frontiers | Research Topics

Polyunsaturated fatty acids and chronic diseases: Population-based study

Edited by
Li Cai, Zheqing Zhang and Muzi Na

Published in
Frontiers in Nutrition
Frontiers in Public Health

ISSN 1664-8714
ISBN 978-2-8325-2719-1
DOI 10.3389/978-2-8325-2719-1

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

Polyunsaturated fatty acids and chronic diseases: Population-based study

Topic editors

Li Cai — Sun Yat-sen University, China

Zheqing Zhang — Southern Medical University, China

Muzi Na — The Pennsylvania State University (PSU), United States

Citation

Cai, L., Zhang, Z., Na, M., eds. (2023). *Polyunsaturated fatty acids and chronic diseases: Population-based study*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2719-1

Table of contents

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 16 May 2022
doi: 10.3389/fnut.2022.897059

Association of Prepregnancy Obesity and Remodeled Maternal-Fetal Plasma Fatty Acid Profiles

*Hai-Tao Yu[1], Wen-Hui Xu[1], Yi-Ru Chen[1], Ye Ji[1], Yi-Wei Tang[1], Yue-Ting Li[1], Jia-Yu Gong[1], Yi-Fei Chen[1], Guo-Liang Liu[2] and Lin Xie[1]**

[1] Department of Nutrition and Food Hygiene, School of Public Health, Jilin University, Changchun, China, [2] Experimental Teaching Center for Preventive Medicine, School of Public Health, Jilin University, Changchun, China

Background: Fatty acids, especially polyunsaturated fatty acid (PUFA), are found abundantly in the brain and are fundamental for a fetus's growth. The fatty acid profiles of mothers and fetuses may be affected by maternal prepregnancy body mass index (pre-BMI), thus affecting fetal growth and development.

Methods: A total of 103 mother-fetus pairs were divided into overweight/obese (OW, $n = 26$), normal weight (NW, $n = 60$), and underweight (UW, $n = 17$) groups according to pre-BMI. Fatty acid profiles in maternal and umbilical cord plasma were analyzed by gas chromatography.

Results: The infant birth BMI *z*-score of the OW group was higher than that of the NW and UW groups ($p < 0.05$). The OW mothers had significantly higher plasma n-6 PUFA and n-6/n-3, but lower docosahexaenoic acid (DHA) and n-3 PUFA ($p < 0.05$). In cord plasma, the proportions of DHA and n-3 PUFA were lower in the OW group ($p < 0.05$), whereas the n-6/n-3 ratio was higher in the OW group ($p < 0.05$). The pre-BMI was negatively correlated with cord plasma DHA in all subjects ($r = -0.303$, $p = 0.002$), and the same negative correlation can be observed in the OW group ($r = -0.561$, $p = 0.004$), but not in the NW and UW groups ($p > 0.05$). The pre-BMI was positively correlated with cord plasma n-6/n-3 in all subjects ($r = 0.325$, $p = 0.001$), and the same positive correlation can be found in the OW group ($r = 0.558$, $p = 0.004$), but not in NW and UW groups ($p > 0.05$).

Conclusions: Maternal pre-BMI was associated with the maternal-fetal plasma fatty acid profiles, whereas the adverse fatty acid profiles are more noticeable in the prepregnancy OW mothers.

Keywords: pre-pregnancy BMI, overweight/obesity, fatty acids, cord blood, DHA

OPEN ACCESS

Edited by:
Zheqing Zhang,
Southern Medical University, China

Reviewed by:
Nurit Argov-Argaman,
Hebrew University of Jerusalem, Israel
Xiao Zhang,
Ningbo University, China

****Correspondence:***
Lin Xie
xielin@jlu.edu.cn

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *15 March 2022*
Accepted: *06 April 2022*
Published: *16 May 2022*

Citation:
Yu H-T, Xu W-H, Chen Y-R, Ji Y, Tang Y-W, Li Y-T, Gong J-Y, Chen Y-F, Liu G-L and Xie L (2022) Association of Prepregnancy Obesity and Remodeled Maternal-Fetal Plasma Fatty Acid Profiles. Front. Nutr. 9:897059. doi: 10.3389/fnut.2022.897059

INTRODUCTION

Obesity is a major public health problem worldwide. The prevalence of obesity in women of reproductive age is increasing. Obesity during pregnancy is associated with adverse pregnancy outcomes and adverse offspring health-related issues such as fetal overgrowth (1–3). Moreover, maternal prepregnancy obesity has been reported to affect neurodevelopment of offspring (4). Also, intrauterine nutrient exposure of the fetus may have a long-term influence on growth and

development, including the possibility of some future metabolic diseases (5). These effects might be related to disturbed polyunsaturated fatty acid (PUFA) compositions in mothers and fetuses. PUFA are involved in the composition of cell membrane phospholipids and are vital in brain and retina development (6, 7). Previous studies in obesity demonstrated a disturbed fatty acid profile (8). Exposure to adverse fatty acids profile *in utero* may affect fetal brain development (9).

Currently, studies on the influence of maternal obesity on the metabolism of infant fatty acids mainly focus on lactation, such as the influence of breast milk fatty acid composition on infant growth and development (10–15). However, the central nervous system undergoes a growth spurt from the third trimester of pregnancy to 18 months postnatally (16). Therefore, the nutritional status of maternal fatty acids during the last trimester of pregnancy is also very important for fetal growth and development. There are few studies on the effect of prepregnancy body mass index (pre-BMI) on the level of fetal fatty acids (17). Most studies focus on the relationship of maternal dietary fatty acids and the fetal development (18, 19). However, many factors affect the absorption of dietary fatty acids and their transport to the fetus, such as genetic factors (20) and the mother's physiological state (21). Genetic variations of the fatty acid desaturase (FADS) and elongase enzymes affect PUFA production. However, it has been reported that maternal BMI changes the effect of different genotypes on fatty acid levels, wherein overweight women were less affected by FADS genetic variants (22). Therefore, maternal pre-BMI may affect maternal and fetal fatty acid metabolism. Consequently, this study aimed to systematically analyze the plasma fatty acid profiles in prenatal and umbilical cord blood in mothers with different pre-BMIs. The association between pre-BMI and maternal and fetal plasma fatty acids was explored.

METHODS

Subjects

Mother-fetus pairs were recruited from 2019 to 2020 at the first hospital of Jilin University, Changchun, China. This study adhered to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Chinese Clinical Trial Registry (ChiCTR2000034179). Written informed consent was obtained from all subjects. Maternal and infantile demographics and physiology characteristics were obtained through questionnaire and hospital medical records. In brief, 103 dyads of healthy pregnancy women were recruited at the last time visit to obstetric clinic before delivery and classified according to pre-BMI, namely, normal weight (NW, BMI = 18.5–23.99 kg/m^2, $n = 60$), overweight/obese (OW, BMI $\geq$ 24 kg/m^2, $n = 26$), and underweight (UW, BMI $<$ 18.5 kg/m^2, $n = 17$). The inclusion criteria were maternal age $\geq$20 years and $\leq$40 years, singleton pregnancy, gestation duration $\geq$37 weeks, and newborn health (Apgar>8). Women were excluded if they were suffering from metabolic diseases (e.g., prepregnancy and gestational diabetes mellitus), HIV-infected disease, pulmonary tuberculosis, other acute infectious diseases, severe heart disease and renal disease, and were taking drugs that affect nutrient metabolism, and infants with congenital and hereditary diseases were excluded. The information on maternal age, pre-BMI, antenatal BMI, gestational weight gain, gestation duration, infant anthropometry, and sex was investigated by questionnaire and medical record.

Dietary Assessment

A valid food frequency questionnaire (FFQ) was carried out by the investigators who were trained uniformly. We gave each participant a face-to-face interview and a semistructured FFQ. Based on 7 different food groups (e.g., meat, eggs, poultry, fish and seafood, fruits, vegetables, milk, etc.,) and 54 different food categories combined with food pictures and standard food mold for food weight evaluation, the questionnaire was used to assess the dietary intake of enrolled subjects during the last trimester. It included specific questions about the cooking styles, cooking oil types, and dosages sources containing docosahexaenoic acid (DHA, C22:6n-3), such as freshwater fish, seafood, and canned tuna. The intake and frequency of food categories per day/week/month were recorded. To better understand the data of DHA intake, in the interview, we also asked participants about the DHA supplement's brand and daily doses. The investigators checked the content of DHA in supplements and calculated the daily doses of DHA. The questionnaire was improved and used by our group to assess the dietary intake, especially fatty acids (23). The intake of energy and five kinds of PUFA dietary intakes were calculated according to the food composition table (24). Finally, 79 questionnaires were collected, 24 subjects did not complete the questionnaire well (OW = 5, NW = 12, UW = 7).

Sample Collection

A total of 5 ml maternal blood samples after an overnight fast was collected either on the morning of admission for surgery in case of primary cesarean sections or at the last visit to the obstetric clinic, no longer than 3 days before delivery. Then, 5 ml cord blood samples were collected at delivery by a maternity nurse. All blood samples were collected in EDTA tubes and centrifuged at 3,500 rpm for 15 min to separate plasma, and stored at −80°C until fatty acids analysis.

Plasma Fatty Acid Analysis

A direct methylation procedure was performed on 100 μl of plasma. Then, 100 μl plasma, 100 μl C17:0 internal standard solution (5 mg/ml), and 600 μl methanol were mixed and vortexed for 30 s, and centrifuged for 5 min at 900 × *g*. The methanol phase was taken to another glass centrifuge tube, mixed with 25 μl sodium methoxide solution, and the solution was mixed for 3 min at room temperature. Also, 75 μl methanol hydrochloride solution was added to terminate reaction. Then, 300 μl *n*-hexane was added, and the mingled solution was mixed for 30 s to extract fatty acid methyl esters (FAMEs), then the upper *n*-hexane phase was transferred to a new glass centrifuge tube. The extraction was blown to dry by nitrogen, and 50 μl *n*-hexane containing 2 g/L butylated hydroxytoluene was added to dissolve the residue for gas chromatography (GC) analysis. The concentrations of 36 plasma fatty acids (μg/ml) were determined

in relation to the peak area of internal standard. Each plasma fatty acid was expressed as a percentage of the total 36 fatty acid concentrations measured.

Plasma FAMEs were detected by using GC-2010Plus gas chromatography (Shimadzu Corp., Kyoto, Japan). The GC was equipped with a SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm; Supelco, Bellefonte, PA). The chromatographic conditions were, namely, high-purity nitrogen was the carrier gas (linear velocity: 1 ml/min), split ratio was 1:50, and the injection volume was 1 μl; the initial temperature of the column box was set at 140°C, held for 5 min, and then the temperature rose to 260°C at the rate of 4°C /min, held for 20 min; the temperature of flame ionization detector was set at 280°C; the flow rate of hydrogen was 40 ml/min, and airflow rate was 500 ml/min. Shimadzu lab solutions chromatography workstation software was used to record the chromatogram, retention time, and peak area. The fatty acid concentrations were calculated by comparing the peak area of internal standard. The levels of plasma fatty acids are expressed as the percentage of total fatty acid (%).

Desaturase Enzyme Indices

Linoleic acid (LA; C18:2n-6) is the precursor of n-6 PUFA, whereas α-linolenic acid (ALA; C18: 3n-3) is the precursor of n-3 PUFA (25). The process by which PUFA are synthesized from LA and ALA in the human body involves Δ6 desaturase (D6D, LA to γ-linolenic acid, ALA to stearidonic acid) and Δ5 desaturase [D5D, dihomo-γ-linolenic acid (DGLA) to arachidonic acid (AA), eicosatetraene acid to eicosapentaenoic acid (EPA)]. Product-to-precursor ratios have been used to represent enzyme indices (26–28). In this study, the D6D index was estimated by DGLA (20:3n-6)/LA (18:2n-6), the D5D index was estimated by AA (20:4n-6)/DGLA (20:3n-6), and the ratio for estimation of the elongase activity was C18:1/C16:1 (29).

Statistical Analysis

The mean and standard error of mean (SEM) were used for normal variables, and quartile was used to describe continuous variables of non-normally distribution. The minimum sample size was calculated according to relevant literature (30). One-way ANOVA and Kruskal-Wallis *H* test were used for the analysis of anthropometric parameters and fatty acids. Chi-square test was used for nonquantitative variables. Pearson and Spearman were used to analyze the association between maternal parameters and infant parameters. Multivariable linear regression was used to analyze the associations between maternal parameters and infant DHA. In linear regression model 1, all significant maternal factors were included, and maternal age and gestation weight gains were adjusted to assess the relative importance without

TABLE 1 | Anthropometric and demographic characteristics of mothers and infants according to maternal prepregnancy BMI categories.

	OW (*n* = 26)	**NW (*n* = 60)**	**UW (*n* = 17)**
Maternal age (years)	30.54 ± 0.75	30.88 ± 0.37	29.35 ± 0.69
Pre-pregnancy BMI (kg/m^2)	**27.28 (25.39, 27.78)**[ac]	**21.06 (20.23, 22.04)**	**17.89 (16.79,18.08)**[b]
Weight gain (kg)	**13.08 ± 1.29**[ac]	**18.25 ± 0.77**	**20.71 ± 1.39**
Antenatal BMI (kg/m^2)	**32.18 ± 0.47**[ac]	**28.16 ± 0.32**	**23.82 ± 1.60**[b]
Gestational duration (weeks)	38.91 ± 0.23	39.18 ± 0.20	39.25 ± 0.26
Delivery modes			
Cesarean (*n*, %)	22 (84.62)	41 (73.21)	11 (68.75)
Vaginal delivery (*n*, %)	4 (15.38)	15 (26.79)	5 (31.25)
Dietary fatty acids intake*			
LA (g/d)	18.89 ± 1.01	18.49 ± 0.72	18.65 ± 1.98
ALA (g/d)	1.98 ± 0.14	1.86 ± 0.11	2.02 ± 0.30
AA (mg/d)	36.54 (28.70, 60.34)	37.75 (28.80, 61.31)	49.70 (23.28, 99.09)
EPA (mg/d)	9.37 (4.05, 17.61)	15.72 (3.87, 53.28)	15.19 (6.23, 18.58)
DHA (mg/d)	18.35 (7.59, 207.16)	43.44 (7.64, 202.61)	18.23 (4.16, 32.26)
Dietary energy intake(kcal/d)*	2,149.10 ± 124.55	2,332.84 ± 95.87	2,291.89 ± 153.24
Infant sex			
Male (*n*, %)	13 (50.00)	39 (66.10)	10 (58.82)
Female (*n*, %)	13 (50.00)	20 (33.90)	7 (41.18)
Infant birth weight(g)	**3,559.36 ± 95.06**[c]	**3,474.07 ± 46.26**	**3,240.59 ± 82.22**[b]
Infant birth length (cm)	50.52 ± 0.37	**50.76 ± 0.18**	**49.65 ± 0.40**[b]
Infant birth BMI z-score	**0.46 ± 0.19**[ac]	**0.03 ± 0.10**	**−0.21 ± 0.22**

BMI, body mass index; OW, mothers with overweight or obese; NW, mothers with normal weight; UW, mothers with underweight; LA, linoleic acid; ALA, alpha-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Data presented as mean ± standard error or P_{50} (P_{25}, P_{75}). Bold font indicates statistical significance at $p < 0.05$.

[a]*OW vs. NW.*

[b]*NW vs. UW.*

[c]*OW vs. UW.*

**24 maternal FFQ missing (OW = 5, NW = 12, UW = 7).*

the influence of dietary fatty acids. In linear regression model 2, dietary fatty acids were adjusted. Statistical significance was set at $p < 0.05$, data were analyzed using SPSS 24.0 software package (SPSS Inc., Chicago, IL, USA), and figures were drawn using R soft.

RESULTS

Anthropometric and Demographic Characteristics

A total of 103 women enrolled and completed the study, and maternal-fetal anthropometric and demographic characteristics are presented in **Table 1**. As expected, maternal prepregnancy and antenatal BMIs were significantly higher in the OW group than the other two groups ($p < 0.001$). The gestational weight gains were significantly lower in the OW group than the other two groups ($p < 0.001$). Infants born to OW and NW mothers presented significantly higher birth weight than infants born to UW mothers ($p = 0.01$, $p = 0.03$). Infants born to OW mothers presented significantly higher birth BMI z-score than NW and UW mothers ($p = 0.034$, $p = 0.012$). Infants born to NW mothers presented higher birth length than infants born to UW mothers ($p = 0.011$).

TABLE 2 | The fatty acid profile in maternal plasma according to maternal prepregnancy BMI.

