia. Cheese intake (OR: 0.434; $p = 0.000536$) and dried fruit intake (OR: 0.322; $p = 0.00716$) decreased the risk of ventral hernia, while cooked vegetable intake (OR: 4.475; $p = 0.0380$) increased the risk of ventral hernia. No causal relationships were found with hernias from other dietary factors.

**Conclusion:** Inguinal, umbilical, and ventral hernias are all related to dietary factors.

KEYWORDS

Mendelian randomization, incisional hernia, dietary intake, umbilical hernia, inguinal hernia

## 1 Introduction

Inguinal hernia, umbilical hernia, and ventral hernia (including incisional hernia and parastomal hernia) are the three types of hernias, according to the 10th revision of the International Classification of Diseases. Inguinal hernias are the most common hernias, and umbilical hernias are also frequent hernias (1). Incisional hernias occur after laparotomies at a rate of 5–20% and more than 30% in high-risk individuals (2). There have been many studies investigating risk factors for hernias (3–9), but few studies have analyzed the effects of dietary factors on hernias (10). Dietary factors are important factors affecting health and disease (11–14). Therefore, this study used the methods of Mendelian randomization (MR) to analyze the effect of dietary factors on hernias.

TABLE 1 Information on exposure and outcome datasets.

| IEU GWAS id | Exposure or outcome | Participants included in analysis |
|---|---|---|
| ieu-b-73 | Alcoholic drinks per week | 335,394 European-descent individuals |
| ukb-b-5779 | Alcohol intake frequency | 462,346 European-descent individuals |
| ukb-b-6324 | Processed meat intake | 461,981 European-descent individuals |
| ukb-b-8006 | Poultry intake | 461,900 European-descent individuals |
| ukb-b-2862 | Beef intake | 461,053 European-descent individuals |
| ukb-b-17627 | Non-oily fish intake | 460,880 European-descent individuals |
| ukb-b-2209 | Oily fish intake | 460,443 European-descent individuals |
| ukb-b-5640 | Pork intake | 460,162 European-descent individuals |
| ukb-b-14179 | Lamb/mutton intake | 460,006 European-descent individuals |
| ukb-b-11348 | Bread intake | 452,236 European-descent individuals |
| ukb-b-1489 | Cheese intake | 451,486 European-descent individuals |
| ukb-b-8089 | Cooked vegetable intake | 448,651 European-descent individuals |
| ukb-b-6066 | Tea intake | 447,485 European-descent individuals |
| ukb-b-3881 | Fresh fruit intake | 446,462 European-descent individuals |
| ukb-b-15926 | Cereal intake | 441,640 European-descent individuals |
| ukb-b-1996 | Salad / raw vegetable intake | 435,435 European-descent individuals |
| ukb-b-5237 | Coffee intake | 428,860 European-descent individuals |
| ukb-b-16576 | Dried fruit intake | 421,764 European-descent individuals |
| ukb-b-8121 | Salt added to food | 462,630 European-descent individuals |
| ukb-b-14898 | Water intake | 427,588 European-descent individuals |
| finn-b-K11_UMBHER | Umbilical hernia | 4,224 European-descent cases and 190,557 European-descent controls |
| finn-b-K11_VENTHER | Ventral hernia | 3,737 European-descent cases and 190,557 European-descent controls |
| finn-b-K11_HERING | Inguinal hernia | 17,096 European-descent cases and 190,557 European-descent controls |

The information of the exposure and outcome datasets. More information about exposures and outcomes is available at the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/). IEU, Integrative Epidemiology Unit; GWAS, Genome-Wide Association Studies.

## 2 Methods

MR identifies the causal relationship between exposures and outcomes by employing genetic variations as instrumental variables (IVs). Three fundamental assumptions must be met for MR to function (15). First, there was no connection between the IVs and any probable confounding factors. Second, there must be robust correlations between the IVs and exposure variables. Third, there are no direct connections between the IVs and outcomes. As a result of using deidentified and freely accessible data from the IEU Open GWAS project, this study was exempt from institutional review board approval.

### 2.1 The selection of IVs and the sources of data

Intake of beef, alcohol, non-oily fish, salad/raw vegetables, water, coffee, fresh fruit, oily fish, dried fruit, cereal, tea, salt, cooked vegetables, cheese, poultry, pork, lamb/mutton, bread, and processed meat were the dietary factors used as exposures in this study. The European-descent participants of dietary factors previously mentioned ranged from 335,394 to 462,630 individuals. The MRC Integrative Epidemiology Unit (IEU) at the University of Bristol funded the IEU open GWAS project, which either directly or indirectly extracted the GWAS data mentioned above from the UK Biobank. The GWAS data of Hernias (including umbilical, ventral, and inguinal hernias) were extracted from the FinnGen biobank. More information on the outcome and exposure datasets is provided in Supplementary Table S1 and Table 1. The IVs that were employed in the study were determined under the following criteria. First, we will immediately delete palindromic and missing SNPs. Second, linkage disequilibrium was at a level of $r2 < 0.001$, the threshold of genome-wide significance $p < 5 \times 10^{-8}$, and the clumping window at 10,000 kb. Third, the F statistics of the IVs must be higher than 10.

### 2.2 Statistical analysis

This study employed the inverse-variance weighted (IVW) method as the primary method for identifying causality. The IVW method, which requires that all SNPs remain valid or horizontal pleiotropy is balanced, offers the strongest power to identify causality (16). The weighted median method and the MR-Egger method were

utilized as supplements to the IVW method, which served as the major method to assess causality in our study. If their results are consistent with the IVW method, the reliability of the IVW method will be greatly improved. Leave-one-out analysis was applied to evaluate the IVW method's robustness. By employing the MR-Egger method, which allows for the existence of nonzero intercepts, the horizontal pleiotropy can be identified. To identify potential outliers, the MR-PRESSO method was used. Cochran's Q test was utilized to assess heterogeneity. The R program (version 4.2.0) and TwoSampleMR package (17) were employed to perform all analyses.

# 3 Results

As shown in Supplementary Table S2, horizontal pleiotropy was detected in the analyses of the effects of fresh fruit intake on umbilical hernia and salt added to food on inguinal hernia ($p < 0.05$). The presence of horizontal pleiotropy indicated that these analyses violated the assumptions of MR and that there were direct associations between IVs and outcomes (18, 19). We will therefore treat them as invalid analyses. Utilizing the MR-PRESSO method, outliers were found in some analyses, but after removing the outliers and repeating the analyses, the results remained largely unchanged. The F statistics of IVs are all larger than 10, indicating that IVs and exposures have strong associations. The results of the MR-PRESSO method and F statistics are shown in the corresponding sections of Supplementary Table S2.

## 3.1 Dietary factors and inguinal hernia

Alcoholic drinks per week was observed to reduce the risk of inguinal hernia only in the IVW method (OR: 0.614; $p = 0.00614$). The intake of non-oily fish was observed to increase the risk of inguinal hernia in the IVW method (OR: 2.945; $p = 0.0214$) and the weighted median method (OR: 4.007; $p = 0.0128$). Lamb/mutton intake was observed to decrease the risk of inguinal hernia only in the MR-Egger method (OR: 0.0735; $p = 0.0420$). The MR-Egger method only complements the IVW method, so there is no causal relationship between lamb/mutton intake and inguinal hernia. Figure 1 shows the results of the leave-one-out analysis of positive dietary factors. Salt added to food is considered as an invalid analysis due to the detection of horizontal pleiotropy. Alcohol intake frequency and the intake of processed meat, poultry, beef, oily fish, pork, bread, cheese, cooked vegetable, tea, fresh fruit, cereal, salad/raw vegetable, coffee, dried fruit, and water were not associated with inguinal hernia in all of the three analysis methods ($p > 0.05$). More analysis results are provided in Supplementary Table S2.

## 3.2 Dietary factors and umbilical hernia

Salt added to food (cooking salt is not included) was observed to increase the risk of umbilical hernia only in the IVW method (OR: 1.841; $p = 0.00267$). Figure 1 shows the results of the leave-one-out analysis of positive dietary factors. Fresh fruit intake is considered as an invalid analysis due to the detection of horizontal pleiotropy. Horizontal pleiotropy was not detected in dried fruit intake after removal of an outlier. Cooked vegetable intake was observed to increase the risk of umbilical hernia only in the weighted median method (OR: 5.038; $p = 0.0470$). The weighted median method only complements the IVW method, so there is no causal relationship between cooked vegetable intake and umbilical hernia. The intake of alcohol, processed meat, non-oily fish, poultry, beef, oily fish, pork, lamb/mutton, bread, cheese, tea, cereal, salad/raw vegetable, coffee, dried fruit, and water were not associated with umbilical hernia in any of the three analysis methods ($p > 0.05$). More analysis results are provided in Supplementary Table S2.