Fatty acids (%)	OW ($n = 26$)	NW ($n = 60$)	UW ($n = 17$)
SFA			
C14:0 (myristic acid)	0.34 (0.25, 0.50)	0.30 (0.24, 0.40)	0.32 (0.29, 0.44)
C15:0 (pentadecylic acid)	0.15 ± 0.02	0.15 ± 0.01	0.14 ± 0.02
C16:0 (palmitic acid)	30.18 ± 0.30	30.41 ± 0.19	29.88 ± 0.38
C18:0 (stearic acid)	9.93 ± 0.23	10.24 ± 0.17	10.14 ± 0.30
∑SFA	41.76 (39.66, 42.73)	40.48 (39.33, 43.21)	40.76 (39.63, 40.76)
MUFA			
C16:1 (palmitoleic acid)	0.57 ± 0.06	0.56 ± 0.04	0.65 ± 0.06
C18:1 (octadecanoenoic acid)	7.17 ± 0.23	6.85 ± 1.14	7.30 ± 0.33
C20:1 (gadoleic acid)	0.16 ± 0.02	0.15 ± 0.01	0.14 ± 0.01
C22:1 (brassidic acid)	0.22 ± 0.08	0.15 ± 0.04	0.19 ± 0.11
∑MUFA	8.64 ± 0.33	8.13 ± 0.19	8.79 ± 0.43
PUFA			
C18:2n-6 (trans linoleic acid, LA)	0.10 ± 0.03	0.17 ± 0.03	0.10 ± 0.03
C18:2n-6 (linoleic acid, LA)	**27.72 ± 0.75**[a]	25.80 ± 0.48	25.92 ± 0.91
C18:3n-6 (gamma-linolenic acid, GLA)	0.13 ± 0.01	0.14 ± 0.02	0.12 ± 0.02
C18:3n-3 (alpha-linolenic acid, ALA)	0.35 ± 0.04	0.42 ± 0.05	0.39 ± 0.05
C20:2n-6 (eicosadienoic acid, EDA)	0.64 ± 0.08	0.56 ± 0.04	0.55 ± 0.04
C20:3n-6 (dihomo-γ-linolenic acid, DGLA)	3.14 ± 0.23	3.48 ± 0.10	3.22 ± 0.17
C20:3n-3 (eicosatrienoic acid)	0.79 ± 0.20	0.73 ± 0.12	0.76 ± 0.09
C20:4n-6 (arachidonic acid, AA)	10.81 ± 0.48	11.66 ± 0.29	11.63 ± 0.65
C22:2n-6 (docosadienoic acid)	0.13 (0.03, 0.76)	0.11 (0.05, 0.32)	0.16 (0.07, 0.31)
C20:5n-3 (eicosapentaenoic acid, EPA)	0.43 (0.34, 0.68)	0.52 (0.38, 0.67)	0.57 (0.42, 0.75)
C22:6n-3 (docosahexaenoic acid, DHA)	**6.07 ± 0.31**[ac]	7.22 ± 0.21	7.12 ± 0.43
∑EFA	**28.08 ± 0.75**[a]	26.22 ± 0.49	26.31 ± 0.89
∑n-6 PUFA	**42.90 ± 0.43**[a]	41.86 ± 0.24	41.79 ± 0.60
∑n-3 PUFA	**7.16 ± 0.39**[a]	8.27 ± 0.21	8.24 ± 0.51
AA/DGLA (D5D)	3.39 ± 0.20	3.49 ± 0.12	3.74 ± 0.26
DGLA/LA (D6D)	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.04
AA/LA	0.41 ± 0.03	0.47 ± 0.02	0.47 ± 0.04
EPA/ALA	1.93 ± 0.46	1.94 ± 0.30	1.87 ± 0.32
C18:1/C16:1 (elongase activity)	11.69 (9.42, 18.15)	12.35 (9.79, 16.36)	12.64 (8.93, 14.59)
n-6: n-3 ratio	**6.57 ± 0.48**[ac]	5.27 ± 0.14	5.39 ± 0.17

BMI, body mass index; OW/OB, mothers with overweight or obese; NW, mothers with normal weight; UW, mothers with underweight. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EFA, essential fatty acid.

Data presented as mean ± standard error or P_{50} (P_{25}, P_{75}). Bold font indicates statistical significance at $p < 0.05$.

[a] *OW vs. NW.*

[b] *NW vs. UW.*

[c] *OB/OW vs. UW.*

Maternal Plasma Fatty Acid Profile

Maternal plasma fatty acids are listed in **Table 2**, and two kinds of fatty acids (C11:0, C23:0) were below the limit of quantification; the proportions of C4:0, C6:0, C8:0, C10:0, C12:0, C13:0, C20:0, C21:0, C22:0, C24:0, C14:1, C15:1, C17:1, C18:1(trans), C24:1 were lower than 0.10%, thus they are not presented in **Table 2**.

OW mothers had significantly lower level of DHA compared with NW and UW mothers ($p = 0.003$, $p = 0.042$). Maternal plasma EFA and n-6 PUFA were significantly higher in the OW group than the NW group ($p = 0.038$, $p = 0.048$). The percentage of n-3 PUFA was lower in the OW group than the NW group ($p = 0.011$). No significant differences were observed in maternal plasma D5D, D6D, and elongase activity indices among the three groups. The indexes of D6D and AA/LA tended to a marginal decrease in OW mothers ($p = 0.054$, $p = 0.084$). The ratio of n-6/n-3 was higher in the OW group than the NW and UW groups ($p = 0.001$, $p = 0.021$).

Umbilical Cord Plasma Fatty Acid Profile

Two kinds of fatty acids in cord plasma (C11:0, C23:0) were below the limit of quantification, and the proportions of C4:0, C6:0, C8:0, C10:0, C12:0, C13:0, C20:0, C21:0, C24:0, C14:1, C15:1, C17:1, C18:1(trans), C24:1, C18:2n-6(trans), C18:3n-3, C20:3n-3 were lower than 0.10%; therefore, they are not presented in **Table 3**.

The proportions of C14:0 and C20:2n-6 in cord plasma were significantly higher in the OW group than that in the NW group ($p = 0.034$, $p = 0.002$). The proportions of C15:0, total saturated fatty acid (SFA), EPA, DHA, and n-3 PUFA were significantly lower in the OW group than that in the NW group ($p = 0.041$, $p = 0.029$, $p = 0.038$, $p = 0.027$, $p = 0.017$). MUFA was higher in the OW group than that in the UW group ($p = 0.011$). The ratio of n-6/n-3 was higher in the OW group than the NW and UW groups ($p < 0.001$, $p = 0.001$).

Association of Maternal Parameters With Infant Parameters

Associations between maternal parameters and the infantile parameters are shown in **Figure 1**. The pre-BMI was positively associated with maternal n-6 PUFA ($r = 0.203$, $p = 0.039$), n-6/n-3 PUFA (r = 0.258, $p = 0.009$), but the prepregnancy BMI was negatively associated with maternal DHA ($r = -0.266$, $p = 0.007$) and n-3 PUFA ($r = -0.255$, $p = 0.009$). Maternal pre-BMI was positively associated with infant birth BMI z-score ($r = 0.274$,

TABLE 3 | The fatty acid profile in fetal cord plasma according to maternal prepregnancy BMI.

Fatty acids (%)	OW($n = 25$)	NW($n = 60$)	UW ($n = 17$)
SFA			
C14:0 (myristic acid)	**0.37 (0.31, 0.44)**[a]	0.33 (0.30, 0.36)	**0.36 (0.31, 0.46)**[b]
C15:0 (pentadecylic acid)	**0.45 (0.09, 0.57)**[a]	0.55 (0.41, 0.66)	0.51 (0.13, 0.69)
C16:0 (palmitic acid)	25.72 ± 0.46	26.38 ± 0.23	26.17 ± 0.65
C18:0 (stearic acid)	12.59 ± 0.26	12.75 ± 0.33	13.11 ± 0.35
C22:0 (behenic acid)	0.43 ± 0.08	0.33 ± 0.02	0.31 ± 0.07
∑SFA	**40.78 ± 0.62**[c]	41.31 ± 0.33	42.90 ± 1.04
MUFA			
C16:1 (palmitoleic acid)	1.22 ± 0.10	1.04 ± 0.07	1.10 ± 0.12
C18:1 (octadecanoenoic acid)	7.27 ± 0.21	7.30 ± 0.11	7.12 ± 0.35
C20:1 (gadoleic acid)	0.38 ± 0.13	0.39 ± 0.08	0.17 ± 0.08
C22:1 (brassidic acid)	0.68 (0.00, 2.51)	0.51 (0.00, 1.83)	0.16 (0.00, 0.45)
∑MUFA	**11.03 (9.43, 14.87)**[c]	9.76 (8.73, 12.98)	9.18 (8.51, 10.02)
PUFA			
C18:2n-6 (linoleic acid, LA)	10.40 ± 0.29	10.91 ± 0.40	10.59 ± 0.41
C18:3n-6 (gamma-linolenic acid, GLA)	0.19 ± 0.02	0.28 ± 0.08	0.15 ± 0.03
C20:2n-6 (eicosadienoic acid, EDA)	**0.89 (0.42, 2.27)**[a]	0.42 (0.20, 0.75)	0.44 (0.37, 0.70)
C20:3n-6 (dihomo-gamma-linolenic acid, DGLA)	5.52 (4.74, 6.35)	5.50 (4.95, 6.17)	5.42 (4.82, 6.15)
C20:4n-6 (arachidonic acid, AA)	19.83 ± 0.54	19.99 ± 0.35	20.07 ± 0.82
C22:2n-6 (docosadienoic acid)	0.54 (0.00, 2.18)	0.33 (0.00, 1.33)	0.00 (0.00, 0.81)
C20:5n-3 (eicosapentaenoic acid, EPA)	**0.41 (0.29, 0.50)**[a]	0.47 (0.36, 0.65)	0.51 (0.31, 0.67)
C22:6n-3 (docosahexaenoic acid, DHA)	**7.82 ± 0.50**[ac]	8.83 ± 0.18	9.16 ± 0.53
∑ EFA	10.42 ± 0.29	10.94 ± 0.40	10.63 ± 0.43
∑ n-6 PUFA	38.90 ± 0.52	38.07 ± 0.33	37.95 ± 1.33
∑ n-3 PUFA	**8.27 ± 0.53**[ac]	9.41 ± 0.19	9.72 ± 0.57
n-6: n-3 ratio	**4.95 (4.42, 6.29)**[ac]	4.15 (3.59, 4.72)	4.00 (3.34, 5.02)

OW, mothers with overweight or obese; NW, mothers with normal weight; UW, mothers with underweight. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EFA, essential fatty acid.

Data presented as mean ± standard error or P_{50} (P_{25}, P_{75}). Bold font indicates statistical significance at $p < 0.05$.

[a] *OW vs. NW.*

[b] *NW vs. UW.*

[c] *OB/OW vs. UW.*

$p = 0.006$) and cord plasma n-6/n-3 PUFA ($r = 0.325$, $p = 0.001$). Maternal pre-BMI was negatively associated with cord plasma DHA ($r = -0.303$, $p = 0.002$) and n-3 PUFA ($r = -0.298$, $p = 0.002$). The gestational weight gain was positively associated with cord plasma SFA ($r = 0.261$, $p = 0.009$). Maternal plasma EPA, DHA, and n-3 PUFA were positively correlated with cord plasma EPA, DHA, and n-3 PUFA ($p < 0.05$). Maternal plasma LA was positively associated with cord plasma LA, n-6 PUFA, and n-6/n-3, but negatively associated with cord plasma DHA and n-3 PUFA ($p < 0.05$).

Subgroup Analysis of Association Between Maternal Pre-BMI and Fetal DHA, n-6/n-3

The negative correlation was found between pre-BMI and cord plasma DHA in all subjects (**Figure 2A**). The subgroup analysis was carried out to explore the association of prepregnancy OW and cord plasma DHA (**Figure 2B**). The pre-BMI was negatively correlated with cord plasma DHA ($r = -0.561$, $p = 0.004$) in the OW group, but there were no correlations between pre-BMI and cord plasma DHA in NW ($r = -0.012$, $p = 0.925$) and UW subjects ($r = -0.414$, $p = 0.098$).

The positive correlation was found between pre-BMI and cord plasma n-6/n-3 PUFA in all subjects (**Figure 3A**). Further subgroup analysis was carried out to explore the association of prepregnancy OW and cord plasma n-6/n-3 PUFA (**Figure 3B**). The pre-BMI was negatively correlated with cord plasma n-6/n-3 ratio ($r = 0.558$, $p = 0.004$) in the OW group, but there were no associations between pre-BMI and cord plasma n-6/n-3 in the NW ($r = 0.041$, $p = 0.756$) and UW groups ($r = 0.439$, $p = 0.078$).

Multiple Linear Regression Analysis of Maternal Parameters and Fetal DHA

A number of confounder factors could influence the association between maternal parameters and fetal parameters. Multiple linear regression analysis was conducted to explore the direct correlation between maternal parameters and cord plasma DHA (**Table 4**). The correlations can be found between maternal pre-BMI, LA, EPA, DHA, EFA, n-6 PUFA, n-3 PUFA, and n-6/n-3 with cord plasma DHA in all subjects (**Figure 1**). Owing to collinearity, pre-BMI, maternal plasma LA, EPA, and DHA were included in the regression model. Multiple linear regression analysis demonstrated the associations between maternal pre-BMI and DHA with cord plasma DHA in all subjects ($p < 0.05$).

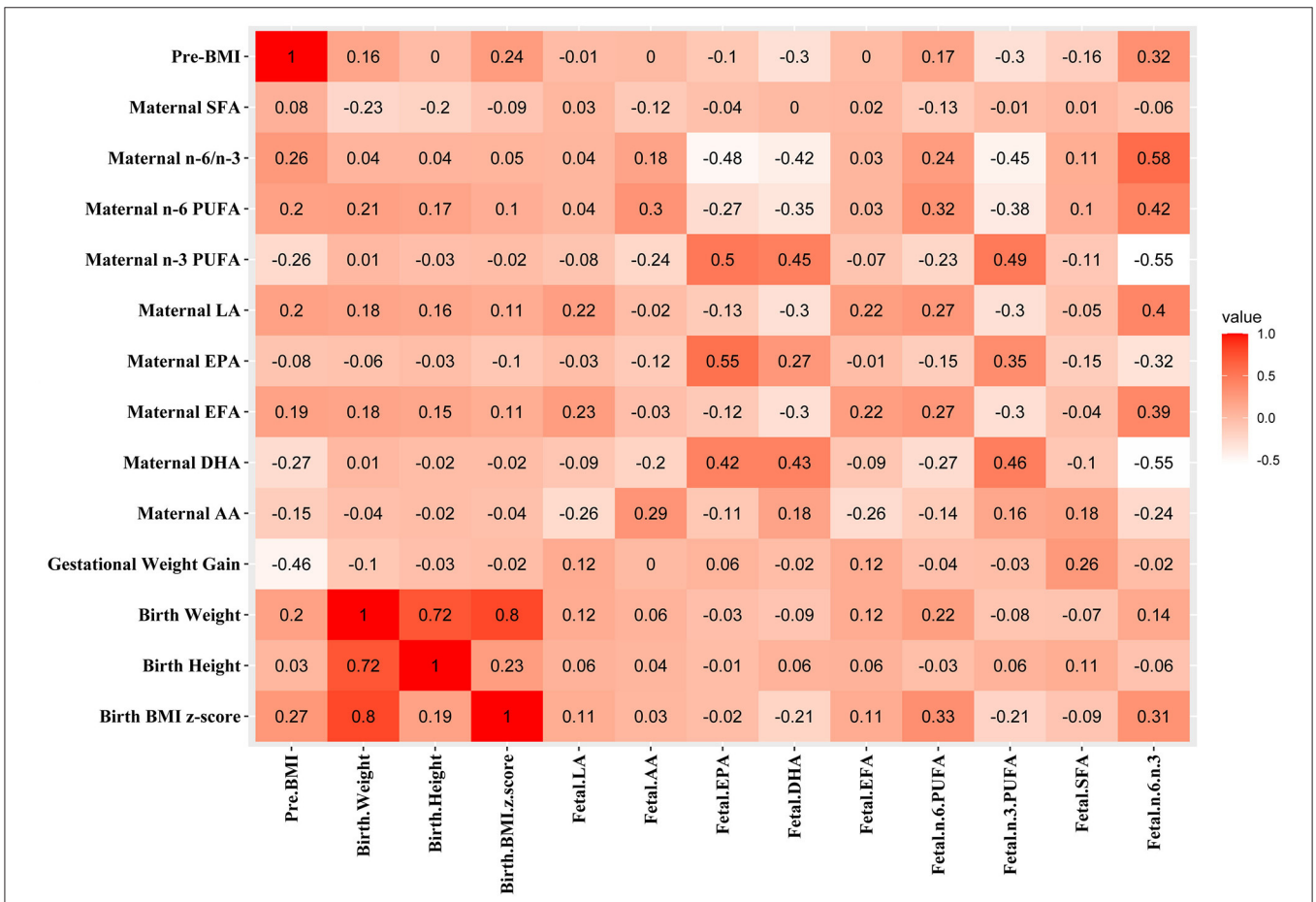


FIGURE 1 | Associations between maternal parameters and fetal parameters. The correlation coefficients are shown in different colors. Red, positive correlation; white, negative correlation.