## 3.3 Dietary factors and ventral hernia

It should be noted that the ventral hernia used in this study is defined according to the 10th revision of the International Classification of Diseases, mainly including incisional hernia and parastomal hernia (20).

Alcohol intake frequency was observed to increase the risk of ventral hernia only in the IVW method (OR: 1.309; $p = 0.0477$).

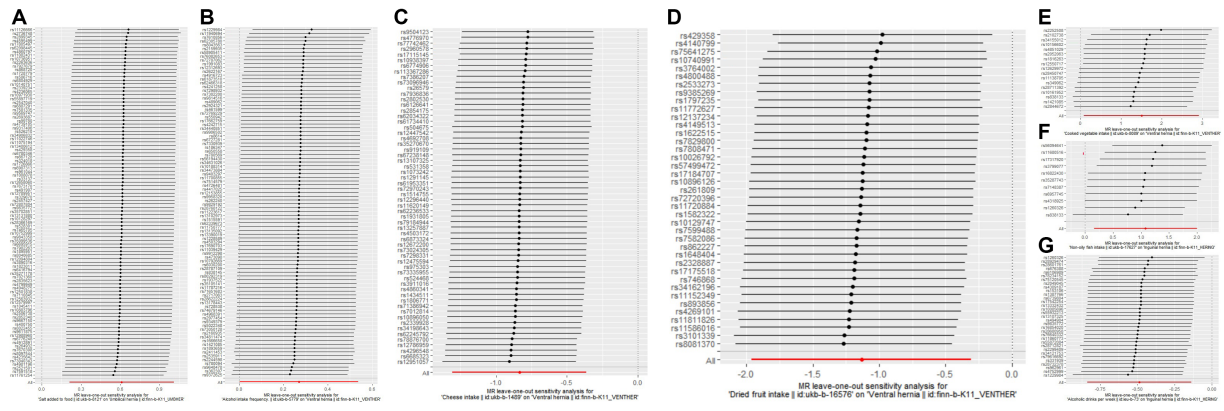



FIGURE 1
The results of leave-one-out analyses for **(A)** salt added to food on umbilical hernia **(B)** alcohol intake frequency on ventral hernia **(C)** cheese intake on ventral hernia **(D)** dried fruit intake on ventral hernia **(E)** cooked vegetable intake on ventral hernia **(F)** non-oily fish intake on inguinal hernia **(G)** alcoholic drinks per week on inguinal hernia.

Cheese intake and dried fruit intake were observed to decrease the risk of ventral hernia in the IVW method (cheese intake OR: 0.434; $p = 0.000536$; dried fruit intake OR: 0.322; $p = 0.00716$) and the weighted median method (cheese intake OR: 0.391; $p = 0.00452$; dried fruit intake OR: 0.239; $p = 0.0107$). Cooked vegetable intake was observed to increase the risk of ventral hernia in the IVW method (OR: 4.475; $p = 0.0380$) and the weighted median method (OR: 5.554; $p = 0.0427$). Figure 1 shows the results of the leave-one-out analysis of positive dietary factors. Non-oily fish intake was observed to increase the risk of ventral hernia only in the weighted median method (OR: 8.941; $p = 0.0224$). The weighted median method only complements the IVW method, so there is no causal relationship between cooked vegetable intake and umbilical hernia. Alcoholic drinks per week, salt added to food, and the intake of processed meat, poultry, beef, oily fish, pork, lamb/mutton, bread, tea, cereal, salad/raw vegetable, coffee, fresh fruit, and water were not associated with ventral hernia in the three analysis methods ($p > 0.05$). More analysis results are provided in Supplementary Table S2.

## 4 Discussion

The associations between nutritional factors and the risk of hernias have not been extensively studied (10), regardless of the fact that diet is an important influencing factor of health (21–23). We only found one study from Turkey that focused on the relationship between dietary factors and hernias, and their study found that dietary factors such as cheese, red meat, chicken, nuts, and bread were associated with inguinal hernia (10). Their study included only 115 people with groin and only used 3-day food consumption records; Therefore, we believe that their study has some limitations. Dietary factors are difficult to measure. The UK Biobank used the frequency to measure dietary factors, more information is provided in Supplementary Table S1. As changing eating habits is difficult, it is highly difficult to use randomized controlled trials to evaluate the effect of dietary factors on hernias. Observational epidemiology is often used to analyze the influence of research factors on study subjects. However, the presence of confounders (24–26), reverse causality (27, 28), and other factors might bias the causal effects that observational epidemiology observed. The introduction of instrumental variables can effectively solve these shortcomings (29, 30). MR, an analysis that uses genetic variation as IVs, is being utilized increasingly frequently. MR sits between observational epidemiology and interventional epidemiology in the hierarchy of evidence (31). In this study, we used MR analysis methods to analyze the effects of 20 dietary factors on three common hernias. The results of this study suggest that alcohol intake has different effects on different hernias. Alcoholic drinks per week reduce the risk of inguinal hernia, alcohol intake frequency increases the risk of abdominal hernia, and alcohol intake does not have any effect on umbilical hernia. In some studies, there was no association between alcohol intake and developing inguinal hernia (32, 33). However, it was found in a different study that individuals with inguinal hernias consumed more alcohol (10). However, less than 1,000 cases of inguinal hernias were included in their analysis, which limited the credibility of their study. Our study included hundreds of thousands of individuals from the UK Biobank and the Finngen Biobank, therefore our study provided new evidence to clarify the relationship between alcohol intake and inguinal hernia. It is worth noting that the causal relationship between drinking frequency and ventral hernia may be influenced by a single SNP, as shown in Figure 1B. In addition, we found that non-oily fish intake increased the risk of inguinal hernia, salt added to food increased the risk of umbilical hernia, cheese intake, and dried fruit intake reduced the risk of ventral hernia, and cooked vegetable intake increased the risk of ventral hernia. It is important to note that neither the causality of non-oily fish consumption on inguinal hernia nor the causality of cooked vegetable intake on ventral hernia are particularly stable; they are affected by a single SNP. More information is shown in Figures 1E,F. We must be particularly careful when interpreting these findings. First, the causal relationship observed by the MR analysis is the consequence of prolonged exposure to dietary factors. Therefore, short-term exposure may not have any clinical effect. Second, the Two-sample MR analysis only revealed the overall effects of exposures on outcomes, not the direct effects. Extremely complex pathways may link exposures and outcomes.

Unavoidably, this study has several restrictions. First, we were incapable of assessing whether there was a U-shaped correlation (for example, as dried fruit intake increases, the risk of ventral hernia rises first and then decreases) between dietary factors and hernias due to continuous data on dietary factors being employed in this study. Second, due to the lack of GWAS data for the two demographics of sex and age, we were unable to conduct stratified analyses. Third, the inability to further divide dietary intake categories prevents a more detailed analysis. Fourth, because our analysis primarily focuses on individuals from Europe, extending our findings to other populations is difficult.

## 5 Conclusion

Alcoholic drinks per week will reduce the risk of inguinal hernia, while alcohol intake frequency will not affect the risk of inguinal hernia. Alcohol intake frequency will increase the risk of ventral hernia, while alcoholic drinks per week will not affect the risk of ventral hernia. Alcohol intake will not affect the risk of umbilical hernia. The intake of non-oily fish will increase the risk of inguinal hernia. Salt added to food will increase the risk of umbilical hernia. Cheese intake and dried fruit intake will decrease the risk of ventral hernia, while cooked vegetable intake will increase the risk of ventral hernia. No causal relationships were found with hernias from other dietary factors.

## 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

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

## Author contributions

YY: Data curation, Methodology, Formal analysis, Project administration, Validation, Funding acquisition, Resources, Visualization, Writing – original draft, Writing – review & editing. BH: Data curation, Methodology, Formal analysis, Project administration, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. YW: Data curation, Methodology, Supervision, Conceptualization, Formal analysis, Project administration, Validation, Investigation, Funding acquisition, Resources, Visualization, Software, Writing – original draft, Writing – review & editing.

## Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

## Acknowledgments

Special thanks to the IEU open GWAS project developed by The MRC Integrative Epidemiology Unit (IEU) at the University of Bristol. Thank them for extracting relevant GWAS summary-level data from published articles, UK Biobank, and FinnGen Biobank.

## 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.2024.1265920/full#supplementary-material

## References

1. Dabbas N, Adams K, Pearson K, Royle G. Frequency of abdominal wall hernias: is classical teaching out of date? *JRSM Short Rep*. (2011) 2:1–6. doi: 10.1258/shorts.2010.010071

2. Reistrup H, Zetner DB, Andresen K, Rosenberg J. Prevention of incisional hernia[J]. *Ugeskr Laeger*. (2018) 180:V02180094.

3. AhmedAlenazi A, Alsharif MM, Hussain MA, et al. Prevalence, risk factors and character of abdominal hernia in Arar City, northern Saudi Arabia in 2017. *Electron Physician*. (2017) 9:4806–11.

4. Abramson JH, Gofin J, Hopp C, Makler A, Epstein LM. The epidemiology of inguinal hernia. A survey in western Jerusalem. *J Epidemiol Community Health*. (1978) 32:59–67. doi: 10.1136/jech.32.1.59

5. Akin ML, Karakaya M, Batkin A, Nogay A. Prevalence of inguinal hernia in otherwise healthy males of 20 to 22 years of age. *J R Army Med Corps*. (1997) 143:101–2. doi: 10.1136/jramc-143-02-06

6. Sorensen LT, Friis E, Jorgensen T, et al. Smoking is a risk factor for recurrence of groin hernia. *World J Surg*. (2002) 26:397–400. doi: 10.1007/s00268-001-0238-6

7. Gislason H, Grønbech JE, Søreide O. Burst abdomen and incisional hernia after major gastrointestinal operations–comparison of three closure techniques. *Eur J. Surg*. (1995) 161:349–54.

8. Gislason H, Søreide O, Viste A. Wound complications after major gastrointestinal operations. The surgeon as a risk factor. *Dig Surg*. (1999) 16:512–4. doi: 10.1159/000018778

9. Niggebrugge AH, Trimbos JB, Hermans J, Steup WH, Van De Velde CJ. Influence of abdominal-wound closure technique on complications after surgery: a randomised study. *Lancet*. (1999) 353:1563–7. doi: 10.1016/S0140-6736(98)10181-2

10. Idiz C, Cakir C. Nutritional status and constipation scoring of inguinal hernia patients: a case-control study. *Hernia*. (2020) 24:1107–12. doi: 10.1007/s10029-019-02075-8

11. Cena H, Calder PC. Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease[J]. *Nutrients*. (2020) 12:334. doi: 10.3390/nu12020334

12. Locke A, Schneiderhan J, Zick SM. Diets for health: goals and guidelines. *Am Fam Physician*. (2018) 97:721–8.

13. Aggarwal M, Bozkurt B, Panjrath G, Aggarwal B, Ostfeld RJ, Barnard ND, et al. Lifestyle modifications for preventing and treating heart failure. *J Am Coll Cardiol*. (2018) 72:2391–405. doi: 10.1016/j.jacc.2018.08.2160

14. Gumbs AA, Gogol M, Spolverato G, Taher H, Chouillard EK. Systematic review of the integrative medicine recommendations for patients with pancreatic Cancer. *Surgeries*. (2021) 2:216–30. doi: 10.3390/surgeries2020022

15. Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. *Stat Methods Med Res*. (2007) 16:309–30. doi: 10.1177/0962280206077743

16. Hartwig FP, 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

17. Hemani G, Zheng J, Elsworth B, et al. The MR-base platform supports systematic causal inference across the human phenome. *eLife*. (2018):7.

18. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. (2014) 23:R89–98. doi: 10.1093/hmg/ddu328

19. Uo Bristol. Horizontal Pleiotropy. (2021-2022). Available at: https://mr-dictionary.mrcieu.ac.uk/term/horizontal-pleiotropy/

20. Organization WH. the 10th revision of the International Classification of Diseases. (2016) Available at: https://icd.who.int/browse10/2016/en#/K43

21. Tilman D, Clark M. Global diets link environmental sustainability and human health. *Nature*. (2014) 515:518–22. doi: 10.1038/nature13959

22. Touger-Decker R. Diet, cardiovascular disease and oral health: promoting health and reducing risk. *J Am Dent Assoc*. (1939) 141:167–70. doi: 10.14219/jada.archive.2010.0135

23. Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, et al. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr*. (1995) 61:1402s–6s. doi: 10.1093/ajcn/61.6.1402S

24. Smith GD, Ebrahim S. Data dredging, bias, or confounding. *BMJ*. (2002) 325:1437–8. doi: 10.1136/bmj.325.7378.1437

25. Lawlor DA, Davey Smith G, Kundu D, Bruckdorfer KR, Ebrahim S. Those confounded vitamins: what can we learn from the differences between observational versus randomised trial evidence? *Lancet*. (2004) 363:1724–7. doi: 10.1016/S0140-6736(04)16260-0

26. Taubes G. Epidemiology Faces Its Limits: The search for subtle links between diet, lifestyle, or environmental factors and disease is an unending source of fear—but often yields little certainty[J]. Science. (1995) 269, 164–169.

27. Fewell Z, Davey Smith G, Sterne JAC. The impact of residual and unmeasured confounding in epidemiologic studies: a simulation study. *Am J Epidemiol*. (2007) 166:646–55. doi: 10.1093/aje/kwm165

28. Sattar N, Preiss D. Reverse causality in cardiovascular epidemiological research: more common than imagined? *Circulation*. (2017) 135:2369–72. doi: 10.1161/CIRCULATIONAHA.117.028307

29. Widding-Havneraas T, Zachrisson HD. A gentle introduction to instrumental variables. *J Clin Epidemiol*. (2022) 149:203–5. doi: 10.1016/j.jclinepi.2022.06.022

30. Greenland S. An introduction to instrumental variables for epidemiologists. *Int J Epidemiol*. (2000) 29:722–9. doi: 10.1093/ije/29.4.722

31. Zuccolo L, Holmes MV. Commentary: Mendelian randomization-inspired causal inference in the absence of genetic data. *Int J Epidemiol*. (2017) 46:962–5. doi: 10.1093/ije/dyw327

32. Ruhl CE, Everhart JE. Risk factors for inguinal hernia among adults in the US population. *Am J Epidemiol*. (2007) 165:1154–61. doi: 10.1093/aje/kwm011

33. Carbonell JF, Sanchez JL, Peris RT, et al. Risk factors associated with inguinal hernias: a case control study. *Eur J Surg*. (1993) 159:481–6.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 27 June 2024
DOI 10.3389/fnut.2024.1342163



OPEN ACCESS

EDITED BY
Giulia Marrone,
University of Rome Tor Vergata, Italy

REVIEWED BY
Javier Carballo,
University of Vigo, Spain
Md Nur Hossain,
Bangladesh Council of Scientific and Industrial Research, Bangladesh
Dimas Rahadian Aji Muhammad,
Sebelas Maret University, Indonesia

*CORRESPONDENCE
Jinguang Yao
✉ yao7760698@126.com

RECEIVED 21 November 2023
ACCEPTED 22 May 2024
PUBLISHED 27 June 2024

CITATION
Wang H, Zhang Z, Wu S, Zhu Y, Liang T, Huang X and Yao J (2024) Dietary patterns suggest that dark chocolate intake may have an inhibitory effect on oral cancer: a Mendelian randomization study. *Front. Nutr.* 11:1342163. doi: 10.3389/fnut.2024.1342163



# Dietary patterns suggest that dark chocolate intake may have an inhibitory effect on oral cancer: a Mendelian randomization study

Hongwei Wang[1,2,3], Zhaoyin Zhang[1,2,3], Sijie Wu[4], Yuanzhi Zhu[2], Tao Liang[2], Xiong Huang[1,2,3] and Jinguang Yao[1,2,3]*

[1]Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi, China, [2]Youjiang Medical University for Nationalities, Baise, China, [3]Department of Tumor Pathology, The Key Laboratory of Molecular Pathology (Hepatobiliary Diseases) of Guangxi, Baise, Guangxi, China, [4]Guilin Medical University, Guilin, Guangxi, China

**Background:** Previous studies reported that variations in dietary intake patterns substantially impact human health, specifically tumorigenesis. However, confounding factors in previous cohort studies have obscured the relationship between dietary differences and the risk of oral cancer (OC).