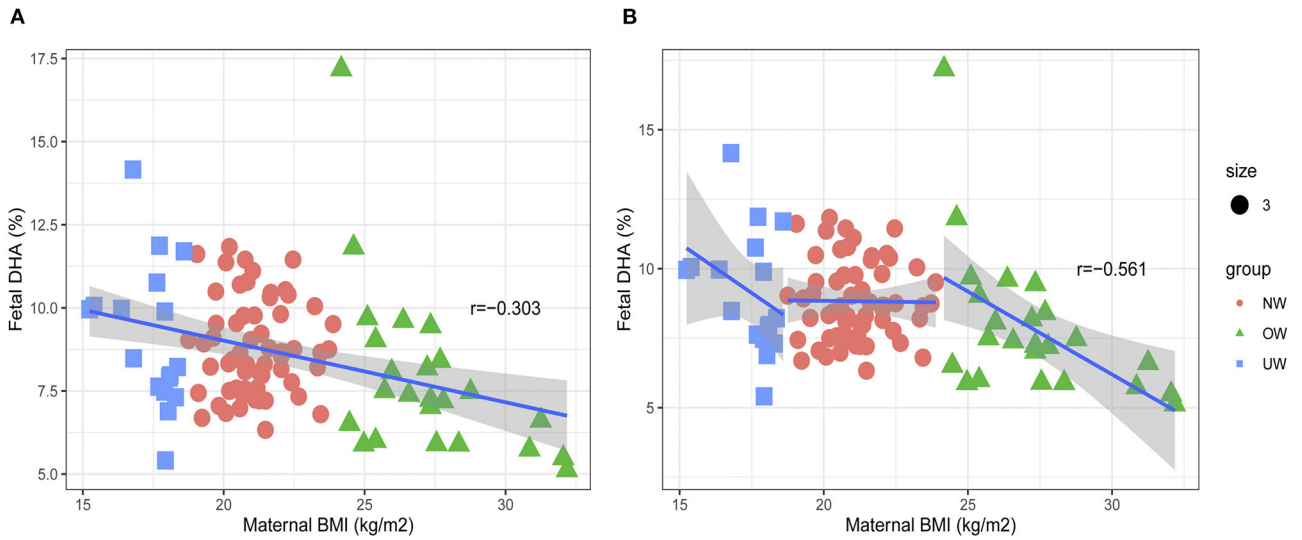


FIGURE 2 | Associations between maternal pre-BMI and fetal DHA. **(A)** The association between maternal pre-BMI and fetal DHA in all subjects. **(B)** The association between maternal pre-BMI and fetal DHA in different groups.

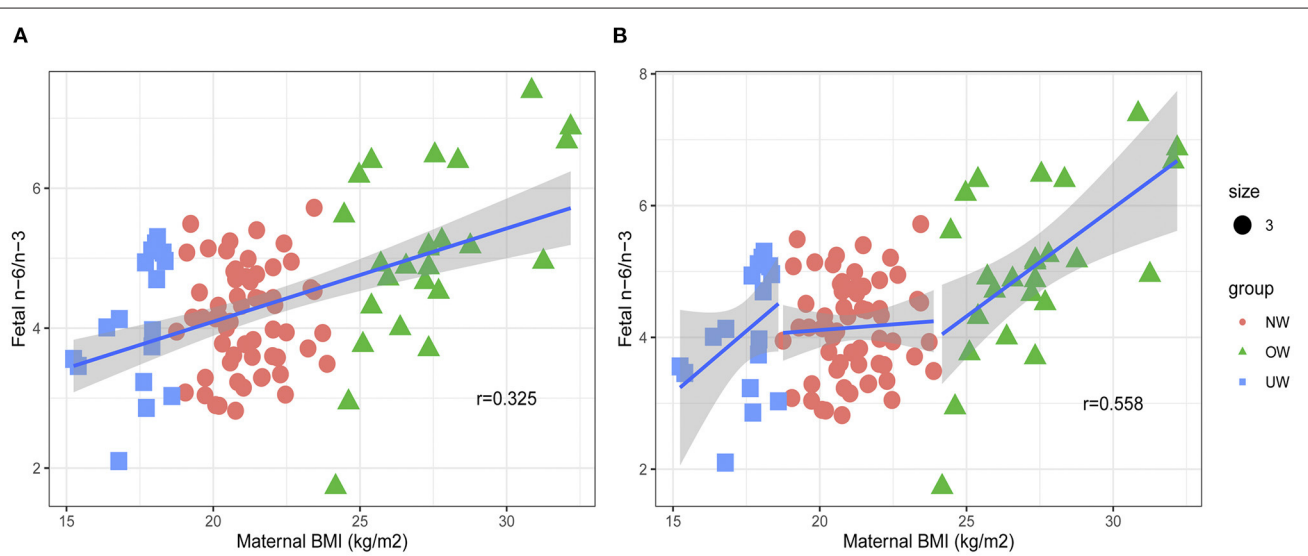


FIGURE 3 | Associations between maternal pre-BMI and fetal n-6/n-3. **(A)** The association between maternal pre-BMI and fetal n-6/n-3 in all subjects. **(B)** The association between maternal pre-BMI and fetal n-6/n-3 in different groups.

In the subgroup analysis, the correlation between maternal pre-BMI and DHA with cord plasma DHA can be observed only in OW mothers ($p < 0.05$), but not in NW subjects.

Multiple Linear Regression Analysis of Maternal Parameters and Cord Plasma n-6/n-3

Multiple linear regression analysis was conducted to explore the direct correlation between maternal parameters and cord plasma n-6/n-3 PUFA (**Table 5**). The correlations can be found between maternal pre-BMI, LA, AA, EPA, DHA, EFA, n-6 PUFA, n-3 PUFA, n-6/n-3, and cord plasma n-6/n-3 in all subjects (**Figure 1**). Owing to collinearity, finally pre-BMI, maternal AA, EPA, DHA, and n-6 PUFA were included in the regression model. Multiple linear regression analysis demonstrated the associations between pre-BMI and maternal DHA with cord plasma n-6/n-3 in all subjects ($p < 0.05$). In the subgroup analysis, maternal pre-BMI was associated with cord plasma n-6/n-3 in OW subjects ($p < 0.05$), but not in NW subjects (**Table 5**).

TABLE 4 | Linear regression analysis on the correlation of maternal parameters and fetal cord plasma DHA percentage.

Maternal	All ($n = 100$)		OW ($n = 25$)		NW ($n = 59$)		UW* ($n = 16$)	
parameters	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*
Model 1[a]								
Pre-BMI	–0.330	**0.002**	–0.632	**<0.001**	–0.128	0.360	0.028	0.925
LA	–0.122	0.199	–0.386	**0.015**	–0.040	0.797	–0.113	0.738
EPA	0.123	0.185	–0.127	0.409	0.102	0.521	–0.510	0.267
DHA	0.290	**0.005**	0.584	**0.001**	0.163	0.314	0.652	0.157
Model 2[b]								
Pre-BMI	–0.327	**0.007**	–0.722	**0.001**	–0.081	0.572	–	–
LA	–0.114	0.305	–0.519	**0.022**	0.119	0.514	–	–
EPA	0.117	0.270	–0.220	0.267	–0.103	0.568	–	–
DHA	0.256	**0.029**	0.618	**0.004**	0.096	0.554	–	–
Model 3[c]								
Pre-BMI	–0.308	**0.013**	–0.717	**0.004**	–0.089	0.564	–	–
LA	–0.081	0.492	–0.456	**0.039**	–0.016	0.935	–	–
EPA	0.155	0.151	–0.158	0.509	0.035	0.844	–	–
DHA	0.252	**0.031**	0.577	**0.014**	0.163	0.359	–	–

Associations were evaluated using linear regression analysis. Beta is corrected values after adjustment. p-Values < 0.05 are highlighted in bold.

Pre-BMI, prepregnancy body mass index; OW, mothers with overweight or obese; NW, mothers with normal weight; UW, mothers with underweight; LA, C18:2n-6, linoleic acid; EPA, C20:5n-3, eicosapentaenoic acid; DHA, C22:6n-3 docosahexaenoic acid.

[a] *The multivariate regression analysis was adjusted for maternal age and gestation weight gains.*

[b] *The multivariate regression analysis was adjusted for maternal age, gestation weight gains, and dietary DHA. Due to the lack of dietary questionnaire, the sample size is reduced in model 2: all (n = 77), OW (n = 20), NW (n = 48), UW (n = 9).*

[c] *The multivariate regression analysis was adjusted for maternal age, gestation weight gains, dietary DHA, and birth BMI z-score.*

**The sample size was small, and regression analysis was not performed.*

DISCUSSION

In this study, we observed that maternal prepregnancy BMIs were associated with maternal-fetal plasma fatty acid profiles. Maternal plasma DHA and n-3 PUFA were lower in the OW group, but n-6 PUFA and n-6/n-3 were higher. The cord plasma EPA, DHA, and n-3 PUFA were lower in the OW group, but n-6/n-3 was higher. Therefore, maternal prepregnancy obesity was associated with an adverse fatty acid profile in both mothers and fetuses. It had been reported that total n-3 PUFA was lower in the obese pregnant mothers, obesity was associated with an adverse fatty acids profile (1), and these results were in agreement with this study.

It had been reported that maternal plasma fatty acid was affected by dietary intake in addition to maternal metabolism (31). In this study, the dietary intake of fatty acids in the OW group did not differ from that in the NW group, but plasma LA was higher and DHA was lower in the OW group. A number of factors could account for this result. First, in addition to dietary intake, the endogenous synthesis of fatty acids also affects maternal fatty acid profile. It has been reported that maternal obesity affects the endogenous synthesis of fatty acids (32), further affecting umbilical cord blood fatty acid profile (33, 34). In this study, endogenous fatty acid synthesis involving the desaturase, the index of δ-6 fatty acid desaturase (AA/LA and DGLA/LA), tended to a marginal decrease in the OW group. The synthesis of subsequent products decreased, resulting in the underutilization of the substrate, then the substrate (LA) was higher in the OW group. In the human body, n-3 and n-6 PUFA share a set of fatty acid synthetase and elongase enzyme system, and there is a competitive relationship between the two pathways. The competition between fatty acid metabolic pathways may lead to changes in fatty acid composition not directly related to the diet. Secondly, methods of dietary assessment in this study require participants to recall their food consumption over the past 3 months. Therefore, there were a number of limitations that affect both accuracy and precision of dietary measurement. Respondents often underreport consumption, especially the OW participants (35). In addition, interviewer bias such as incorrect portion size estimations also account for the accuracy and precision of the dietary measurement. Relative intakes of individual fatty acids in the diet are therefore extremely difficult to estimate from reported dietary intakes. Thirdly, the lack of some dietary information also affected the accuracy of the results in this study. Besides, we performed this study in an inland city. Most pregnant women have the low dietary DHA intake (about 20 mg/day) (36), and part of pregnant women (about 30% in this study) intake DHA through a dietary supplement. Therefore, DHA dietary intake varies widely among individuals. Therefore, we observed the higher median of DHA intake in the OW group; it does not significantly differ from the other two groups. Although maternal plasma DHA was affected by both dietary and maternal metabolism, given these limitations, it is likely that the associations between dietary fatty intake and plasma fatty acids are limited by biases of dietary assessment in this study. Therefore, there has been considerable interest in using blood fatty acid composition as biological markers of fatty

TABLE 5 | Linear regression analysis on the correlation of maternal parameters and fetal cord plasma n-6/n-3.

Maternal	All ($n = 100$)		OW ($n = 25$)		NW ($n = 59$)		UW* ($n = 16$)	
parameters	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*
Model 1[a]								
Pre-BMI	0.429	**<0.001**	0.686	**<0.001**	0.250	0.055	–0.146	0.483
AA	–0.139	0.080	–0.163	0.253	–0.123	0.329	0.080	0.714
EPA	–0.092	0.268	0.041	0.766	–0.235	0.111	0.827	**0.018**
DHA	–0.332	**0.001**	–0.420	**0.009**	–0.323	0.051	–0.905	**0.011**
n-6 PUFA	0.091	0.330	0.231	0.090	–0.171	0.296	0.435	0.074
Model 2[b]								
Pre-BMI	0.432	**<0.001**	0.697	**0.002**	0.355	**0.022**	–	–
AA	–0.111	0.235	–0.031	0.898	–0.116	0.452	–	–
EPA	–0.087	0.372	–0.014	0.939	–0.178	0.321	–	–
DHA	–0.282	**0.015**	–0.295	0.208	–0.263	0.166	–	–
n-6 PUFA	0.158	0.164	0.331	0.075	–0.127	0.518	–	–
Model 3[c]								
Pre-BMI	0.387	**0.001**	0.643	**0.008**	0.284	0.060	–	–
AA	–0.090	0.326	–0.172	0.521	–0.096	0.515	–	–
EPA	–0.139	0.156	–0.063	0.770	–0.184	0.286	–	–
DHA	–0.318	**0.006**	–0.326	0.199	–0.440	**0.031**	–	–
n-6 PUFA	0.070	0.598	0.316	0.116	–0.273	0.178	–	–

Associations were evaluated using linear regression analysis. Beta is corrected values after adjustment. p-Values < 0.05 are highlighted in bold.

Pre-BMI, prepregnancy body mass index; OW, mothers with overweight or obese; NW, mothers with normal weight; UW, mothers with underweight; AA, 20:4n-6, arachidonic acid; EPA, C20:5n-3, eicosapentaenoic acid; DHA, C22:6n-3 docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

[a] *The multivariate regression analysis was adjusted for maternal age and gestation weight gains.*

[b] *The multivariate regression analysis was adjusted for maternal age, gestation weight gains, and dietary total fatty acid. Due to the lack of dietary questionnaire, the sample size reduced in model 2: all (n = 77), OW (n = 20), NW (n = 48), and UW (n = 9).*

[c] *The multivariate regression analysis was adjusted for maternal age, gestation weight gains, dietary total fatty acid, and birth BMI z-score.*

**The sample size was small, and regression analysis was not performed.*

acids intake to reflect on dietary assessment (37). Compared to the dietary survey to assess maternal fatty acids intake (38), it is more accurate to investigate the relationship between maternal plasma fatty acids and fetal growth and development (39).

Previously, many researchers have focused on the relationship of prepregnancy obesity and fatty acids profile of breast milk, which could directly affect the infant's growth, body composition, and cognitive development (10, 11, 13). However, intrauterine development is also a key period. Fatty acids such as DHA are stored in maternal fat and are available when fetal fat accretion and brain growth increase exponentially during late pregnancy (40). Therefore, maternal fatty acids profile in this period is crucial for the fetus. This study investigated the association of maternal pre-BMI and maternal-fetal fatty acids profiles among different pre-BMI subgroups. There is a direct negative correlation between pre-BMI and cord plasma DHA and a direct positive correlation between pre-BMI and cord plasma n-6/n-3 in OW subgroup, but not in the NW and UW groups. We could conclude that the associations of maternal pre-BMI and maternal-fetal fatty acids profiles are not linear, and the statistical difference could be mainly attributed to the OW group. It is well known that maternal obesity is an important factor that affects the metabolism of fatty acids in mothers and fetuses (22). The body fat content causes different physiological statuses in mothers, influencing maternal metabolism and the placenta's transport function (41–43), consequently affecting fatty acid supply to the fetus.

It has been reported that maternal obesity modifies fatty acid profile, resulting in low n-3 and elevated n-6 PUFA levels in maternal circulation during pregnancy (44, 45). These modifications of the fatty acid profile are associated with a pro-inflammatory state and oxidative stress with short- and long-term consequences in the fetus and neonate. These changes confer a higher risk of developing obesity and its complications to the offspring (44). In this study, maternal plasma n-6/n-3 ratio was higher in OW mothers; correspondingly, the ratio of n-6/n-3 was higher in fetal cord plasma. Animal studies have found that maternal obesity may induce changes in the body fat composition or lead to obesity in offspring, which may be related to the increases in n-6/n-3 (46), and the decrease in n-6/n-3 had a protective influence on the development of offspring obesity (47). There are two critical periods in the fat development of infants, namely, before birth and the first year of life. The nutritional exposures during this time have permanent consequences on the regulation of body fat mass throughout life (12). It had been reported that a higher ratio of n-6/n-3 in umbilical cord blood was associated with a high subscapular skinfold thickness at 3 years of age (48). Therefore, a fetus exposed to high levels of n-6/n-3 ratio may have an increased risk for childhood obesity and even adult obesity. These conclusions were in agreement with our results. Moreover, the pre-BMI was positively associated with birth BMI *z*-score and cord plasma n-6/n-3 in this study. It had been reported that infants born to mothers with prepregnancy obesity had a higher weight and

length at birth (49). Birth weight and birth BMI *z*-score are the risk factors of offspring adult obesity and metabolism diseases; therefore, maternal prepregnancy obesity could be related to the offspring's growth and increase the risk of offspring obesity. In short, we found the associations of maternal prepregnancy obesity and adverse maternal-fetal fatty acid profiles, and the influence of maternal prepregnancy obesity on the offspring's growth and development requires further research. Besides, more DHA intake by diet is required in China, especially prepregnancy OW women.