**Materials and methods:** We developed an outcome dataset from genome-wide association studies (GWAS) data on three OCs within the GAME-ON project, using GWAS-META merging. We extracted 21 dietary exposures, including 10 dietary patterns, 6 vitamins, and 5 micronutrients, from the UK Biobank database, using the inverse variance weighting method as the primary statistical method. Sensitivity analysis was conducted to detect heterogeneity and pleiotropy. Serum metabolite concentrations were adjusted using multivariate Mendelian randomization.

**Results:** Of the 10 analyzed dietary patterns, 8 showed no significant association with the risk of developing OC. Consumption of dark chocolate (inverse variance weighted [IVW]: Odds ratio (OR) = 0.786, 95% confidence interval [CI]: 0.622–0.993, $p$ = 0.044) and sweet pepper exhibited an inverse relationship with OC risk (IVW: OR = 0.757, 95% CI: 0.574–0.997, $p$ = 0.048). Reverse MR analysis revealed no reverse causality. Furthermore, no significant correlation was observed between the intake of 6 vitamins and 5 micronutrients and the risk of developing OC. After using multivariable MR to adjust for serum caffeine, linoleate, theophylline, and theobromine metabolism levels, consuming dark chocolate was unrelated to a decreased risk of OC. After adjusting each serum metabolite individually, the observed $p$-values deviated from the original values to varying degrees, indicating that the components of dark chocolate could have different effects. Among these components, theophylline demonstrated the most significant inhibitory effect.

**Conclusion:** This study demonstrated a causal relationship between the intake of dark chocolate and sweet peppers and a lower risk of OC. The components of dark chocolate could have different effects.

KEYWORDS

**dark chocolate intake, sweet peppers intake, dietary intake, oral cancer, Mendelian randomization**

# 1 Introduction

Oral cancer (OC) is a common malignancy of the head and neck region. Globally, it accounts for approximately 405,000 new cases annually (1). Tobacco use and alcohol consumption are well-documented etiological factors of OC (2, 3). In China, betel nut consumption is a common etiological factor for OC (4). An OC usually has no apparent symptoms in the early stages, making early and prompt diagnostic intervention and routine medical evaluations difficult. Patients with advanced stages of OC frequently present with symptoms, including ulcers, lumps, and lymph node metastases in the neck region. Consequently, their chances of survival at the time of diagnosis are significantly reduced (5). Numerous strategies for early OC screening have been developed to facilitate earlier detection and treatment. However, there is a critical need for more precise and effective screening approaches (6). Various dietary intakes are implicated in disease development (7, 8). Therefore, clarifying the relationship between various dietary intakes and OC is crucial to preventing disease and changing the lifestyle habits of high-risk populations.

The degree to which dietary intake affects OC remains unknown (9). Unlike single-nutrient studies that are often influenced by various confounding factors, studies of dietary intake patterns offer a more accurate reflection of the body's condition in everyday life by summarizing and analyzing individual nutrients (10). This study employed the Mendelian randomization (MR) method to clarify these potential associations and comprehensively investigate the plausible link between various diets and OC.

The MR is a method to investigate possible associations between an exposure factor (dietary intake) and an outcome (OC). The association between exposure and outcome was analyzed at the genetic level using data sourced from public databases. One major advantage of MR is its ability to reduce potential confounders in clinical research and substantially reduce the possibility of reverse causation (11). This improves the precision, reliability, and validity of the findings.

Food often contains multiple components that can have individual or synergistic effects. Investigating the primary components of a food may be more effective in determining its mechanism of action. Although MR analysis has numerous benefits, it cannot identify the roles of individual components because of its inherent limitations. Conversely, multivariable MR (MVMR) is a method for investigating the direct or mediated effects of two or more exposures on outcomes (12). Consequently, by considering each internal component of a nutrient, MVMR enables a direct assessment of its impact on an outcome, making it easier to investigate the role of each component.

## 1.1 Method design

This study used a two-sample MR design to investigate the relationship between dietary intake and OC. In MR studies, single nucleotide polymorphisms (SNPs) utilized as instrumental variables (IVs) must satisfy three core criteria (11): First, SNPs must be strongly associated with the exposure factors. Second, SNPs should not be associated with confounding variables other than exposure factors. Third, SNPs must not be directly associated with the outcome, in this case, OC. Moreover, reverse MR analysis was employed to investigate the potential reverse causality effects of OC on positive outcomes. Additionally, MVMR was used to determine the direct impact of positive exposures on OC.

## 1.2 Data source

This study included 21 exposure factors, including 10 dietary intakes (oily fish, non-oily fish, alcohol, tea, coffee, cooked vegetables, salad/raw vegetables, fresh fruit, dark chocolate, and sweet pepper), 6 vitamins (vitamin B12, vitamin C, vitamin B6, vitamin D, vitamin E, and carotene), and 5 trace minerals (zinc, copper, calcium, magnesium, and potassium). Specific information about the diet in our study can be found in the publicly available information on the UK Biobank.[1] We combined genome-wide association studies (GWAS) data on OC from three geographic regions using a GWAS-meta-analysis approach. The data obtained from the GAME-ON consortium included 2,989 cases and 6,583 controls, with >90% of participants predominantly of European ancestry (>70% CEU) (13). Furthermore, Chen et al. (14) and the GWAS catalog[2] provide serum metabolite levels of theobromine, theophylline, caffeine, and linoleate (18:2n6). Other GWAS datasets are available through the Integrative Epidemiology Unit Open GWAS project.[3] Table 1 shows the details of all the data.

## 1.3 Selection of instrumental variables

We identified SNPs associated with exposure factors that met a significance threshold ($p < 5 \times 10^{-6}$). SNPs were selected to be free of linkage disequilibrium to ensure independence, with an $r^2 < 0.001$ within a 10,000 KB window. Surrogate SNPs were not

1 https://biobank.ctsu.ox.ac.uk/crystal/label.cgi?id=100052
2 https://www.ebi.ac.uk/gwas/
3 https://gwas.mrcieu.ac.uk/

TABLE 1 The GWAS data source details in our study.

| Phenotype | Data source | GWAS ID | PMID | Case | Control | Sample size | Ancestry |
|---|---|---|---|---|---|---|---|
| Oral cancer | GAME-ON | NA | 27749845 | 2989 | 6583 | 9572 | >90% of participants were European |
| Non-oily fish intake | MRC-IEU | ukb-b-17627 | NA | NA | NA | 460880 | European |
| Oily fish intake | MRC-IEU | ukb-b-2209 | NA | NA | NA | 460443 | European |
| Alcohol intake frequency. | MRC-IEU | ukb-b-5779 | NA | NA | NA | 462346 | European |
| Tea intake | MRC-IEU | ukb-b-6066 | NA | NA | NA | 447485 | European |
| Coffee intake | MRC-IEU | ukb-b-5237 | NA | NA | NA | 428860 | European |
| Salad / raw vegetable intake | MRC-IEU | ukb-b-1996 | NA | NA | NA | 435435 | European |
| Fresh fruit intake | MRC-IEU | ukb-b-3881 | NA | NA | NA | 446462 | European |
| Cooked vegetable intake | MRC-IEU | ukb-b-8089 | NA | NA | NA | 448651 | European |
| Dark chocolate intake | MRC-IEU | ukb-b-16139 | NA | NA | NA | 64945 | European |
| Sweet pepper intake | MRC-IEU | ukb-b-10932 | NA | NA | NA | 64949 | European |
| Vitamin B12 | MRC-IEU | ukb-b-19524 | NA | NA | NA | 64979 | European |
| Vitamin C | MRC-IEU | ukb-b-19390 | NA | NA | NA | 64979 | European |
| Vitamin B6 | MRC-IEU | ukb-b-7864 | NA | NA | NA | 64979 | European |
| Vitamin D | MRC-IEU | ukb-b-18593 | NA | NA | NA | 64979 | European |
| Vitamin E | MRC-IEU | ukb-b-6888 | NA | NA | NA | 64979 | European |
| Carotene | MRC-IEU | ukb-b-16202 | NA | NA | NA | 64979 | European |
| Zinc | NA | ieu-a-1079 | 23720494 | NA | NA | 2603 | European |
| Copper | NA | ieu-a-1073 | 23720494 | NA | NA | 2603 | European |
| Calcium levels | NA | ebi-a-GCST90025990 | 34226706 | NA | NA | 400792 | European |
| Magnesium | MRC-IEU | ukb-b-7372 | NA | NA | NA | 64979 | European |
| Potassium | MRC-IEU | ukb-b-17881 | NA | NA | NA | 64979 | European |
| Theobromine levels | NA | GCST90199644 | 36635386 | NA | NA | 8137 | European |
| Theophylline levels | NA | GCST90199647 | 36635386 | NA | NA | 7822 | European |
| Caffeine levels | NA | GCST90200436 | 36635386 | NA | NA | 8005 | European |
| Linoleate (18:2n6) levels | NA | GCST90200354 | 36635386 | NA | NA | 8260 | European |





10.3389/fnut.2024.1342163

considered. SNPs with F-statistic values <10 were regarded as weak IVs and were excluded. If the SNP was not found in the resulting GWAS dataset, it was excluded. Subsequently, we estimated that positive stranded alleles and SNPs with palindromes were eliminated. The qualifying SNPs were utilized in the subsequent analytical phase. The conditions and protocols mentioned above were used for inverse MR and MVMR analyses.