A major limitation of this study was that only 103 mother-fetus pairs were collected; a larger sample size would provide further evidence to support the conclusions. The lack of some dietary information also affected the accuracy of the conclusion. Moreover, as this was a cross-sectional study, only the birth information of infants was collected. The offspring's follow-up growth and development information need to be collected in future studies. In conclusion, the pre-BMI was associated with the maternal-fetal plasma fatty acid profiles, whereas the adverse fatty acid profiles are more noticeable in the prepregnancy OW mothers.

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

The studies involving human participants were reviewed and approved by Chinese Clinical Trial Registry (ChiCTR2000034179). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

H-TY: carrying out the study, analyzing the data, and writing the article. W-HX, Y-RC, and YJ: collecting sample. Y-WT, Y-TL, J-YG, and Y-FC: fatty acids detection. G-LL: methodology. LX: designing the study. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Graduate Innovation Fund of Jilin University (No. 101832020CX27) and Health Commission of Jilin Province (No. 2019J027).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.897059/full#supplementary-material

REFERENCES

1. Vidakovic AJ, Jaddoe VWV, Gishti O, Felix JF, Williams MA, Hofman A, et al. Body mass index, gestational weight gain and fatty acid concentrations during pregnancy: the Generation R Study. *Eur J Epidemiol.* (2015) 30:1175–85. doi: 10.1007/s10654-015-0106-6
2. Catalano PM. The impact of gestational diabetes and maternal obesity on the mother and her offspring. *J Dev Orig Health Dis.* (2010) 1:208–15. doi: 10.1017/S2040174410000115
3. Prieto-Sanchez MT, Blanco-Carnero JE, Ruiz-Palacios M, Pagan A, Ruiz-Alcaraz AJ. Larque, E. Increased alkaline phosphatase in cord blood of obese diabetic mothers is associated to polyunstaurated fatty acid levels. *Ann Nutr Metab.* (2019) 75:153–62. doi: 10.1159/000504404
4. Daraki V, Roumeliotaki T, Koutra K, Georgiou V, Kampouri M, Kyriklaki A et al. Effect of parental obesity and gestational diabetes on child neuropsychological and behavioral development at 4 years of age: the Rhea mother-child cohort, Crete, Greece. *Eur Child Adolesc Psychiatry.* (2017) 26:703–14. doi: 10.1007/s00787-016-0934-2
5. Voortman T, Tielemans MJ, Stroobant W, Schoufour JD. Kiefte-de Jong JC, Steenweg-de Graaff J, et al. Plasma fatty acid patterns during pregnancy and child's growth, body composition, and cardiometabolic health: the Generation R Study. *Clin Nutr.* (2018) 37:984–92. doi: 10.1016/j.clnu.2017.04.006
6. Ostadrahimi A, Salehi-Pourmehr H, Mohammad-Alizadeh-Charandabi S, Heidarabady S, Farshbaf-Khalili A. The effect of perinatal fish oil supplementation on neurodevelopment and growth of infants: a randomized controlled trial. *Eur J Nutr.* (2018) 57:2387-97. doi: 10.1007/s00394-017-1512-1
7. Sambra V, Echeverria F, Valenzuela A, Chouinard-Watkins R, Valenzuela R. Docosahexaenoic and arachidonic acids as neuroprotective nutrients throughout the life cycle. *Nutrients.* (2021) 13:986. doi: 10.3390/nu13030986
8. Fekete K, Gyorei E, Lohner S, Verduci E, Agostoni C, Decsi T. Long-chain polyunsaturated fatty acid status in obesity: a systematic review and meta-analysis. *Obes Rev.* (2015) 16:488–97. doi: 10.1111/obr.12280
9. Barrera C, Valenzuela R, Chamorro R, Bascunan K. SandovalJ, Sabag N, et al. The impact of maternal diet during pregnancy and lactation on the fatty acid composition of erythrocytes and breast milk of chilean women. *Nutrients.* (2018) 10:839. doi: 10.3390/nu10070839
10. de la Garza Puentes A, Marti Alemany A, Chisaguano AM, Goyanes RM, Castellote AI, Torres-Espinola FJ, et al. The effect of maternal obesity on breast milk fatty acids and its association with infant growth and cognition-the PREOBE follow-up. *Nutrients.* (2019) 11:2154. doi: 10.3390/nu11092154
11. Nuss H, Altazan A, Zabaleta J, Sothern M, Redman L. Maternal pre-pregnancy weight status modifies the influence of PUFAs and inflammatory biomarkers in breastmilk on infant growth. *PLoS ONE.* (2019) 14:e0217085. doi: 10.1371/journal.pone.0217085
12. Rudolph MC, Young BE, Lemas DJ, Palmer CE, Hernandez TL, Barbour LA, et al. Early infant adipose deposition is positively associated with the n-6 to n-3 fatty acid ratio in human milk independent of maternal BMI. *Int J Obes.* (2017) 41:510–7. doi: 10.1038/ijo.2016.211
13. Ellsworth L, Perng W, Harman E, Das A, Pennathur S, Gregg B. Impact of maternal overweight and obesity on milk composition and infant growth. *Matern Child Nutr.* (2020) 16:e12979. doi: 10.1111/mcn.12979

14. Makela J, Linderborg K, Niinikoski H, Yang BR, Lagstrom H. Breast milk fatty acid composition differs between overweight and normal weight women: the STEPS Study. *Eur J Nutr.* (2013) 52:727–35. doi: 10.1007/s00394-012-0378-5
15. Armand M, Bernard JY, Forhan A, Heude B, Charles MA. Maternal nutritional determinants of colostrum fatty acids in the EDEN mother-child cohort. *Clin Nutr.* (2018) 37:2127–36. doi: 10.1016/j.clnu.2017.10.007
16. Zhao JP, Levy E, Shatenstein B, Fraser WD, Julien P, Montoudis A, et al. Longitudinal circulating concentrations of long-chain polyunsaturated fatty acids in the third trimester of pregnancy in gestational diabetes. *Diabet Med.* (2016) 33:939–46. doi: 10.1111/dme.12978
17. Cinelli G, Fabrizi M, Rava L. degli Atti MC, Vernocchi P, Vallone C, et al. Influence of maternal obesity and gestational weight gain on maternal and foetal lipid profile. *Nutrients.* (2016) 8:368. doi: 10.3390/nu8060368
18. Basak S, Vilasagaram S, Duttaroy AK. Maternal dietary deficiency of n-3 fatty acids affects metabolic and epigenetic phenotypes of the developing fetus. *Prostaglandins Leukot Essent Fatty Acids.* (2020) 158:102109. doi: 10.1016/j.plefa.2020.102109
19. Gustafsson HC, Holton KF, Anderson AN, Nousen EK, Sullivan CA, Loftis JM, et al. Increased maternal prenatal adiposity, inflammation, and lower omega-3 fatty acid levels influence child negative affect. *Front Neurosci.* (2019) 13:1035. doi: 10.3389/fnins.2019.01035
20. Carvalho GQ, Pereira-Santos M, Marcon LD, Louro ID, Peluzio MCG, Santos DB. Maternal polymorphisms in the FADS1 and FADS2 genes modify the association between PUFA ingestion and plasma concentrations of omega-3 polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids.* (2019) 150:38–46. doi: 10.1016/j.plefa.2019.09.004
21. Mishra JS, Zhao H, Hattis S, Kumar S. Elevated glucose and insulin levels decrease DHA transfer across human trophoblasts via SIRT1-dependent mechanism. *Nutrients.* (2020) 12:1271. doi: 10.3390/nu12051271
22. Rosenfeld CS. de la Garza Puentes A, Montes Goyanes R, Chisaguano Tonato AM, Torres-Espínola FJ, Arias García M, et al. Association of maternal weight with FADS and ELOVL genetic variants and fatty acid levels- The PREOBE follow-up. *PLoS ONE.* (2017) 12:e0179135. doi: 10.1371/journal.pone.0179135
23. Wu Y, Wang Y, Tian H, Lu T, Yu M, Xu WH, et al. DHA intake interacts with ELOVL2 and ELOVL5 genetic variants to influence polyunsaturated fatty acids in human milk. *J Lipid Res.* (2019) 60:1043–9. doi: 10.1194/jlr.M090951
24. YX Yang. *China Food Composition Tables (Standard Edition)*. Beijing: Peking University Medical Press (2019).
25. Uauy R, Mena P, Wegher B, Nieto S, Salem N. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. *Pediatr Res.* (2000) 47:127–35. doi: 10.1203/00006450-200001000-00022
26. Matsumoto A, Kawabata T, Kagawa Y, Shoji K, Kimura F, Miyazawa T, et al. Associations of umbilical cord fatty acid profiles and desaturase enzyme indices with birth weight for gestational age in Japanese infants. *Prostaglandins Leukot Essent Fatty Acids.* (2021) 165:102233. doi: 10.1016/j.plefa.2020.102233
27. Hammouda S, Ghzaiel I, Khamlaoui W, Hammami S, Mhenni SY, Samet S, et al. Genetic variants in FADS1 and ELOVL2 increase level of arachidonic acid and the risk of Alzheimer's disease in the Tunisian population. *Prostaglandins Leukot Essent Fatty Acids.* (2020) 160:102159. doi: 10.1016/j.plefa.2020.102159
28. Olga L, van Diepen JA, Bobeldijk-Pastorova I, Gross G, Prentice PM, Snowden SG, et al. Lipid ratios representing SCD1, FADS1, and FADS2 activities as candidate biomarkers of early growth and adiposity. *EBioMedicine.* (2021) 63:103198. doi: 10.1016/j.ebiom.2020.103198
29. Venäläinen T, Ågren J, Schwab U, de Mello VD, Eloranta A-M, Laaksonen DE, et al. Cross-sectional associations of plasma fatty acid composition and estimated desaturase and elongase activities with cardiometabolic risk in Finnish children—The PANIC study. *J Clin Lipidol.* (2016) 10:82–91. doi: 10.1016/j.jacl.2015.09.004
30. Zornoza-Moreno M, Fuentes-Hernandez S, Carrion V, Alcantara-Lopez MV, Madrid JA, Lopez-Soler C, et al. Is low docosahexaenoic acid associated with disturbed rhythms and neurodevelopment in offsprings of diabetic mothers? *Eur J Clin Nutr.* (2014) 68:931–7. doi: 10.1038/ejcn.2014.104
31. Rodríguez Y, Christophe AB. Effect of diabetes mellitus and different treatments on plasma and erythrocyte phospholipid fatty acid composition in type 2 diabetics. *Ann Nutr Metab.* (2004) 48:335–42. doi: 10.1159/000081201
32. Ferchaud-Roucher V, Barner K, Jansson T, Powell TL. Maternal obesity results in decreased syncytiotrophoblast synthesis of palmitoleic acid, a fatty acid with anti-inflammatory and insulin-sensitizing properties. *FASEB J.* (2019) 33:6643–54. doi: 10.1096/fj.201802444R
33. Donahue SM, Rifas-Shiman SL, Olsen SF, Gold DR, Gillman MW, Oken E. Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels. *Prostaglandins Leukot Essent Fatty Acids.* (2009) 80:289–96. doi: 10.1016/j.plefa.2009.02.007
34. Liu K, Ye K, Han Y, Sheng J, Jin Z, Bo Q, et al. Maternal and cord blood fatty acid patterns with excessive gestational weight gain and neonatal macrosomia. *Asia Pac J Clin Nutr.* (2017) 26:291–7. doi: 10.6133/apjcn.012016.11
35. Lichtman SW, Pisarska K, Berman ER, Pestone M, Dowling H, Offenbacher E, et al. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N Engl J Med.* (1992) 327:1893–8. doi: 10.1056/NEJM199212313272701
36. Zhou YB Li HT, Trasande L, Wang LL, Zhang YL Si KY, et al. A correlation study of DHA intake estimated by a FFQ and concentrations in plasma and erythrocytes in mid- and late pregnancy. *Nutrients.* (2017) 9:1256. doi: 10.3390/nu9111256
37. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res.* (2008) 47:348–80. doi: 10.1016/j.plipres.2008.03.003
38. Hakola L, Takkinen HM, Niinistö S, Ahonen S, Erlund I, Rautanen J, et al. Maternal fatty acid intake during pregnancy and the development of childhood overweight: a birth cohort study. *Pediatric Obes.* (2017) 12(Suppl. 1):26–37. doi: 10.1111/ijpo.12170
39. Saadatian-Elahi M, Slimani N, Chajès V, Jenab M, Goudable J, Biessy C, et al. Plasma phospholipid fatty acid profiles and their association with food intakes: results from a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr.* (2009) 89:331–46. doi: 10.3945/ajcn.2008.26834
40. Bergmann RL, Haschke-Becher E, Klassen-Wigger P, Bergmann KE, Richter R, Dudenhausen JW, et al. Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake and of their infants. *Ann Nutr Metab.* (2008) 52:157–66. doi: 10.1159/000129651
41. Gazquez A, Prieto-Sanchez MT, Blanco-Carnero JE, Ruiz-Palacios M, Nieto A, van Harskamp D, et al. Altered materno-fetal transfer of 13C-polyunsaturated fatty acids in obese pregnant women. *Clin Nutr.* (2020) 39:1101–7. doi: 10.1016/j.clnu.2019.04.014
42. Segura MT, Demmelmair H, Krauss-Etschmann S, Nathan P, Dehmel S, Padilla MC, et al. Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. *Placenta.* (2017) 57:144–51. doi: 10.1016/j.placenta.2017.07.001
43. Dubé E, Gravel A, Martin C, Desparois G, Moussa I, Ethier-Chiasson M, et al. Modulation of fatty acid transport and metabolism by maternal obesity in the human full-term placenta. *Biol Reprod.* (2012) 14:1–11. doi: 10.1095/biolreprod.111.098095
44. Alvarez D, Munoz Y, Ortiz M, Maliqueo M, Chouinard-Watkins R, Valenzuela R. Impact of maternal obesity on the metabolism and bioavailability of polyunsaturated fatty acids during pregnancy and breastfeeding. *Nutrients.* (2020) 13:19. doi: 10.3390/nu13010019
45. Scifres CM, Catov JM, Simhan HN. The impact of maternal obesity and gestational weight gain on early and mid-pregnancy lipid profiles. *Obesity.* (2014) 22:932–8. doi: 10.1002/oby.20576
46. Muhlhausler BS, Ailhaud GP. Omega-6 polyunsaturated fatty acids and the early origins of obesity. *Curr Opin Endocrinol Diabetes Obes.* (2013) 20:56–61. doi: 10.1097/MED.0b013e32835c1ba7
47. Heerwagen MJR, Stewart MS. de la Houssaye BA, Janssen RC, Friedman JE. Transgenic increase in N-3/N-6 fatty acid ratio reduces maternal obesity-associated inflammation and limits adverse developmental programming in mice. *PLoS ONE.* (2013) 8:e67791. doi: 10.1371/journal.pone.0067791
48. Donahue SMA, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. *Am J Clin Nutr.* (2011) 93:780–8. doi: 10.3945/ajcn.110.005801
49. Poston L, Caleyachetty R, Cnattingius S, Corvalán C, Uauy R, Herring S, et al. Preconceptional and maternal obesity: epidemiology

and health consequences. *Lancet Diabetes Endocrinol.* (2016) 4:1025–36. doi: 10.1016/S2213-8587(16)30217-0

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.

frontiers | Frontiers in Nutrition

SYSTEMATIC REVIEW
published: 19 May 2022
doi: 10.3389/fnut.2022.889576

Effect of High Ratio of n-6/n-3 PUFAs on Depression: A Meta-Analysis of Prospective Studies

*Yuanyuan Wang[1], Lirong Dong[2], Da Pan[1], Dengfeng Xu[1], Yifei Lu[1], Shiyu Yin[1], Shaokang Wang[1], Hui Xia[1], Wang Liao[1,3] and Guiju Sun[1,3]**

[1] Key Laboratory of Environmental Medicine and Engineering of Ministry of Education, Department of Nutrition and Food Hygiene, School of Public Health, Southeast University, Nanjing, China, [2] Department of Integrated Service and Management, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China, [3] China-DRIs Expert Committee on Macronutrients, Beijing, China

Objective: The aim of this systematic review and meta-analysis was to examine the association between high ratio of n-6/n-3 polyunsaturated fatty acids (PUFAs) and depression.

Methods: The authors conducted a meta-analysis of research articles on the association of high ratio of n-6/n-3 PUFAs with the risk of depression published in the online article database on PubMed, Embase, Cochrane library as of December 2021. Pooled odds ratios (OR) were calculated using random effects models. Publication bias was assessed visually by funnel plots and statistically by the Egger's and Begg's tests.

Results: Finally, 12 studies included in this systematic review and meta-analysis with a total of 66,317 participants (including 4,173 individuals with depression condition). The pooled results showed that high ratio of n-6/n-3 PUFAs might be positively associated with depression [OR = 1.21, 95% confidence intervals (CIs): 1.04∼1.41]. The I^2 test indicated that there was a substantial statistical heterogeneity across the included studies (I^2 = 54.38%, P = 0.01). Subgroup analysis showed that high ratio of n-6/n-3 PUFAs in blood had no significant association with depression (OR = 1.15, 95%CI: 0.88∼1.50), while high ratio of n-6/n-3 PUFAs in dietary supplements was positively associated with depression (OR = 1.32, 95%CI: 1.16∼1.51).

Conclusion: This meta-analysis confirmed the association between high ratio of n-6/n-3 PUFAs and the risk of depression. High ratio of n-6/n-3 PUFAs in dietary supplementation was positively associated with depression, but had no significant association in the blood. This study suggested that lowering the dietary intake of the ratio of n-6/n-3 PUFAs would be beneficial in the prevention of depression.

Keywords: depression, n-3, n-6, polyunsaturated fatty acids, prospective study

OPEN ACCESS

Edited by:
Zheqing Zhang,
Southern Medical University, China

Reviewed by:
Fang Fang Zeng,
Jinan University, China
Guoxun Chen,
The University of Tennessee, Knoxville, United States

****Correspondence:***
Guiju Sun
gjsun@seu.edu.cn

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *04 March 2022*
Accepted: *25 April 2022*
Published: *19 May 2022*

Citation:
Wang Y-y, Dong L-r, Pan D, Xu D-f, Lu Y-f, Yin S-y, Wang S-k, Xia H, Liao W and Sun G (2022) Effect of High Ratio of n-6/n-3 PUFAs on Depression: A Meta-Analysis of Prospective Studies. Front. Nutr. 9:889576. doi: 10.3389/fnut.2022.889576

INTRODUCTION

Depression is a common mental illness that involves a complex interplay of social, psychological and biological factors. A continuous and prolonged depressed mood is the main clinical feature (1). Depression can have a profound impact on all aspects of life with researches showing a strong link between depression and health, including tuberculosis and cardiovascular disease

(2, 3). In addition, it can disrupt sleep and appetite (4). Depression is reported to be the leading cause of disability worldwide and contributes significantly to the global burden of disease (5–7). According to the World Health Organization (WHO), an estimated 5% of adults worldwide suffer from depression (1). The prevalence of depression in the United States was between 5% and 10% and may be higher in some specific settings (8). Meanwhile, the latest results from the China Mental Health Survey indicated that the prevalence of depression in China was 3.6%, with women being more likely than men at all stages (9). Unfortunately, mental illnesses like depression are getting worse. One of the main reasons is the impact of the COVID-19 pandemic on the mental health of the global population (10, 11). How to prevent and treat depression has become a major area of research for researchers worldwide. Positive effects on depressive symptoms or depression through dietary changes have been demonstrated in many observational and clinical studies (12–15).

The n-3 polyunsaturated fatty acids (PUFAs) and n-6 PUFAs are important fatty acids required by the human body and mainly provided by dietary intake. They are named due to the presence of the first unsaturated bond in the third and sixth positions of the methyl end of the carbon chain, respectively. N-3 PUFAs mainly include alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while n-6 PUFAs mainly include linoleic acid (LA) and arachidonic acid (AA). Recent articles have shown that n-3 PUFAs have myriad health benefits on cardiovascular disease, diabetes, cancer, depression and various mental disorders, age-related cognitive decline, periodontal disease and rheumatoid arthritis (16). Since n-3 and n-6 fatty acids can be converted to share the same family of enzymes, there is competitive metabolism between the n-3 and n-6 fatty acid families (17). Therefore, seeking the optimal balance of the ratio of n-6 to n-3 PUFAs seems to have more health benefits. However, the results of the effects of different ratios of n-6/n-3 PUFAs on depression are inconsistent. Some studies showed that high ratio of n-6/n-3 PUFAs was not associated with depression. A French study on the association between n-3 PUFAs and depression found that n-6/n-3 ratio was not associated with depression, either from cross-sectional data or from cohort data (18). However, there are studies that hold the opposite opinion. An article prospectively examining depression in 54,632 US women from the Nurses' Health Study found that the risk of depression decreased as the n-3/n-6 ratio increased, while intake of long-chain n-3 fatty acids from fish was not associated with risk of depression (19). They suggested that it was the n-6/n-3 ratio rather than n-3 fatty acids alone that played a role in depression (19, 20). Besides, in a meta-analysis, a higher ratio of n-6/n-3 PUFAs was positively associated with depression (21). However, this meta-analysis only focused on the gestational population and the results could not be extrapolated to the whole population, which would affect the overall prevention and treatment policy of depression. Therefore, examining the relationship between the ratio of n-6/n-3 PUFAs and depression in the whole population has become the focus of this study, which can provide a scientific basis for the primary and secondary prevention of depression.

MATERIALS AND METHODS

Search Strategy

We conducted a systematic search on the databases such as PubMed, Embase, and Cochrane library up to December 2021. We used the following key words for the literature search: ("depression" or "depressive symptoms" or "depressive symptom" or "symptom, depressive" or "symptoms, depressive" or "emotional depression" or "depression, emotional") AND ("n-6: n-3 fatty acid ratio" or "n-6/n-3 PUFAs" or "n-3 PUFAs" or "omega-3 fatty acid" or "n-6 PUFAs" or "omega-6 fatty acid" or "α-linolenic acid" or "DHA" or "EPA" or "arachidonic acid" or "linoleic acid" or "fish oil" or "fish"). All indexed studies were retrieved and the reference list of identified publications was reviewed for other relevant studies.

Eligibility Criteria

The criteria of the inclusion in this study were as follows: (1) this study was limited to English-language publications; (2) studies included only prospective cohort studies; (3) human studies; (4) participants had a clear ratio of n-6/n-3 PUFAs by dietary supplementation or biochemical testing for fatty acids; (5) only original studies were included in this study while those studies that were non-original studies (reviews, editorials or commentaries), abstracts, unpublished studies and duplicate studies were excluded.

Data Extraction

In this study, dietary intake and blood levels of n-3 and n-6 PUFAs were considered the primary exposures, and risk of depression was considered the primary outcome. Adjusted effect sizes were extracted where available. Only total n-3 and n-6 PUFAs content was chosen to be calculated in this study. Risk estimates (ORs or HRs or RRs) were pooled prior to data analysis.

In addition, the following characteristics of eligible articles were extracted: name of first author, date of publication, source of study, numbers of participants completing the study, length of cohort study, type of exposure (dietary intake, blood fatty acids), outcome of interest (depressive status), reported risk assessment related to depression [including ORs, RRs, HRs and their 95% confidence intervals (95%CIs)]. Each step was assessed by two independent investigators. In the event of inconsistent results, the final decision was made primarily by the investigators.

Quality Assessment of Studies

The Newcastle-Ottawa Scale (NOS) was used to determine the quality of included articles. According to the STAR scoring system, each prospective study is awarded a maximum of nine points based on criteria in three domains: selection (maximum 4 points), comparability (maximum 2 points) and assessment of results (maximum 3 points). According to the NOS, one to three stars indicate low quality, four to six stars indicate moderate quality and seven to nine stars indicate high quality. Quality was assessed independently by the two authors and any disagreements were resolved through discussion.

Statistical Analysis

In this meta-analysis of prospective cohort studies, log ORs and standard errors (SEs) were calculated using ORs, RRs and HRs and their 95% CIs. At first, a fixed-effects model was used to drive the overall effect sizes. If there was significant between-studies heterogeneity, the random-effects model (DerSimonian–Laird) was applied as an alternative. Cochrane Q test and I^2 were used to measure potential sources of heterogeneity across studies. In this study, $I^2 > 50$ was used as an indicator of heterogeneity among studies. Subgroup analyses were performed using random effects models for the following criteria: source of n-3 and n-6 PUFAs (food intake or blood), quality assessment score ($> 6/ \leq 6$) and covariates such as gender, BMI, energy, smoking, alcohol consumption.

Sensitivity analysis was performed to elucidate the stability of findings and to ascertain whether the final pooled effect sizes were affected by a single or several publications. In addition, plausible publication bias was specified visually by funnel plot and confirmed by the statistical evidence of Egger's test. Data analyses were performed on Stata version 16.0 (Stata Corp., College Station, TX). P values of two sides were considered significant at the level of <0.05.

RESULTS

Literature Search

We identified 5,739 articles from the original search by keywords. Of these, 1,951 articles were excluded because they were duplicates. Then, 3,644 articles were excluded because there was no relevant study design (non-prospective cohort study) or non-human studies. 144 articles were left for full-text examination. Through checking the full-text, twelve eligible papers with 66,317 participants (including 4,173 individuals with depression condition) were included in the current meta-analysis. The process of the literature search is presented in **Figure 1**.

Study Characteristics

Twelve prospective studies were finally selected for inclusion in the current systematic review. Characteristics of each study are provided in **Table 1**. Publication date varied between 2006 and 2021. Four of the included studies were conducted in Europe (18, 22–24), four in Asia (25–28), and four in America (19, 20, 29, 30). Six studies were conducted on women, one study was conducted on men, while five studies included both genders. None of the studies considered the gender-specific association between n-6/n-3 and the risk of depression. Six of the included

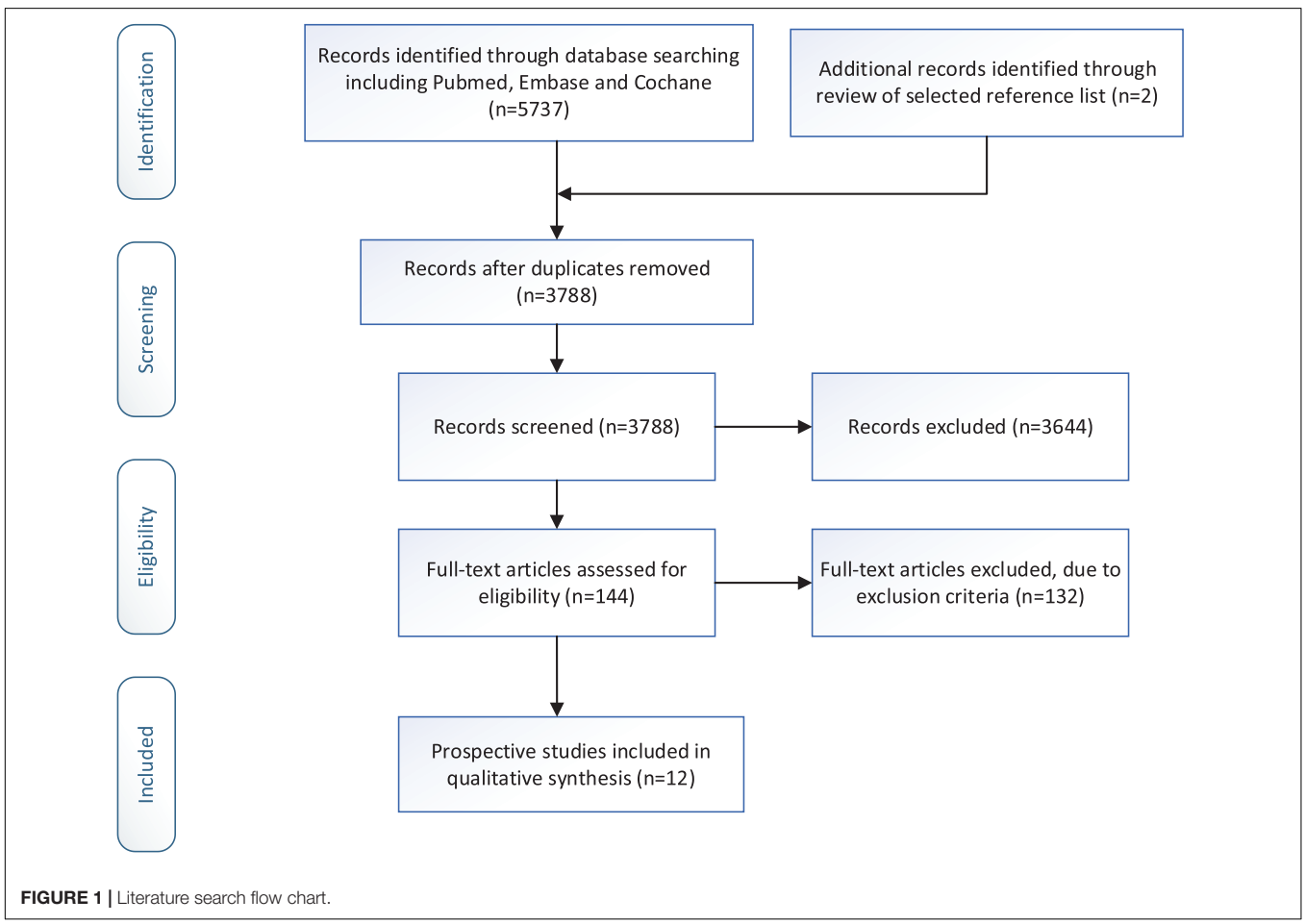


FIGURE 1 | Literature search flow chart.

TABLE 1 | Characteristics of the included studies.

Study source	Population	Follow-up duration (years)	Participants/case	Exposure measurement	Odds ratio (95%CI)
Miyake, et al. (25)	The Osaka Maternal and Child Health Study in Japan	2–9 months postpartum	865/121	Food intake n-6: LA, AA n-3: ALA, EPA, DHA	1.03 (0.6∼1.82)
Lucas, et al. (19)	Women from the Nurses' Health Study in United States	10	54632/2823	Food intake n-6: LA, AA n-3: ALA, EPA, DHA	1.35 (1.11∼1.64)
Ruusunen, et al. (22)	Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study cohort in Finland	18	2077/46	Blood n-6: LA, AA n-3: ALA, EPA, DHA, DPA	0.97 (0.49∼2.00)
Kesse-Guyot, et al. (18)	Participants from the Supplementation with Antioxidant Vitamins and Minerals (SU.VI.MAX) Study in France	10.8	1235/140	Food intake n-6: LA, AA n-3: ALA, EPA, DPA, DHA	0.98 (0.58∼1.65)
da Rocha and Kac (20)	A prospective observational cohort of pregnant women in Brazil	at least 30 days post-partum	106/28	Food intake n-6: total n-6 PUFA n-3: total n-3 PUFA	2.50 (1.21∼5.14)
Chong, et al. (26)	Mothers from the Growing Up in Singapore Toward healthy Outcomes (GUSTO) mother-offspring cohort study in Singapore	3 mothers postgratum	698/72	Blood n-6: AA n-3: DHA, EPA, DPA	0.70 (0.09∼5.14)
Matsuoka, et al. (28)	Participants from Japan Public Health Center-based Prospective Study (JPHC Study)	10	1181/99	Food intake n-6: LA, AA n-3: ALA, EPA, DHA, DPA	1.16 (0.64∼2.08)
Pinto, et al. (23)	Pregnant women from a prospective observational cohort in Brazil	30–36 gestational weeks	138/24	Blood n-6: LA, γ linolenic acid, AA, eicosatrienoic acid, docosatetraenoic acid, docosapentaenoic acid n-3: ALA, EPA, DPA, DHA	1.40 (1.09∼1.79)
Horikawa, et al. (27)	Participants from the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA) in Japan	8.1	2335/515	Food intake n-6: AA, LA n-3: DHA, EPA, ALA	1.36 (1.10∼1.69)
Hoge, et al. (29)	Pregnant women from a prospective observational cohort in Belgian	one year after delivery	71/17	Blood n-6: total n-6 PUFA n-3: total n-3 PUFA	2.31 (1.20∼4.45)
Thesing et al. (24)	Participants from the Netherlands Study of Depression and Anxiety and the Depression Evaluation Longitudinal Therapy Assessment studies in Netherlands	8	474/165	Blood n-6: LA, γ linolenic acid, Eicosadienoic acid, Homogamma-Linolenic Acid, AA, Docosadienoic acid, Docosatetraenoic acid, Docosapentaenoic acid n-3: ALA, EPA, DPA, DHA	0.90 (0.76∼1.06)
Mongan, et al. (30)	Participants from the Avon Longitudinal Study of Parents and Children (ALSPAC) in United Kingdom	7	2505/157	Blood n-6: total n-6 PUFA n-3: total n-3 PUFA	1.02 (0.79∼1.32)

studies were conducted on dietary intake, while six of the included studies were conducted on blood biochemistry tests. The studies' sample size ranged from 71 to 54,632. In total, 66,317 individuals, with depression (n = 4173) were entered in the current systematic review.

The risk assessment of bias for each study through NOS was shown in **Table 2**. In terms of selection of populations, the exposed cohort in most studies were underrepresented and only represented a certain group of people. Whereas the non-exposed population was from the same population as the exposed population. Besides, all studies were highly comparable between groups. As for outcome measures, most of the studies had strict outcome measures.

Ultimately, we found that four of the twelve included in this meta-analysis were of moderate quality and eight were of high quality.

TABLE 2 | Quality assessment of the included cohort studies.