### 1.4 Statistical analysis

In our MR analysis, we employed five approaches: inverse variance weighted (IVW), weighted median, MR-Egger, weighted mode, and simple mode. These approaches aimed to investigate the potential association between dietary intake and the risk of developing OC. The value of $p < 0.05$ was deemed to be statistically significant. The directional consistency of associations, as represented by the inverse variance weighted IVW, weighted median, and MR-Egger methods, proved to be useful. The IVW was selected as the primary reference due to its excellent accuracy in identifying causality (15). The MR-Egger method was particularly responsive to horizontal pleiotropy and heterogeneity in the outcomes. The other three outcomes, weighted median, and two more served as supplementary approaches to the MR analysis. Results with inconsistent odds ratios (ORs) or evidence of horizontal pleiotropy were excluded from the analyses performed using the three MR methods: IVW, MR-Egger, and weighted median. The ORs were used to assess the impact of dietary factors on the risk of OC.

## 2 Results

**Two-sample MR results:** We used five MR statistics to assess the association between 21 exposures and the risk of OC. Figure 1 depicts the IVW results for the 21 exposures.

Of the 10 dietary patterns analyzed, 8 dietary intakes showed no significant association with OC risk (Supplementary Table 1). However, dark chocolate intake was inversely associated with OC risk (IVW: OR = 0.786, 95% confidence interval [CI]: 0.622–0.993, $p = 0.044$). Furthermore, the MR-Egger intercept test did not indicate evidence of horizontal pleiotropy ($p > 0.05$) (Supplementary Figure 1). The consistency of these findings was further confirmed by the leave-one-out method, suggesting the stability of the results (Supplementary Figure 2).

Similarly, sweet pepper intake was found to reduce the risk of OC (IVW: OR = 0.757, 95% CI: 0.574–0.997, $p = 0.048$). The MR-Egger intercept test did not indicate the presence of horizontal pleiotropy ($p > 0.05$) (Supplementary Figure 3). Moreover, the leave-one-out analysis revealed no outliers (Supplementary Figure 4). For both significant findings, the direction of the ORs obtained through the other three MR methods was consistent. This indicated that the results were more accurate (Table 2).

Regarding the other 6 vitamins (vitamin B12, vitamin C, vitamin B6, vitamin D, vitamin E, and carotene) and 5 trace elements (zinc, copper, calcium, magnesium, and potassium), the MR analysis indicated that the intake of these nutrients was not associated with an increased risk of OC (Supplementary Table 1).

**Reverse MR results:** We conducted a reverse MR analysis to determine whether the intake of dark chocolate and sweet pepper affects the risk of developing OC. The analysis revealed that dietary intake does not inversely affect OC development. Furthermore, the sensitivity analysis revealed no evidence of heterogeneity or pleiotropy. The leave-one-out analysis demonstrated consistent results, highlighting the stability of our findings (Supplementary Figures 5, 6 and Supplementary Table 2).

**MVMR results:** Given that numerous components in dark chocolate are known to have direct or indirect cancer-inhibitory bioactivity (16), we aimed to investigate their impact on oral carcinogenesis. Although trace elements in dark chocolate are known as active components (16), our two-sample MR analysis demonstrated that these factors do not influence the development of OC, leading to their exclusion from further analysis. Consequently, we employed MVMR to adjust serum metabolite levels for caffeine, linoleate, theophylline, and theobromine. This analysis indicated that dark chocolate intake was not associated with the risk of developing OC (Table 3).

We adjusted each ingredient separately in the MVMR analysis to elucidate the role of these factors in the suppression of OC by dark chocolate. After adjusting for each serum metabolite individually, we observed a decrease in $p$-values compared to the original values, specifically when adjusting for linoleate levels. This suggested a potential reduction in the inhibitory effect of dark chocolate on OC associated with linoleate levels. Conversely, adjusting for theobromine levels or theophylline levels resulted in higher $p$-values than the original values, with theophylline levels demonstrating a more pronounced increase. These findings suggest that theophylline may be the primary inhibitory factor in dark chocolate, while theobromine has a relatively limited inhibitory effect (Table 4).

Despite our efforts to understand how sweet pepper may influence oral carcinogenesis, limitations in data availability restricted further investigation in this area.

## 3 Discussion

We found that dark chocolate and sweet pepper intake may exert a significant inhibitory effect on the development of OC. The MR analysis did not reveal any considerable association between OC risk and the remaining 8 dietary intakes (oily fish, non-oily fish, alcohol, tea, coffee, cooked vegetables, salad/raw vegetables, fresh fruit), 6 vitamins (vitamin B12, vitamin C, vitamin B6, vitamin D, vitamin E, and carotene), and 5 trace minerals (zinc, copper, calcium, magnesium, and potassium).

Considering the variety of vitamins and micronutrients in the daily diet, pooling and analyzing individual nutrients can significantly help eliminate the confounding factors often present in studies focused on specific nutrients. The findings of various studies on the association between dietary intake and OC are inconsistent (9). Concerning fish consumption, our findings are consistent with those of previous studies (17–19), which found no clear association between oily and non-oily fish intake and the development of OC. Although alcohol is considered potentially carcinogenic (20), its association with OC is unknown (21). Furthermore, the impact of tea and coffee consumption on OC

10.3389/fnut.2024.1342163

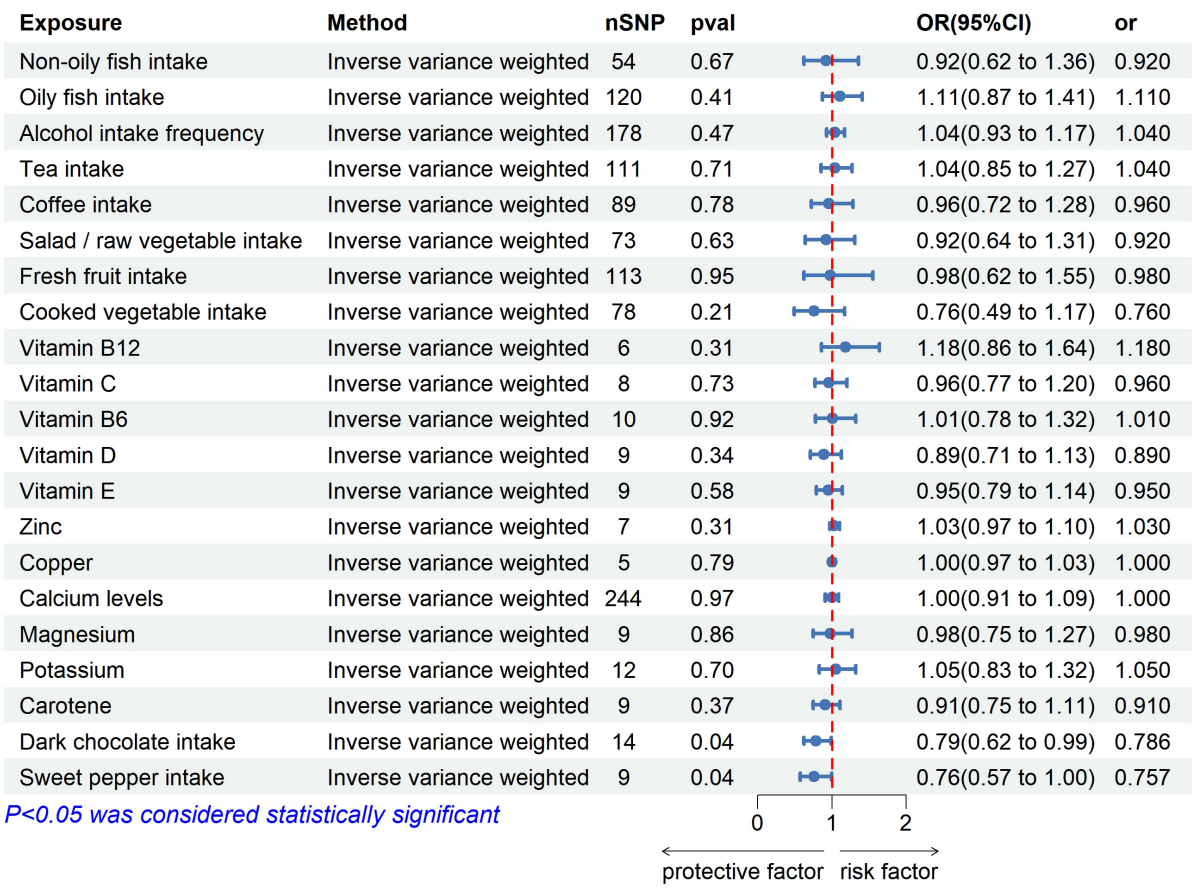



FIGURE 1
Inverse variance weighted results of MR for 21 dietary intakes and OC.