Study design	Selection (☆ ☆ ☆ ☆)	Comparability (☆ ☆)	Exposure or Outcome (☆ ☆ ☆)	Stars	Quality scores
Cohort studies	1) Representativeness of the exposed cohort? ☆ 2) Selection of the non-exposed cohort? ☆ 3) Evaluating exposure? ☆ 4) Outcomes of interest were not present at study start? ☆	1) Study controls for the most important factor? ☆ 2) Study controls for any additional factors? ☆	1) How to ascertain outcome? ☆ a) Independent blindness b) Record linkage 2) Follow-up till outcomes happened? ☆ 3) Adequacy of follow up? ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆ (9)	High quality: 8–9 stars, Moderate quality: 6–7 stars.
Included cohort studies					
Miyake, et al. (25)	1) × : female cohort, 2) ☆, 3) × , 4) ☆	1) ☆, 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆	Moderate
Lucas, et al. (19)	1) × : female cohort, 2) ☆, 3) ☆, 4) ☆	1) ☆, 2) ☆	1) ☆, 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Ruusunen, et al. (22)	1) × : male cohort; 2) ☆, 3) ☆, 4) ☆	1) ☆; 2) ☆	1) ☆, 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Kesse-Guyot, et al. (18)	1) ☆, 2) ☆, 3) × , 4) × : no statement	1) ☆; 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆	Moderate
da Rocha and Kac (20)	1) × : female cohort, 2)☆, 3) ☆, 4) × : no statement	1) ☆; 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆	Moderate
Chong, et al. (26)	1) × : female cohort, 2) ☆, 3) ☆, 4) ☆	1) ☆, 2) ☆	1) ×, 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Matsuoka, et al. (28)	1) ☆, 2) ☆, 3) ☆, 4) × : no statement	1) ☆, 2) ☆	1) ☆, 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Pinto, et al. (23)	1) × : female cohort, 2) ☆, 3) ☆, 4) ☆	1) ☆, 2) ☆	1) ☆, 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Horikawa et al. (27)	1) ☆, 2) ☆, 3) ☆, 4) ☆	1) ☆, 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Hoge et al. (29)	1) × : female cohort, 2)☆, 3) ☆, 4) × : no statement	1) ☆, 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆	Moderate
Thesing et al. (24)	1) ☆, 2) ☆, 3) ☆, 4) ☆	1) ☆, 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High
Mongan et al. (30)	1) ☆, 2) ☆, 3) ☆, 4) ☆	1) ☆, 2) ☆	1) × , 2) ☆, 3) ☆	☆ ☆ ☆ ☆ ☆ ☆ ☆ ☆	High

Effect of High Ratio of n-6/n-3 PUFAs on Depression

Twelve studies from independent cohorts reported an association between high ratio of n-6/n-3 PUFAs and risk of depression, with 66,317 participants and 4,173 depression events. The forest plot of depression is shown in **Figure 2**. The pooled results from the forest plot showing in **Figure 2**, demonstrated that high ratio of n-6/n-3 PUFAs was positively associated with depression (OR = 1.21, 95%CI: 1.04∼1.41). The I^2 test indicated that there was a substantial statistical heterogeneity across the included trials (I^2 = 54.38%, P = 0.01). Moreover, as shown in **Figure 3**, six studies, which detected n-3 and n-6 PUFAs in blood, indicated that high ratio of n-6/n-3 PUFAs had no significant association with depression (OR = 1.15, 95%CI: 0.88∼1.50), with a substantial statistical heterogeneity across the included trials (I^2 = 67.30%, P = 0.01). However, six studies conducting on dietary intake showed that high ratio of n-6/n-3 PUFAs was positively associated with depression (OR = 1.32, 95%CI: 1.16∼1.51), with a non-significant heterogeneity between studies (I^2 = 0.00%, P = 0.38).

Sensitivity Analysis and Subgroup Analysis

Sensitivity analysis showed that the effect of high ratio of n-6/n-3 PUFAs on depression was not changed by removing any one of the studies at a time. When studies with one or more high risks of bias were excluded, the overall effect size was not significantly changed for depression.

To more precisely identify the relationship between high ratio of n-6/n-3 PUFAs and depression, we performed further substantification analysis of the screened studies (shown in **Table 3**). We found that in America and Asia, the high ratio of n-6/n-3 PUFAs showed a significantly increasing effect on depression (OR = 1.44, 95CI%: 1.24∼1.67; OR = 1.29, 95CI%: 1.07∼1.56, respectively), with a substantial statistical heterogeneity across the included trials (I^2 = 0.00%, P = 0.19; I^2 = 0.00%, P = 0.72, respectively). However, in European countries, the high ratio of n-6/n-3 PUFAs had no significant effect on depression (OR = 0.94, 95CI%: 0.82∼1.07). The significant positive association with high ratio of n-6/n-3 PUFAs and the risk of depression was observed in pregnant women

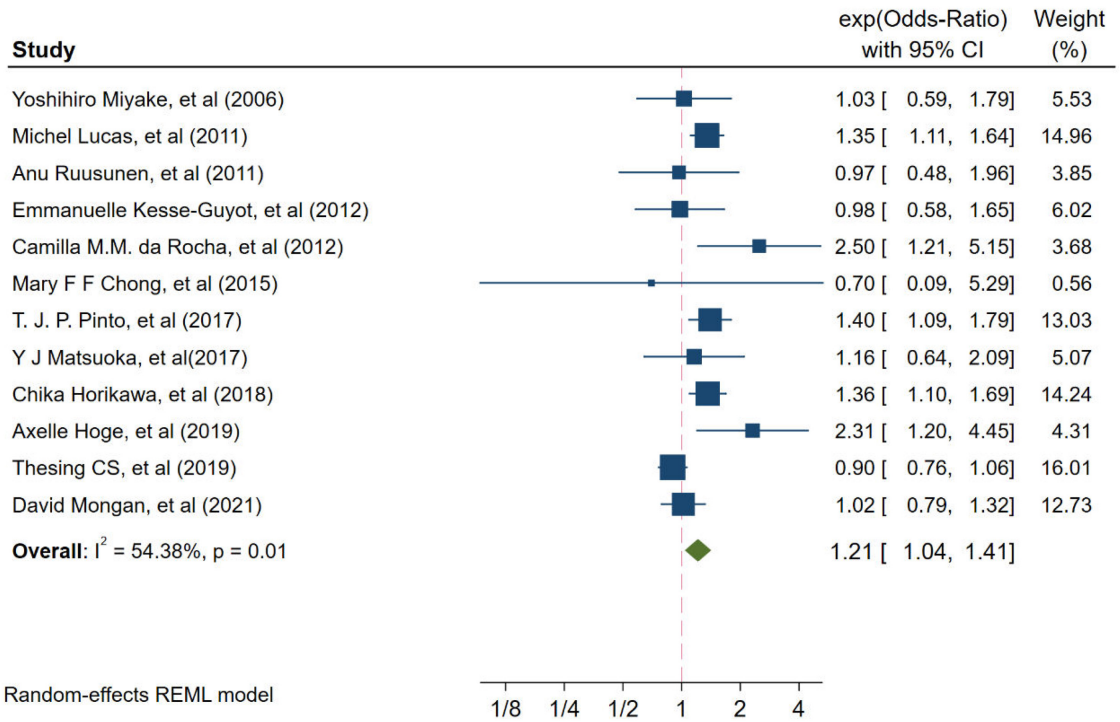


FIGURE 2 | Odds ratios of depression for highest vs. lowest category of ratio of n-6/n-3 PUFAs. Overall odds ratios calculated with random effects model.

(OR = 1.53, 95CI%: 1.10∼2.13, I^2 = 36.46%, P = 0.19), while it was not significant for high ratio of n-6/n-3 PUFAs among studies in healthy people (OR = 1.12, 95CI%: 0.94∼1.32). Additionally, high ratio of n-6/n-3 PUFAs was associated with depression in studies that adjusted for BMI. Besides, the significant positive association was observed in unadjusted model with education, smoke and drink compared with those studies with such adjustment, while there was no significant relationship between high ratio of n-6/n-3 PUFAs and depression in both unadjusted and adjusted model with energy and education.

Publication Bias

There was no significant evidence of publication bias as indicated by the results from Begg's test and Egger's test for the relationship between high ratio of n-6/n-3 PUFAs and depression (P_{Begg} = 0.451, P_{Egger} = 0.581).

DISCUSSION

A growing number of researchers recommend that the intake of n-6 PUFAs should be considered alongside n-3 PUFAs (31–33). The current conflicting findings on the relationship between high ratio of n-6/n-3 PUFAs and depression is not conducive to the development of strategies related to the treatment of depression. Therefore, the aim of this meta-analyses was to examine the relationship between high ratio of n-6/n-3 PUFAs and depression in the whole population.

In the final twelve cohort studies included, we concluded that a high ratio of n-6/n-3 PUFAs was indeed positively associated with depression. A study in Japan examining the relationship between n-3 unsaturated fatty acids and the tendency to depression in healthy people showed that 22.1% of people suffered from depressive conditions over an average follow-up of 8.1 years, and that high proportions of n-6/n-3 increased the risk of developing depressive symptoms (27). The results of the maternal population study also showed that a higher ratio of n-6/n-3 PUFAs was associated with a higher risk of depressive symptoms in the first year after delivery (29). Several potential mechanisms have been proposed regarding the association between high ratio of n-6/n-3 PUFAs, one of which is the inflammatory response (34). Depression has been associated with activation of the inflammatory response with this association being bidirectional (35, 36). For some depressed patients, inflammation promotes the onset of depression; meanwhile depression stimulates a greater cytokine response to stress (37). Therefore, depression can be alleviated by decreasing the inflammatory response. EPA and DHA are often considered to have anti-inflammatory effects and may promote the reduction of inflammation. Studies have shown that the anti-inflammatory effects of EPA and DHA were enhanced when the intake of AA was reduced. It indicates that the ratio of n-6/n-3 PUFAs seems to be more sensitive to the inflammatory response and thus to depression (38). A randomized controlled trial revealed that an increased ratio of n-6/n-3 PUFAs was associated with major depression and increased production of pro-inflammatory cytokines in students. This study found that lowering the n-6/n-3 PUFAs ratio resulted

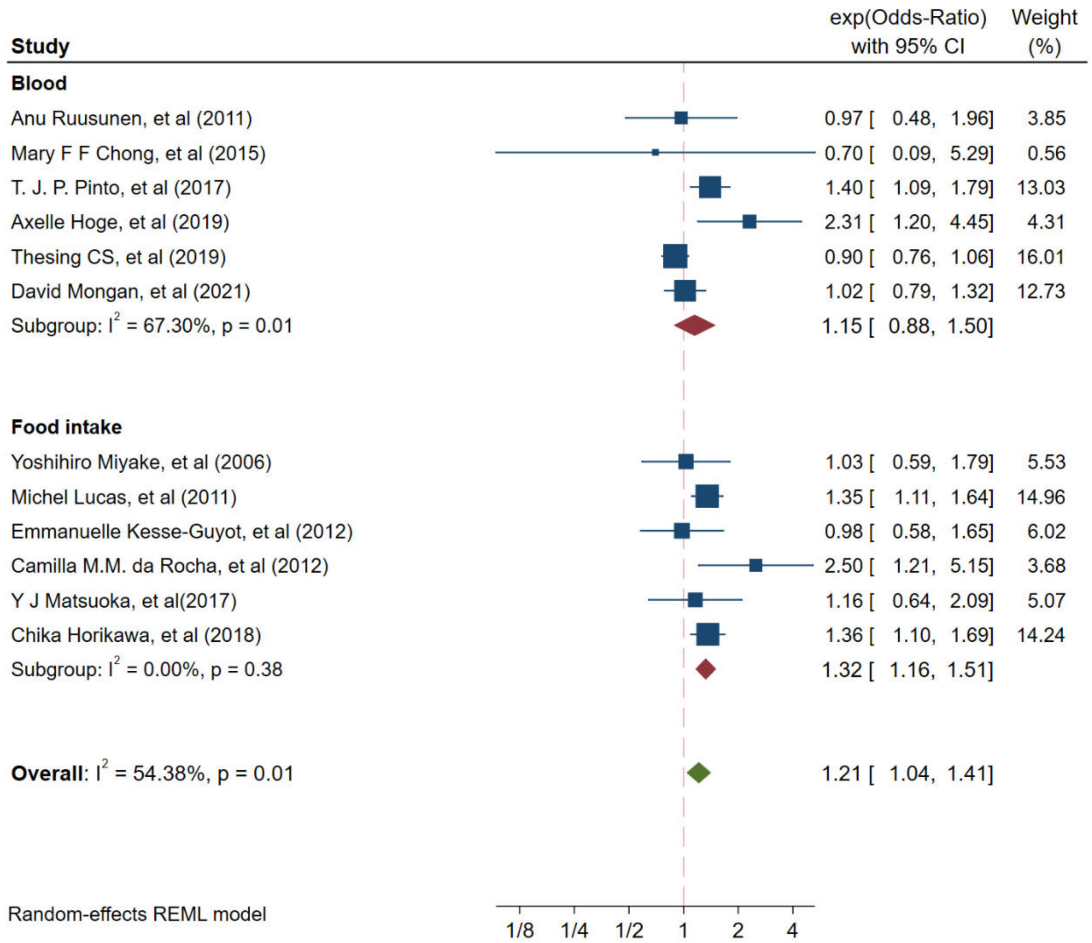


FIGURE 3 | Odds ratios of depression for highest vs. lowest category of ratio of n-6/n-3 PUFAs from different resources. Overall odds ratios calculated with random effects model.

in lower anxiety and stimulated reductions in IL-6 and tumor necrosis factor alpha (TNF-α) production, as well as small differences in serum TNF-α (39). However, the interactions regarding n-3 and n-6 fatty acids in the context of inflammation are complex and still need to be justified by a large number of studies. From the public health point of view, lowering the n-6/n-3 PUFAs ratio in the diet and maintaining the dynamic interactions between n-3 and n-6 PUFAs (PUFAs balance), which according to some studies are certainly better indicators of health effects than individual PUFA concentrations, are both relevant for depression prevention (40).

In subgroup analyses, we found that low ratio of dietary-derived n-6/n-3 PUFAs supplementation significantly reduced depression. In a cross-sectional study from Japan, a significant negative association was found between the low ratio of n-3/n-6 PUFAs in the dietary intake of overweight and obese women and depressive symptoms (41). Similar results were found in depression during pregnancy. A study showed that pregnant women with higher than recommended dietary intakes of total fatty acids and high ratio of n-6/n-3 were at higher risk of developing depressive symptoms (42). However, low ratio of blood-derived n-6/n-3 PUFAs in our study did not significantly reduce depression. Some studies have shown that a higher n-6/n-3 PUFAs ratio in the blood was positively associated with depression (21, 43–45). Depression and the n-6/n-3 PUFAs ratio acted together to enhance pro-inflammatory cytokines beyond the contribution provided by either variable alone, and as depressive symptoms increased, higher ratio of n-6/n-3 PUFAs was associated with progressively higher levels of TNF-α and IL-6 (44). The potential reason for this was that the small sample size of ratio of n-6/n-3 PUFAs derived from blood produced higher heterogeneity. Besides, we should consider that dietary intake of PUFAs may not fully reflect the amount of fatty acids in the blood. A prospective study showed that PUFAs determined at baseline in red blood cells and in the diet were differentially associated with cognitive function and cognitive impairment (46). A study from the United States also noted that both plasma EPA and DHA concentrations were significantly predicted by dietary intake of these fatty acids. However, plasma docosapentaenoic acid (DPA) levels were not related to dietary intake of DPA (47). Therefore, our next study sought to explore the relationship between the ratio of n-6/n-3 PUFAs in the diet and blood, clarify

TABLE 3 | Subgroup analyses of high ratios of n-6/n-3 PUFAs and risk of depression (highest vs. lowest category).

Subgroup	No. of studies	Odds risk (95% CI)	I^2%	*P* value
Regions:				
America	4	1.44 (1.24∼1.67)	0.00	0.19
Asia	3	1.29 (1.07∼1.56)	0.00	0.72
Europe	4	0.94 (0.82∼1.07)	0.00	0.88
Population:				
Pregnant women	5	1.53 (1.10∼2.13)	36.46	0.19
Healthy people	6	1.12 (0.94∼1.32)	56.53	0.03
Covariate method:				
Adjustment for age	8	1.15 (0.99∼1.33)	53.18	0.03
No adjustment for age	3	2.24 (1.40∼3.59)	0.00	0.50
Adjustment for BMI	7	1.20 (1.02∼1.40)	22.39	0.45
No adjustment for BMI	4	1.39 (0.96∼2.01)	82.56	0.00
Adjustment for energy	2	1.26 (0.97∼1.65)	22.58	0.26
No adjustment for energy	9	1.22 (1.01∼1.47)	59.93	0.01
Adjustment for education	4	1.07 (0.84∼1.36)	61.91	0.03
No adjustment for education	7	1.32 (1.11∼1.57)	32.47	0.13
Adjustment for smoking	7	1.11 (0.95∼1.30)	51.46	0.05
No adjustment for smoking	4	1.72 (1.18∼2.52)	34.37	0.23
Adjustment for drinking	4	1.07 (0.86∼1.35)	53.31	0.06
No adjustment for drinking	7	1.33 (1.10∼1.63)	43.59	0.09
Risk expression				
Hazard/rate ratio	2	1.10 (0.73∼1.65)	88.73	0.00
Odds ratio	7	1.27 (1.02∼1.58)	38.82	0.11
Relative risk	2	1.32 (1.09∼1.59)	0.00	0.37
Quality assessment				
High	6	1.17 (0.99∼1.37)	58.12	0.02
Moderate	5	1.49 (0.91∼2.43)	61.58	0.05

the underlying mechanisms of this relationship, and figure out the relationship between the ratio of n-6/n-3 PUFAs in the diet and blood and depression.

STRENGTHS AND LIMITATIONS

Current research findings on the relationship between high ratio of n-6/n-3 PUFAs and depression is conflicting, which will influence the formulation of policies related to the prevention and treatment of depression. To our knowledge, this is the first study that focuses on the relationship between high ratio of n-6/n-3 PUFAs and depression. This study included a high-quality grade of cohort studies from a variety of countries, so there is a high degree of confidence in the pooled results. In addition, we conducted a series of subgroup and meta-regression analyses to explore sources of heterogeneity and thus improve the accuracy of the results for the studies of interest. The robustness of our results was supported by sensitivity analyses. There was no significant publication bias in our study through the use of the Begg's test and the Egger's test.

However, there are some limitations in the meta-analysis. Firstly, different assessment methods were used in the included studies, mainly derived from fatty acids in dietary intake and blood, but this was resolved by subgroup analysis. Secondly, language bias may have arisen as we excluded articles that were not in English. However, we selected articles covering most of non-English speaking Europe and Asia, with a limited number of cohort studies from other countries. Finally, n-3 and n-6 unsaturated fatty acids were also calculated differently in different studies; for example, some studies included EPA and DHA content as total n-3 unsaturated fatty acid content, which undoubtedly biased the results. Therefore, future studies should be more standardized in their calculation of fatty acids, and it is hoped that large, high-quality, long-term randomized controlled trials will also be conducted to provide more reliable clinical evidence.

CONCLUSION

This study had significant public health implications. Our meta-analysis found a positive association between high ratio of n-6/n-3 PUFAs and depression and this positive association was only present in high ratio of n-6/n-3 PUFAs in dietary supplementation but not in blood. This study suggests that lowering the dietary intake of the ratio of n-6/n-3 PUFAs would be beneficial in the prevention of depression.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

YW designed the study and wrote the manuscript. YW and LD searched and reviewed the relevant trials and collected the data. DP and DX played a role as a consultant. YL and SY helped employ search strategies. HX and WL performed statistical analysis. SW was responsible for the quality assessments for the studies. GS was the corresponding author. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the National Natural Science Foundation of China (No. 81872618) and CNS Research Fund for DRI.

ACKNOWLEDGMENTS

The authors thank all participants, researchers and collaborators included in this meta-analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.889576/full#supplementary-material

REFERENCES

1. World Health Organization. (2022). Available online at: https://www.who.int/health-topics/depression#tab=tab_1 (accessed February 10, 2022).
2. Meng R, Yu C, Liu N, He M, Lv J, Guo Y, et al. Association of depression with all-cause and cardiovascular disease mortality among adults in China. *JAMA Netw Open.* (2020) 3:e1921043. doi: 10.1001/jamanetworkopen.2019.21043
3. Zhang K, Wang X, Tu J, Rong H, Werz O, Chen X. The interplay between depression and tuberculosis. *J Leukoc Biol.* (2019) 106:749–57. doi: 10.1002/JLB.MR0119-023R
4. Park LT, Zarate CA Jr. Depression in the primary care setting. *N Engl J Med.* (2019) 380:559–68. doi: 10.1056/NEJMcp1712493
5. Smith K. Mental health: a world of depression. *Nature.* (2014) 515:181. doi: 10.1038/515180a
6. Breslow AS, Tran NM, Lu FQ, Alpert JE, Cook BL. Depression treatment expenditures for adults in the USA: a systematic review. *Curr Psychiatry Rep.* (2019) 21:105. doi: 10.1007/s11920-019-1083-3
7. Gbd 2016 DALYs and Hale Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet.* (2017) 390:1260–344. doi: 10.1016/S0140-6736(17)32130-X
8. McCarron RM, Shapiro B, Rawles J, Luo J. Depression. *Ann Intern Med.* (2021) 174:Itc65–80. doi: 10.7326/AITC202105180
9. Lu J, Xu X, Huang Y, Li T, Ma C, Xu G, et al. Prevalence of depressive disorders and treatment in China: a cross-sectional epidemiological study. *Lancet Psychiatry.* (2021) 8:981–90. doi: 10.1016/S2215-0366(21)00251-0
10. Taquet M, Holmes EA, Harrison PJ. Depression and anxiety disorders during the COVID-19 pandemic: knowns and unknowns. *Lancet.* (2021) 398:1665–6. doi: 10.1016/S0140-6736(21)02221-2
11. Covid-19 Mental Disorders Collaborators. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *Lancet.* (2021) 398:1700–12. doi: 10.1016/S0140-6736(21)02143-7
12. Ljungberg T, Bondza E, Lethin C. Evidence of the importance of dietary habits regarding depressive symptoms and depression. *Int J Environ Res Public Health.* (2020) 17:1616. doi: 10.3390/ijerph17051616
13. Quirk SE, Williams LJ, O'Neil A, Pasco JA, Jacka FN, Housden S, et al. The association between diet quality, dietary patterns and depression in adults: a systematic review. *BMC Psychiatry.* (2013) 13:175. doi: 10.1186/1471-244X-13-175
14. Lassale C, Batty GD, Baghdadli A, Jacka F, Sánchez-Villegas A, Kivimäki M, et al. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. *Mol Psychiatry.* (2019) 24:965–86. doi: 10.1038/s41380-018-0237-8
15. Parletta N, Zarnowiecki D, Cho J, Wilson A, Bogomolova S, Villani A, et al. A mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: a randomized controlled trial (HELFIMED). *Nutr Neurosci.* (2019) 22:474–87. doi: 10.1080/1028415X.2017.1411320
16. Shahidi F, Ambigaipalan P. Omega-3 and their health benefits. *Annu Rev Food Sci Technol.* (2018) 9:345–81. doi: 10.1146/annurev-food-111317-095850
17. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. *Prog Lipid Res.* (2008) 47:147–55. doi: 10.1016/j.plipres.2007.12.004
18. Kesse-Guyot E, Touvier M, Andreeva VA, Jeandel C, Ferry M, Hercberg S, et al. Cross-sectional but not longitudinal association between n-3 fatty acid intake and depressive symptoms: results from the SU.VI.MAX 2 study. *Am J Epidemiol.* (2012) 175:979–87. doi: 10.1093/aje/kwr472
19. Lucas M, Mirzaei F, O'Reilly EJ, Pan A, Willett WC, Kawachi I, et al. Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: a 10-y prospective follow-up study. *Am J Clin Nutr.* (2011) 93:1337–43. doi: 10.3945/ajcn.111.011817
20. Da Rocha CM, Kac G. High dietary ratio of omega-6 to omega-3 polyunsaturated acids during pregnancy and prevalence of post-partum depression. *Matern Child Nutr.* (2012) 8:36–48. doi: 10.1111/j.1740-8709.2010.00256.x
21. Lin PY, Chang CH, Chong MF, Chen H, Su KP. Polyunsaturated fatty acids in perinatal depression: a systematic review and meta-analysis. *Biol Psychiatry.* (2017) 82:560–9. doi: 10.1016/j.biopsych.2017.02.1182
22. Ruusunen A, Virtanen JK, Lehto SM, Tolmunen T, Kauhanen J, Voutilainen S. Serum polyunsaturated fatty acids are not associated with the risk of severe depression in middle-aged Finnish men: Kuopio ischaemic heart disease risk factor (KIHD) study. *Eur J Nutr.* (2011) 50:89–96. doi: 10.1007/s00394-010-0118-7
23. Pinto TJ, Vilela AA, Farias DR, Lepsch J, Cunha GM, Vaz JS, et al. Serum n-3 polyunsaturated fatty acids are inversely associated with longitudinal changes in depressive symptoms during pregnancy. *Epidemiol Psychiatr Sci.* (2017) 26:157–68. doi: 10.1017/S204579601500116X
24. Thesing CS, Lok A, Milaneschi Y, Assies J, Bockting CLH, Figueroa CA, et al. Fatty acids and recurrence of major depressive disorder: combined analysis of two Dutch clinical cohorts. *Acta Psychiatr Scand.* (2020) 141:362–73. doi: 10.1111/acps.13136
25. Miyake Y, Sasaki S, Yokoyama T, Tanaka K, Ohya Y, Fukushima W, et al. Risk of postpartum depression in relation to dietary fish and fat intake in Japan: the Osaka maternal and child health study. *Psychol Med.* (2006) 36:1727–35. doi: 10.1017/S0033291706008701
26. Chong MF, Ong YL, Calder PC, Colega M, Wong JX, Tan CS, et al. Long-chain polyunsaturated fatty acid status during pregnancy and maternal mental health in pregnancy and the postpartum period: results from the GUSTO study. *J Clin Psychiatry.* (2015) 76:e848–56. doi: 10.4088/JCP.14m09191
27. Horikawa C, Otsuka R, Kato Y, Nishita Y, Tange C, Rogi T, et al. Longitudinal association between n-3 long-chain polyunsaturated fatty acid intake and depressive symptoms: a population-based cohort study in Japan. *Nutrients.* (2018) 10:1655. doi: 10.3390/nu10111655
28. Matsuoka YJ, Sawada N, Mimura M, Shikimoto R, Nozaki S, Hamazaki K, et al. Dietary fish, n-3 polyunsaturated fatty acid consumption, and depression risk in Japan: a population-based prospective cohort study. *Transl Psychiatry.* (2017) 7:e1242. doi: 10.1038/tp.2017.206
29. Hoge A, Tabar V, Donneau AF, Dardenne N, Degée S, Timmermans M, et al. Imbalance between omega-6 and Omega-3 polyunsaturated fatty acids in early pregnancy is predictive of postpartum depression in a belgian cohort. *Nutrients.* (2019) 11:876. doi: 10.3390/nu11040876
30. Mongan D, Healy C, Jones HJ, Zammit S, Cannon M, Cotter DR. Plasma polyunsaturated fatty acids and mental disorders in adolescence and early adulthood: cross-sectional and longitudinal associations in a general population cohort. *Transl Psychiatry.* (2021) 11:321. doi: 10.1038/s41398-021-01425-4
31. Lands B. Historical perspectives on the impact of n-3 and n-6 nutrients on health. *Prog Lipid Res.* (2014) 55:17–29. doi: 10.1016/j.plipres.2014.04.002
32. Li N, Yue H, Jia M, Liu W, Qiu B, Hou H, et al. Effect of low-ratio n-6/n-3 PUFA on blood glucose: a meta-analysis. *Food Funct.* (2019) 10:4557–65. doi: 10.1039/c9fo00323a
33. Wei Y, Meng Y, Li N, Wang Q, Chen L. The effects of low-ratio n-6/n-3 PUFA on biomarkers of inflammation: a systematic review and meta-analysis. *Food Funct.* (2021) 12:30–40. doi: 10.1039/d0fo01976c
34. Husted KS, Bouzinova EV. The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. *Medicina (Kaunas).* (2016) 52:139–47. doi: 10.1016/j.medici.2016.05.003
35. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med.* (2013) 11:200. doi: 10.1186/1741-7015-11-200
36. Beurel E, Toups M, Nemeroff CB. The bidirectional relationship of depression and inflammation: double trouble. *Neuron.* (2020) 107:234–56. doi: 10.1016/j.neuron.2020.06.002
37. Kiecolt-Glaser JK, Derry HM, Fagundes CP. Inflammation: depression fans the flames and feasts on the heat. *Am J Psychiatry.* (2015) 172:1075–91. doi: 10.1176/appi.ajp.2015.15020152
38. Innes JK, Calder PC. Omega-6 fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids.* (2018) 132:41–8. doi: 10.1016/j.plefa.2018.03.004
39. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. *Brain Behav Immun.* (2011) 25:1725–34. doi: 10.1016/j.bbi.2011.07.229

40. Lands B, Bibus D, Stark KD. Dynamic interactions of n-3 and n-6 fatty acid nutrients. *Prostaglandins Leukot Essent Fatty Acids.* (2018) 136:15–21. doi: 10.1016/j.plefa.2017.01.012
41. Tsujiguchi H, Thi Thu Nguyen T, Goto D, Miyagi S, Kambayashi Y, Hara A, et al. Relationship between the intake of n-3 polyunsaturated fatty acids and depressive symptoms in elderly Japanese people: differences according to sex and weight status. *Nutrients.* (2019) 11:775. doi: 10.3390/nu11040775
42. Sousa TM, Santos LCD. Dietary fatty acids, omega-6/omega-3 ratio and cholesterol intake associated with depressive symptoms in low-risk pregnancy. *Nutr Neurosci.* (2022) 25:642–7. doi: 10.1080/1028415X.2020.1792618
43. Kiecolt-Glaser JK, Belury MA, Porter K, Beversdorf DQ, Lemeshow S, Glaser R. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. *Psychosom Med.* (2007) 69:217–24. doi: 10.1097/PSY.0b013e3180313a45
44. Horikawa C, Otsuka R, Kato Y, Nishita Y, Tange C, Kakutani S, et al. Cross-sectional association between serum concentrations of n-3 long-chain PUFA and depressive symptoms: results in Japanese community dwellers. *Br J Nutr.* (2016) 115:672–80. doi: 10.1017/S0007114515004754
45. Mamalakis G, Jansen E, Cremers H, Kiriakakis M, Tsibinos G, Kafatos A. Depression and adipose and serum cholesteryl ester polyunsaturated fatty acids in the survivors of the seven countries study population of crete. *Eur J Clin Nutr.* (2006) 60:1016–23. doi: 10.1038/sj.ejcn.1602413
46. Bigornia SJ, Scott TM, Harris WS, Tucker KL. Prospective associations of erythrocyte composition and dietary intake of n-3 and n-6 PUFA with measures of cognitive function. *Nutrients.* (2018) 10:1253. doi: 10.3390/nu10091253
47. Richter CK, Bisselou KS, Nordgren TM, Smith L, Appiah AK, Hein N, et al. n-3 Docosapentaenoic acid intake and relationship with plasma long-chain n-3 fatty acid concentrations in the United States: NHANES 2003-2014. *Lipids.* (2019) 54:221–30. doi: 10.1002/lipd.12146

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.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 07 July 2022
doi: 10.3389/fnut.2022.948604

Association of Dietary Intake and Biomarker of α-Linolenic Acid With Incident Colorectal Cancer: A Dose-Response Meta-Analysis of Prospective Cohort Studies

Ze-Bin Dai[1,2†], Xiao-Li Ren[2,3†], Yi-Lang Xue[1], Ya Tian[2], Bing-Bing He[1], Chang-Long Xu[1] and Bo Yang[2,3*]*

[1] The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China, [2] Institute of Lipids Medicine, Wenzhou Medical University, Wenzhou, China, [3] Department of Preventive Medicine, School of Public Health and Management, Wenzhou Medical University, Wenzhou, China

OPEN ACCESS

Edited by:
Li Cai,
Sun Yat-sen University, China

Reviewed by:
Shanshan Bian,
Second Hospital of Tianjin Medical University, China
Yuandi Xi,
Capital Medical University, China

***Correspondence:**
Bo Yang
yb@wmu.edu.cn
Chang-Long Xu
xchlong@163.com

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

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: 17 February 2022
Accepted: 14 June 2022
Published: 07 July 2022

Citation:
Dai Z-B, Ren X-L, Xue Y-L, Tian Y, He B-B, Xu C-L and Yang B (2022) Association of Dietary Intake and Biomarker of α-Linolenic Acid With Incident Colorectal Cancer: A Dose-Response Meta-Analysis of Prospective Cohort Studies. Front. Nutr. 9:948604. doi: 10.3389/fnut.2022.948604

Background and Objective: There is keen interest in better understanding the impacts of alpha-linolenic acid (ALA), a plant-derived n-3 fatty acid, in ameliorating the development of cancer; however, results of several prospective cohorts present an inconsistent association between ALA intake and the incident colorectal cancer (CRC). We aimed to investigate the summary association of dietary intake and biomarkers of ALA with CRC risk based on the prospective cohorts.

Methods: Pertinent prospective cohorts were identified in Cochrane Library, PubMed, and EMBASE from inception to February 2022. Study-specific risk ratios (RRs) with 95% confidence intervals (CIs) for comparing the top with the bottom quartiles of ALA levels were combined using a random-effects model. Nonlinear dose-response relationships of ALA levels in diet and blood with CRC risk were assessed using the restricted cubic spline models, respectively.

Results: Over the duration of follow-up with a median of 9.3 years ranging from 1 to 28 years, 12,239 CRC cases occurred among 861,725 participants from 15 cohorts (11 studies on diet and 5 studies on biomarkers including 4 on blood and 1 on adipose tissue). The summary RR was 1.03 (95% CI: 0.97, 1.10; I^2: 0.00%) for dietary intake and 0.83 (95% CI: 0.69, 0.99; I^2: 0.00%) for biomarker. Each 0.1% increase in the levels of ALA in blood was associated with a 10% reduction in risk of CRC (summary RR: 0.90, 95% CI: 0.80, 0.99; I^2: 38.60%), whereas no significant dose-response association was found between dietary intake of ALA and the incident CRC (p for non-linearity = 0.18; p for linearity = 0.24).