TABLE 2 Results of MR analysis of Dark chocolate intake and Sweet pepper intake with oral cancer.

| Exposure | Outcome | SNP | Method | OR | 95% CI | P-value | egger_intercept | Q_df(Q_pval) |
|---|---|---|---|---|---|---|---|---|
| Dark chocolate intake | Oral cancer | 14 | MR Egger | 0.759 | 0.487 - 1.179 | 0.244 | 0.002 | 13 (0.838) |
| | | | Weighted median | 0.739 | 0.543 - 1.005 | 0.054 | | 12 (0.781) |
| | | | Inverse variance weighted | 0.786 | 0.622 - 0.993 | **0.044** | | |
| | | | Simple mode | 0.71 | 0.50 - 1.01 | 0.08 | | |
| | | | Weighted mode | 0.75 | 0.55 - 1.01 | 0.08 | | |
| Sweet pepper intake | Oral cancer | 9 | MR Egger | 0.598 | 0.325 - 1.100 | 0.142 | 0.012 | 8 (0.612) |
| | | | Weighted median | 0.694 | 0.472 - 1.018 | 0.062 | | 7 (0.588) |
| | | | Inverse variance weighted | 0.757 | 0.574 - 0.997 | **0.048** | | |
| | | | Simple mode | 0.65 | 0.39 - 1.10 | 0.15 | | |
| | | | Weighted mode | 0.70 | 0.47 - 1.04 | 0.12 | | |

MR, Mendelian randomization; OR, odds ratio; 95% CI, 95% confidence interval; Heterogeneity, Heterogeneity Test; Pleiotropy, Pleiotropy Test; Q_pval, horizontal pleiotropy *P*-value for Q-test.

TABLE 3 Multivariable MR results of causal links between Dark chocolate and oral cancer after adjusting for specific serum metabolites.

| Exposure | Outcome | Adjustment of metabolites | Method | Beta | Se | *P*-value | OR | 95% CI | Heterogeneity test | Egger (intercept) |
|---|---|---|---|---|---|---|---|---|---|---|
| Dark chocolate | Oral cancer | Caffeine levels | MVMR-IVW | −0.2 | 0.146 | 0.168 | 1.22 | 0.61–1.10 | 0.1896 | |
| | | Linoleate levels | | | | | | | | |
| | | Theobromine levels | MVMR-Egger | −0.134 | 0.167 | 0.422 | 0.87 | 0.55–1.20 | 0.1826 | 0.397 |
| | | Theophylline levels | | | | | | | | |

MR, Mendelian randomization; OR, odds ratio; 95% CI, 95% confidence interval; Heterogeneity, Heterogeneity Test; Pleiotropy, Pleiotropy Test.

TABLE 4 Multivariable MR results of causal links between Dark chocolate and oral cancer after adjusting for single serum metabolites.

| Exposure | Outcome | Adjustment of metabolites | Method | Beta | Se | *P*-value | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| Dark chocolate | Oral cancer | Caffeine levels | MVMR-IVW | −0.22 | 0.11 | 0.04 | 0.8 | 0.65–0.99 |
| Dark chocolate | Oral cancer | Linoleate levels | MVMR-IVW | −0.24 | 0.11 | 0.02 | 0.78 | 0.64–0.97 |
| Dark chocolate | Oral cancer | Theobromine levels | MVMR-IVW | −0.23 | 0.12 | 4.80E−02 | 0.79 | 0.63–1.00 |
| Dark chocolate | Oral cancer | Theophylline levels | MVMR-IVW | −0.24 | 0.15 | 9.93E−02 | 0.79 | 0.59–1.05 |

MVMR, multivariate Mendelian randomization; OR, odds ratio; IVW, Inverse Variance Weighted; 95% CI, 95% confidence interval.

is controversial (22). Our findings imply that their role may not be considerable, which is consistent with the findings of previous studies conducted by Tverdal et al. (23) and Hildebrand et al. (24). Fruits and vegetables, which are rich in vitamins, enhance antioxidant and anti-inflammatory responses, potentially inhibiting tumor growth (25). However, our findings did not demonstrate any substantial inhibitory effect of cooked vegetables, salad/raw vegetables, or fresh fruit intake on OC, inconsistent with the findings of previous studies (26, 27). Moreover, our analysis of vitamins and OC revealed no association between the intake of six common vitamins and the risk of OC, confirming our findings. Furthermore, although numerous studies (28) have highlighted the health benefits of micronutrients, our study did not find any substantial role of micronutrients in the risk of OC. We believe that the inconsistency between our study's results and those of other experiments can be attributed to the following factors: 1. Previous observational studies may contain confounding factors, whereas our study, based on Mendelian Randomization (MR) analysis of Genome-Wide Association Studies (GWAS), minimizes the effects of confounding and reverse causation. 2. Our study is limited to European ancestries only. Variations in ancestry can influence disease susceptibility, thereby contributing to differences in results. Our findings suggest that while no direct association was found, dietary intake may still have some influence on the development of OC. These findings require further validation in future research.

Our findings indicated that dark chocolate intake may have a significant inhibitory effect on OC. Currently, studies that specifically investigate the impact of dark chocolate on OC are rare. Although some studies have proposed that various components of dark chocolate, including micronutrients, can have varying effects on overall health (16), their specific implications for OC are unknown. In this study, MVMR analysis suggested that caffeine, linoleate, theophylline, and theobromine levels may each have distinct roles in inhibiting oral carcinogenesis associated with consuming dark chocolate, particularly when controlling for several common components. Cocoa, the main ingredient in dark chocolate, contains a high percentage of methylxanthine compounds, mainly theobromine and caffeine (29). These substances are known for their potent antioxidant effects, aiding in the scavenging of free radicals, reducing DNA damage and oxidative stress, and thus potentially preventing cancer at its onset (30). Contrary to our initial expectations, our MVMR analysis revealed that caffeine in dark chocolate may not inhibit OC as anticipated. Furthermore, while theobromine has demonstrated inhibitory effects on OC, these effects are limited. Notably, theophylline in dark chocolate demonstrated a stronger inhibitory effect despite the lower percentage of theophylline in cocoa (31). Theophylline, a naturally occurring methylxanthine, is known to impact adenosine activation and downstream signaling pathways of cAMP by inhibiting cAMP phosphodiesterase activity (32). The activation of the cAMP signaling pathway plays a crucial role in the proliferation and growth of cancer cells. Notably, theophylline also inhibits the activation of NF-κB and the release of inflammatory factors such as IL-6 (33), which are implicated in creating a tumor-favorable inflammatory microenvironment that promotes cancer development and progression. Additionally, in breast cancer, theophylline has been shown to induce cell cycle arrest in the G2/M phase through the phosphorylation of cell cycle proteins B1 and Cdc2 (34). Therefore, it is evident that theophylline plays a distinct role in cancer inhibition, which aligns with the findings of our study. Dark chocolate contains a high percentage of linoleate (18:2n6) (35). These linoleic acids decrease cholesterol levels of low-density lipoproteins and fasting blood glucose concentrations by improving insulin sensitivity (36, 37). Metabolic disorders, including diabetes mellitus and obesity, are associated with an increased risk of oral carcinogenesis (38, 39). Contrary to the anticipated findings, our findings demonstrated that the inhibitory effect of dark chocolate on OC was reduced after adjusting for linoleate levels. This suggests that linoleate levels might not fulfill their anticipated role but rather may slightly reduce the inhibitory effect of dark chocolate. Further

studies are required to clarify its exact role in the impact of dark chocolate.

The above findings suggest that the biological activity of dark chocolate is not attributable to a single ingredient. Furthermore, dark chocolate is specifically abundant in total polyphenols and flavonoids (40), which are known to have antioxidant properties. These may be crucial in inhibiting the development of OC (41, 42). However, the data restrictions prevented us from fully investigating and analyzing this aspect. Additionally, dark chocolate intake has been associated with improved mood (43), suggesting an additional psychological benefit. Our findings indicate that the consumption of sweet pepper may substantially inhibit the development of OC. Sweet pepper are rich in an array of phytochemicals including polyphenols, flavonoids, carotenoids, capsaicinoids, and dihydrocapsaicin. Han et al. (44) observed that capsaicinoids inhibit the activation of the NF-κB pathway and activator protein-1 (AP-1), suggesting a potential role in cancer and inflammation prevention. Further research indicates that capsaicin activates the AMPK/mTOR signaling pathway to induce autophagy in renal cancer cells, thereby reducing tumor proliferation, invasion, and epithelial-mesenchymal transition (EMT) (45). Additionally, capsaicin facilitates the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun, while inhibiting the Hedgehog/GLI pathway, exhibiting antiproliferative and anticancer properties (46). In cases of oral cancer, capsaicin has been shown to suppress tumor cell proliferation and induce apoptosis. (47). Interestingly, capsiate, another compound in sweet pepper, is effective in inhibiting tumor activity (48). Despite our efforts to further understand the underlying mechanisms, the limitations of our data prevented a more in-depth investigation.

This is a novel study on the association between various dietary patterns and OC using multiple MR analysis methods. To our knowledge, this is the first study to uncover this relationship through MR. The lack of studies that examine the relationship between dietary intake and OC highlights the importance of our findings. These findings lay the foundation for future larger-scale clinical studies and basic research to investigate this association more thoroughly. Moreover, our research bridges the gap in understanding the association between dark chocolate and OC risk. It investigates the potential mechanisms by which dark chocolate components can suppress OC, laying the foundation for future fundamental research. Our results provide insights into the role of different dietary intakes in the development of OC, offering guidance for individuals at elevated risk of OC to adjust their diets in a clinically meaningful way.

There are limitations to our study. First, to capture a wide range of associations between dietary variety and OC, we employed a significance threshold of $p < 5 \times 10^{-6}$ rather than the more conventional $p < 5 \times 10^{-8}$. Although our datasets were solely of European origin, it is crucial to conduct further studies that include diverse regions to investigate these associations globally. Second, the nature of the MR analysis prevented us from determining a dose-response relationship between various intakes and OC at this stage. Third, we did not categorize outcomes by clinical, pathological, site, or other clinical features to investigate the relationship between diet and each subtype. While we have identified possible mechanisms of action for dark chocolate, limited data prevented definitive conclusions on the role of all its ingredients.

## 4 Conclusion

This study demonstrated a causal association between the intake of dark chocolate and sweet peppers and a decreased risk of OC. The components of dark chocolate could have different effects.

## Data availability statement

All GWAS data are available by accessing the open GWAS database (https://gwas.mrcieu.ac.uk/) or GWAS Catalog (https://www.ebi.ac.uk/gwas/), further inquiries can be directed to the corresponding author.

## Ethics statement

All data for the study were obtained from publicly available databases that had been approved by the Ethics Committee, so no additional information was required. The studies were conducted in accordance with the local legislation and institutional requirements.

## Author contributions

HW: Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft. ZZ: Conceptualization, Software, Visualization, Writing – original draft. SW: Conceptualization, Data curation, Validation, Writing – original draft. YZ: Writing – original draft. TL: Formal analysis, Validation, Writing – original draft. XH: Resources, Data curation, Writing – original draft. JY: Writing review and editing, Supervision, Project administration.

## Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

## Acknowledgments

We thank Home for Researchers editorial team (www.home-for-researchers.com) for language editing service.

## 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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1342163/full#supplementary-material

## References


1. Montero PH, Patel SG. Cancer of the oral cavity. *Surg Oncol Clin N Am.* (2015) 24:491–508.

2. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res.* (1988) 48:3282–7.

3. Ford PJ, Rich AM. Tobacco use and oral health. *Addiction.* (2021) 116:3531–40.

4. Nagao T, Warnakulasuriya S. Screening for oral cancer: Future prospects, research and policy development for Asia. *Oral Oncol.* (2020) 105:104632. doi: 10.1016/j.oraloncology.2020.104632

5. Bagan J, Sarrion G, Jimenez Y. Oral cancer: Clinical features. *Oral Oncol.* (2010) 46:414–7.

6. Warnakulasuriya S, Kerr AR. Oral cancer screening: Past, present, and future. *J Dent Res.* (2021) 100:1313–20.

7. Yin L, Yan H, Chen K, Ji Z, Zhang X, Ji G, et al. Diet-derived circulating antioxidants and risk of digestive system tumors: A Mendelian randomization study. *Nutrients.* (2022) 14:3724.

8. Yuan S, Chen J, Ruan X, Sun Y, Zhang K, Wang X, et al. Smoking, alcohol consumption, and 24 gastrointestinal diseases: Mendelian randomization analysis. *Elife.* (2023) 12:e84051.

9. Rodriguez-Molinero J, Miguelanez-Medran BDC, Puente-Gutierrez C, Delgado-Somolinos E, Martin Carreras-Presas C, Fernandez-Farhall J, et al. Association between oral cancer and diet: An update. *Nutrients.* (2021) 13:1299.

10. Liese AD, Krebs-Smith SM, Subar AF, George SM, Harmon BE, Neuhouser ML, et al. The dietary patterns methods project: Synthesis of findings across cohorts and relevance to dietary guidance. *J Nutr.* (2015) 145:393–402. doi: 10.3945/jn.114.205336

11. Sekula P, Del Greco MF, Pattaro C, Kottgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol.* (2016) 27:3253–65.

12. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol.* (2019) 48:713–27.

13. Lesseur C, Diergaarde B, Olshan AF, Wunsch-Filho V, Ness AR, Liu G, et al. Genome-wide association analyses identify new susceptibility loci for oral cavity and pharyngeal cancer. *Nat Genet.* (2016) 48:1544–50. doi: 10.1038/ng.3685

14. Chen Y, Lu T, Pettersson-Kymmer U, Stewart ID, Butler-Laporte G, Nakanishi T, et al. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. *Nat Genet.* (2023) 55:44–53. doi: 10.1038/s41588-022-01270-1

15. Hartwig FP, 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

16. Samanta S, Sarkar T, Chakraborty R, Rebezov M, Shariati MA, Thiruvengadam M, et al. Dark chocolate: An overview of its biological activity, processing, and fortification approaches. *Curr Res Food Sci.* (2022) 5:1916–43. doi: 10.1016/j.crfs.2022.10.017

17. Perloy A, Maasland DHE, van den Brandt PA, Kremer B, Schouten LJ. Intake of meat and fish and risk of head-neck cancer subtypes in the Netherlands cohort study. *Cancer Causes Control.* (2017) 28:647–56. doi: 10.1007/s10552-017-0892-0

18. Sanchez MJ, Martinez C, Nieto A, Castellsague X, Quintana MJ, Bosch FX, et al. Oral and oropharyngeal cancer in Spain: Influence of dietary patterns. *Eur J Cancer Prev.* (2003) 12:49–56. doi: 10.1097/00008469-200302000-00008

19. Garavello W, Lucenteforte E, Bosetti C, La Vecchia C. The role of foods and nutrients on oral and pharyngeal cancer risk. *Minerva Stomatol.* (2009) 58:25–34.

20. Boffetta P, Hashibe M. Alcohol and cancer. *Lancet Oncol.* (2006) 7:149–56.

21. Reidy J, McHugh E, Stassen LF. A review of the re-lationship between alcohol and oral cancer. *Surgeon.* (2011) 9:278–83.

22. He T, Guo X, Li X, Liao C, Yin W. Association between coffee intake and the risk of oral cavity cancer: A meta-analysis of observational studies. *Eur J Cancer Prev.* (2020) 29:80–8. doi: 10.1097/CEJ.0000000000000515

23. Tverdal A, Hjellvik V, Selmer R. Coffee intake and oral-oesophageal cancer: Follow-up of 389,624 Norwegian men and women 40-45 years. *Br J Cancer.* (2011) 105:157–61. doi: 10.1038/bjc.2011.192

24. Hildebrand JS, Patel AV, McCullough ML, Gaudet MM, Chen AY, Hayes RB, et al. Coffee, tea, and fatal oral/pharyngeal cancer in a large prospective US cohort. *Am J Epidemiol.* (2013) 177:50–8. doi: 10.1093/aje/kws222

25. Cirmi S, Navarra M, Woodside JV, Cantwell MM. Citrus fruits intake and oral cancer risk: A systematic review and meta-analysis. *Pharmacol Res.* (2018) 133:187–94. doi: 10.1016/j.phrs.2018.05.008

26. Chainani-Wu N. Diet and oral, pharyngeal, and esophageal cancer. *Nutr Cancer.* (2002) 44:104–26.

27. Turati F, Rossi M, Pelucchi C, Levi F, La Vecchia C. Fruit and vegetables and cancer risk: A review of southern European studies. *Br J Nutr.* (2015) 113:S102–10. doi: 10.1017/S0007114515000148

28. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med.* (2005) 26:235–44.

29. Ashihara H, Kato M, Crozier A. Distribution, biosynthesis and catabolism of methylxanthines in plants. *Handb Exp Pharmacol.* (2011) 200:11–31.

30. Saraf S, Kaur CD. Phytoconstituents as photoprotective novel cosmetic formulations. *Pharmacogn Rev.* (2010) 4:1–11. doi: 10.4103/0973-7847.65319

31. Hurst WJ, Glinski JA, Miller KB, Apgar J, Davey MH, Stuart DA. Survey of the trans-resveratrol and trans-piceid content of cocoa-containing and chocolate products. *J Agric Food Chem.* (2008) 56:8374–8. doi: 10.1021/jf801297w

32. Hashemi-Niasari F, Rabbani-Chadegani A, Razmi M, Fallah S. Synergy of theophylline reduces necrotic effect of berberine, induces cell cycle arrest and PARP, HMGB1, Bcl-2 family mediated apoptosis in MDA-MB-231 breast cancer cells. *Biomed Pharmacother.* (2018) 106:858–67. doi: 10.1016/j.biopha.2018.07.019

33. Ichiyama T, Hasegawa S, Matsubara T, Hayashi T, Furukawa S. Theophylline inhibits NF-kappa B activation and I kappa B alpha degradation in human pulmonary epithelial cells. *Naunyn Schmiedebergs Arch Pharmacol.* (2001) 364:558–61. doi: 10.1007/s00210-001-0494-x

34. Chang YL, Hsu YJ, Chen Y, Wang YW, Huang SM. Theophylline exhibits anti-cancer activity via suppressing SRSF3 in cervical and breast cancer cell lines. *Oncotarget.* (2017) 8:101461–74. doi: 10.18632/oncotarget.21464

35. de Oliveira LN, de Jesus Coelho Castro R, de Oliveira MA, de Oliveira LF. Lipid characterization of white, dark, and milk chocolates by FT-Raman spectroscopy and capillary zone electrophoresis. *J AOAC Int.* (2015) 98:1598–607. doi: 10.5740/jaoacint.15-083

36. Darand M, Hajizadeh Oghaz M, Hadi A, Atefi M, Amani R. The effect of cocoa/dark chocolate consumption on lipid profile, glycemia, and blood pressure in diabetic patients: A meta-analysis of observational studies. *Phytother Res.* (2021) 35:5487–501. doi: 10.1002/ptr.7183

37. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, et al. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent

vasodilation in hypertensives. *Hypertension.* (2005) 46:398–405. doi: 10.1161/01.HYP.0000174990.46027.70

38. Ramos-Garcia P, Roca-Rodriguez MDM, Aguilar-Diosdado M, Gonzalez-Moles MA. Diabetes mellitus and oral cancer/oral potentially malignant disorders: A systematic review and meta-analysis. *Oral Dis.* (2021) 27:404–21. doi: 10.1111/odi.13289

39. Gaudet MM, Kitahara CM, Newton CC, Bernstein L, Reynolds P, Weiderpass E, et al. Anthropometry and head and neck cancer:a pooled analysis of cohort data. *Int J Epidemiol.* (2015) 44:673–81.

40. Oracz J, Zyzelewicz D. Antioxidants in Cocoa. *Antioxidants (Basel).* (2020) 9:1230.

41. Febrianto NA, Wang S, Zhu F. Chemical and biological properties of cocoa beans affected by processing: A review. *Crit Rev Food Sci Nutr.* (2022) 62:8403–34.

42. Jalil AM, Ismail A. Polyphenols in cocoa and cocoa products: Is there a link between antioxidant properties and health? *Molecules.* (2008) 13:2190–219. doi: 10.3390/molecules13092190

43. Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. *Antioxid Redox Signal.* (2011) 15:2779–811.

44. Han SS, Keum YS, Seo HJ, Chun KS, Lee SS, Surh YJ. Capsaicin suppresses phorbol ester-induced activation of NF-kappaB/Rel and AP-1 transcription factors in mouse epidermis. *Cancer Lett.* (2001) 164:119–26. doi: 10.1016/s0304-3835(01)00378-0

45. Que T, Ren B, Fan Y, Liu T, Hou T, Dan W, et al. Capsaicin inhibits the migration, invasion and EMT of renal cancer cells by inducing AMPK/mTOR-mediated autophagy. *Chem Biol Interact.* (2022) 366:110043. doi: 10.1016/j.cbi.2022.110043

46. Bai H, Li H, Zhang W, Matkowskyj KA, Liao J, Srivastava SK, et al. Inhibition of chronic pancreatitis and pancreatic intraepithelial neoplasia (PanIN) by capsaicin in LSL-KrasG12D/Pdx1-Cre mice. *Carcinogenesis.* (2011) 32:1689–96. doi: 10.1093/carcin/bgr191

47. Mosqueda-Solis A, Lafuente-Ibanez de Mendoza I, Aguirre-Urizar JM, Mosqueda-Taylor A. Capsaicin intake and oral carcinogenesis: A systematic review. *Med Oral Patol Oral Cir Bucal.* (2021) 26:e261–8. doi: 10.4317/medoral.24570

48. Gupta R, Kapoor B, Gulati M, Kumar B, Gupta M, Singh SK, et al. Sweet pepper and its principle constituent capsiate: Functional properties and health benefits. *Crit Rev Food Sci Nutr.* (2022) 62:7370–94. doi: 10.1080/10408398.2021.1913989

# Frontiers in Nutrition

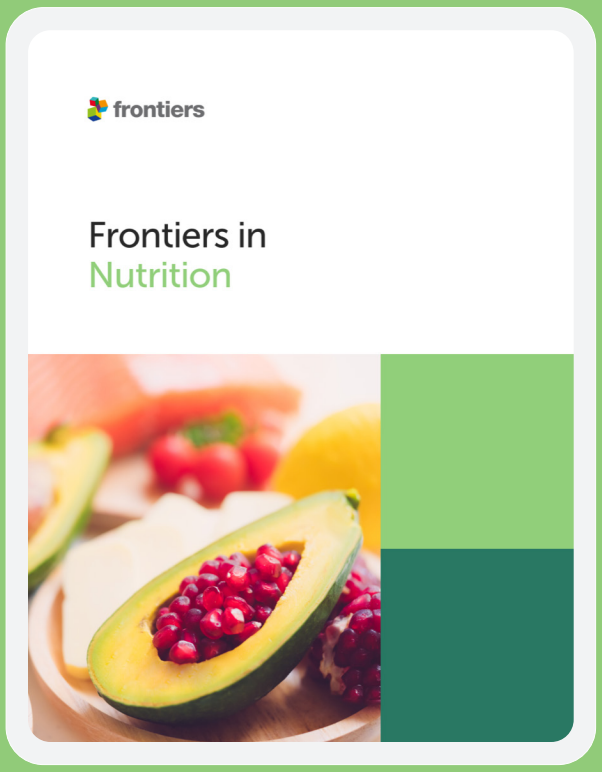
**Explores what and how we eat in the context of health, sustainability and 21st century food science**

A multidisciplinary journal that integrates research on dietary behavior, agronomy and 21st century food science with a focus on human health.

---

## Discover the latest Research Topics

See more →




**Frontiers**

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

**Contact us**

+41 (0)21 510 17 00
frontiersin.org/about/contact