Conclusions: Blood levels of ALA were inversely and linearly associated with the risk of CRC, which suggested that increased intake of ALA to improve circulating levels was beneficial for CRC prevention.

Keywords: omega-3 fatty acids, colorectal cancer, meta-analysis, biomarker, linolenic acid

INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer diagnosed in women and the third most in men, and currently ranks as the fourth most deadly cancer worldwide with nearly 900,000 deaths annually (1). The incidence of colorectal cancer worldwide was predicted to be 2.5 million new cases in 2035 (2). As a result, the primary prevention of CRC has always been an important public health priority.

Dietary factors have been shown to play an important role in the prevention of CRC (3). At cellular and animal model levels, dietary n-3 polyunsaturated fatty acids (PUFAs) were proved to be implicated in the several biological mechanisms underlying the antineoplastic effects of alpha-linolenic acid (ALA, 18:3n-3), including suppression of nuclear factor-κB (NF-κB), activation of AMPK/SIRT1, modulation of cyclooxygenase activity, and upregulation of the novel anti-inflammatory lipid mediators identified recently such as protectins, maresins, and resolvins (3, 4).

Alpha-linolenic acid, as a plant-based member of n-3 PUFAs, can be derived from vegetable oils (5). Population-based epidemiological studies have reported the protective effect of ALA on obesity-related diseases such as diabetes and cardiovascular disease (6–8). Nevertheless, the associations with CRC risk were found to be inconsistent in several prior cohorts using food ALA as interest exposure, and two previous meta-analyses reported a null association estimation (9, 10). Given the possibility of a measurement error or report bias in most of the observational cohorts using dietary questionnaires to estimate ALA intake, it was difficult to accurately assess the real intake of individual fatty acids (11). Moreover, there may be a disturbance of gut microbiota in the individuals vulnerable to CRC, which might have resulted in an overestimation of the exact level of ALA *in vivo* (12, 13).

In contrast to dietary questionnaires, biomarker measurements provide objective assessments of ALA exposure in diet, which reflect both on the biologically relevant process and dietary consumption, as well as are free of memory errors, recall bias, or inaccuracies in food databases (14). One prior meta-analysis included three cohorts only to conclude a null association with blood levels of ALA (15), which may have been influenced by the limited number of eligible studies. So far, the relationships between ALA biomarkers and CRC risk remain unclear, as various prospective cohorts reported inconsistent results (16–18). One study found that the levels of ALA in adipose tissue (AT) had an inverse association with the incident CRC (16), whereas the other studies showed that circulating ALA was inversely associated with colon cancer but not rectum cancer (17) and had a null association with CRC risk (18).

To further address the role of the plant-based n-3 fatty acid in preventing the development of CRC, we conducted a meta-analysis to summarize the updated evidence on the relationship between ALA intake and the incident CRC. The novelty of the present study was to quantitatively evaluate a dose-response association of ALA levels in the diet and human biospecimens (blood and AT) with CRC risk using the available data from the more comprehensive perspective studies.

MATERIALS AND METHODS

Literature Search

We identified 20,195 potential studies from PubMed, EMBASE, and Cochrane Library databases up through Feb 2022, and the search strategy we have predefined was listed in the literature searching section of **Supplementary Materials**. We also searched for the published meta-analyses from the above-mentioned databases and checked their reference lists to identify the relevant publications that might have been missed. The present study was conducted and reported following the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (**Supplementary Table 2**) (19).

Eligibility Criteria

To assess the association of ALA in diet and human tissues and the risk of CRC, the inclusion criteria were: (1) Participants: Adults of any age across different countries; (2) Exposure: levels of ALA intake estimated by dietary records, food frequency questionnaires, or quantitative determining the compositions or concentrations of ALA in circulating blood and adipose tissue (AT); (3) Outcomes: Evaluating the incident CRC as an endpoint, presented as multivariate-adjusted risk ratio (RR) or hazard ration (HR) with 95% confidence interval (CI); (4) Study design: Prospective cohort study, nested case-control study, and case-cohort study.

Data Extraction

The following data were extracted by the two independent reviewers from each original study using a standardized extraction form: first author, publication year, study design (prospective cohort/nested case-control/case cohort), study location (America/Europe/Asia), cohort name, sample size (number of cases/participants), baseline age (median value, year), gender, duration of follow-up (median value, year), cancer location (colon/rectum), exposure measurements, types of interest exposure (diet or biomarker), multivariate-adjusted RRs (HRs) with 95% CI for all category levels of ALA in diet or human tissues, and the potential confounders adjusted. The study quality of each included study was evaluated by using the 9-stars Newcastle-Ottawa Scale (NOS) (20) (**Supplementary Table 3**).

Data Synthesis

If an original study provided HRs with 95% CIs for the incident CRC, the HR value was assumed to approximate the RR value. All the included studies provided RR (HR) for ALA intake (diet or biomarker) based on various categories (e.g., tertiles, quartiles, or quintiles) or per SD difference in exposure. To achieve a consistent approach to the present meta-analysis, the RRs (HRs) were first transformed to involve comparisons between the top and the bottom quartiles of baseline diet or biomarker of ALA using methods described previously (21, 22). In brief, log risk estimates were transformed with the comparison between the top and bottom quartiles being equivalent to 2.54 times the logRRs for per 1-SD increase. These scaling methods assume that the exposure is normally distributed and the association with the risk of CRC is log-linear. The conversion factor of 2.54 is the difference in the medians of the top and bottom quartiles of

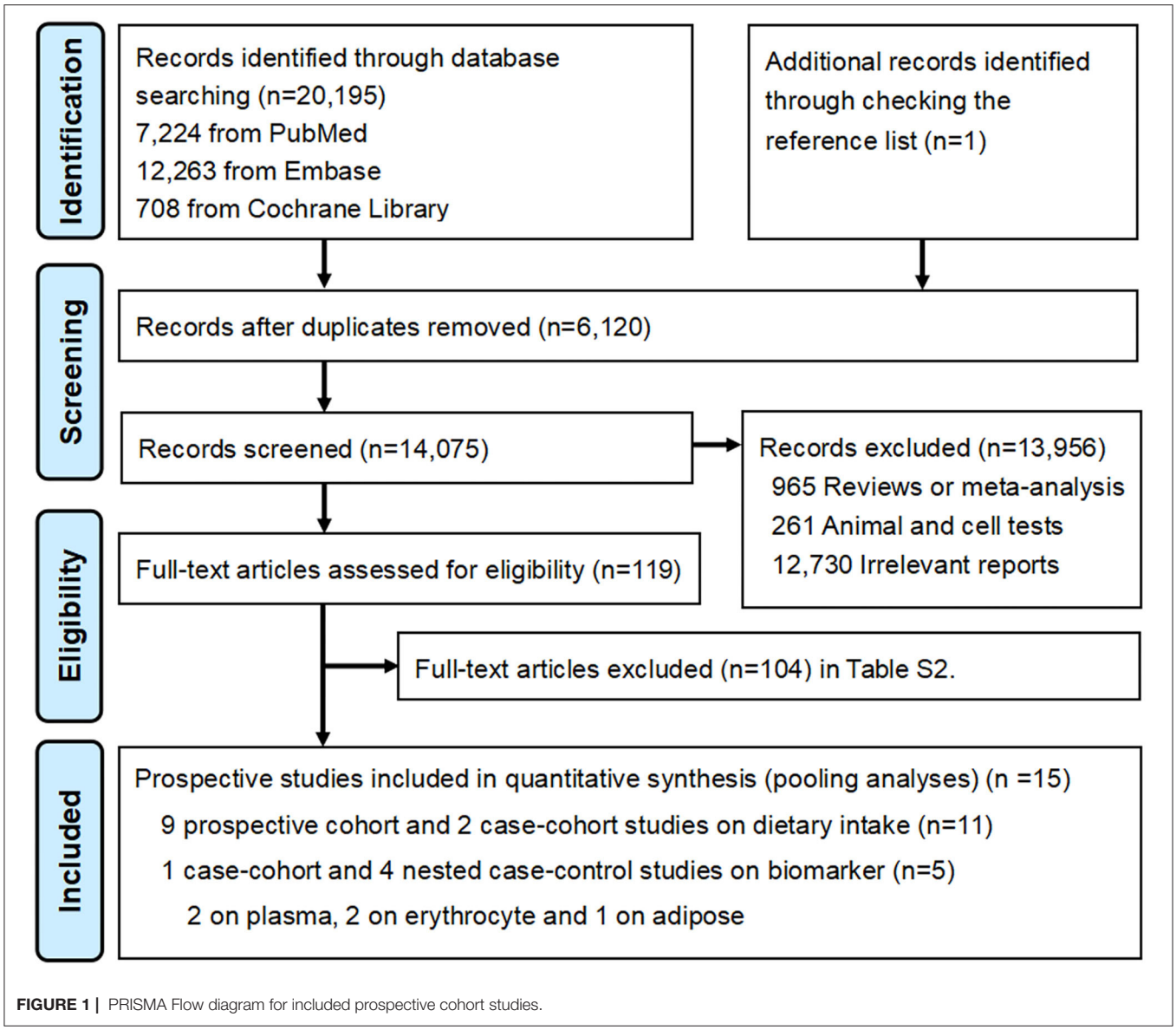


FIGURE 1 | PRISMA Flow diagram for included prospective cohort studies.

the standard normal distribution; other conversions were used for differences in medians of extreme tertiles (2.18) or quintiles (2.80). The standard errors (SEs) of log RRs were calculated using reported data on precision and were similarly standardized.

Statistical Analysis

Multivariate-adjusted RRs (HRs) comparing the top with the bottom quartiles of ALA intake (diet and biomarker) in each study were first transformed to their logarithm (logRRs), and their corresponding 95% CIs were used to calculate the standard errors (selogRRs). Summary RRs (SRRs) with 95% CIs as the overall risk estimate for the top vs. bottom quartiles of ALA intake was calculated using a random-effects model described by DerSimonian and Laird (23), which considers both within-study and between-study variability. Heterogeneity across studies was evaluated with the Q test and I^2 statistic (24). We defined an I^2 value >50% as indicative of heterogeneity according to Cochrane Handbook. Stratified analysis was performed to identify the possible sources of heterogeneity based on living region (America/Europe vs. Asia), baseline age ($<$ 60 vs. $\geq$ 60, yr), gender (man vs. women), median duration of follow-up ($\leq$ 9.3 vs. $>$ 9.3, yr), cancer location (colon vs. rectum), quality scores (7 vs. 8–9), study design (prospective cohort vs. nested case-control/case-cohort), biomarker types (adipose vs. blood), and multiple adjustments (yes vs. no). A univariate meta-regression with restricted maximum likelihood was performed to measure if summary RR significantly differed between each stratum analyzed. Sensitivity analyses were performed to evaluate the possible influence of individual studies on the summary results. A possibility of publication bias was qualitatively

TABLE 1 | Baseline characteristics of the individual prospective cohort studies.

First author, published year	Location (cohort name)	Design	Cases/ Participants	Age (median, yr), gender	Follow-up (median, yr)	Exposure of interest		Outcomes		QS
						Measurement	Exposure range (top vs. bottom)	Endpoints	RR (95% CI)	
Pietinen et al. (30)	America (ATBCS)	PC	185/27,111	57.1, Male	8.0	Diet (FFQ)	Median of top quartile range vs. bottom in subjects: 2.4 vs. 1.0, g/day	CRC	1.40 (0.90, 2.10)	9
Terry et al. (31)	Europe	PC	460/61,463	52.0, Female	9.6	Diet (FFQ)	Median of top quartile range vs. bottom in subjects: 0.70 vs. 0.45, g/day	CRC	0.99 (0.75, 1.32)	8
								CC	0.90 (0.63, 1.28)	
								RC	1.11 (0.70, 1.78)	
Brink et al. (32)	Europe (NLCS)	CH	608/120,852	61.3, Both	4.4	Diet (FFQ)	Median of top quartile range vs. bottom in subjects: 1.8 vs. 0.70, g/day	CC	1.01 (0.75, 1.36)	9
								RC	0.91 (0.58, 1.44)	
Daniel et al. (33)	America (CPS-II)	PC	452/43,108	70.3, Male	6.0	Diet (FFQ)	Range of top quartile vs. bottom in subjects: ≥1.26 vs. <0.82, g/day	CRC	0.87 (0.66, 1.04)	9
		PC	417/55,972	68.5, Female	6.0	Diet (FFQ)	Range of top quartile vs. bottom in subjects: ≥1.19 vs. <0.78, g/day	CRC	1.38 (1.02, 1.85)	
Murff et al. (34)	Asia (SWHS)	PC	396/73,242	52.5, Female	9.0	Diet (FFQ)	Median of top quintile range vs. bottom in subjects: 1.44 vs. 0.58, g/day	CRC	1.16 (0.66, 2.06)	9
								CC	1.40 (0.58, 3.37)	
								RC	0.64 (0.22, 1.89)	
Sasazuki et al. (35)	Asia (JPHCS)	PC	774/41,382	56.9, Male	9.3	Diet (FFQ)	Median of top quintile range vs. bottom in subjects: 2.76 vs. 1.21, g/day	CC	0.84 (0.56, 1.28)	9
								RC	1.10 (0.61, 1.98)	
		PC	494/47,192	57.4, Female	9.3	Diet (FFQ)	Median of top quintile range vs. bottom in subjects: 2.64 vs. 1.35, g/day	CC	1.01 (0.65, 1.57)	
								RC	1.02 (0.50, 2.06)	
Song et al. (36)	America (NHS & HPFS)	PC	987/47,143	53.9, Male	20.6	Diet (FFQ)	Range of top quartile vs. bottom in subjects: ≥1.30 vs. <0.90, g/day	CRC	0.89 (0.70, 1.13)	7
								CC	0.96 (0.72, 1.30)	
								RC	0.68 (0.41, 1.15)	
		PC	1,469/76,386	50.4, Female	23.8	Diet (FFQ)	Range of top quartile vs. bottom in subjects: ≥1.20 vs. <0.90, g/day	CRC	1.05 (0.86, 1.29)	
								CC	1.09 (0.87, 1.37)	
								RC	0.84 (0.52, 1.37)	
Hodge et al. (37)	Europe (MCCS)	CH	395/41,514	58.5, Both	9.0	Diet (FFQ)	Range of top quintile vs. bottom in subjects: ≥1.13 vs. <0.66, g/day	CRC	1.09 (0.77, 1.53)	8
		CH	395/41,514	58.5, Both	9.0	Plasma (GLC)	Range of top quintile vs. bottom in subjects: ≥0.21 vs. <0.10, %	CRC	0.96 (0.69, 1.33)	

(Continued)

TABLE 1 | Continued

First author, published year	Location (cohort name)	Design	Cases/ Participants	Age (median, yr), gender	Follow-up (median, yr)	Exposure of interest		Outcomes		QS
						Measurement	Exposure range (top vs. bottom)	Endpoints	RR (95% CI)	
Shin et al. (38)	Europe (WLH Cohort)	PC	344/48,233	39.7, Female	21.3	Diet (FFQ)	Range of top quartile vs. bottom in subjects: 1.16-4.47 vs. 0.12-0.84, g/day	CRC	1.17 (0.86, 1.59)	9
								CC	0.96 (0.65, 1.41)	
								RC	1.61 (0.98, 2.69)	
Nguyen et al. (10)	Asia (SMHS)	PC	876/59,986	55.1, Male	9.8	Diet (FFQ)	Not available data	CRC	1.15 (0.92, 1.43)	9
								CC	0.98 (0.74, 1.31)	
								RC	1.45 (1.03, 2.05)	
Wan et al. (39)	America (NHS & HPFS)	PC	2,726/ 111,234	53.2, Both	24.3	Diet (FFQ)	Top quintile vs. bottom in subjects: not available	CRC	1.01 (0.90, 1.15)	7
Kojima et al. (16)	Asia (JACC Study)	NCC	83/324	60.5, Male	7.1	Adipose (GLC)	Range of top quartiles vs. bottom in subjects: >1.07 vs. <0.69, %	CRC	0.39 (0.16, 0.91)	9
			86/326	62.4, Female	7.1	Adipose (GLC)	Range of top quartile vs. bottom in subjects: >1.10 vs. <0.71, %	CRC	2.16 (0.87, 5.47)	
Cottet et al. (18)	Europe (E3N)	NCC	328/947	57.5, Female	9.0	Erythrocyte (GLC)	Range of top tertiles vs. bottom in subjects: >0.12 vs. <0.10, %	CRC	0.71 (0.49, 1.03)	8
Butler et al. (17)	Asia (SCHS)	NCC	350/700	59.7, Both	3.3	Plasma (GC-MS)	Range of top quartile vs. bottom in subjects: >3.8 vs. <1.9, umol/L	CC	0.41 (0.23, 0.73)	9
								RC	1.70 (0.84, 3.43)	
Wang et al. (40)	America (NHS & HPFS)	NCC	809/4,610	57.1, Both	20.0	Erythrocyte (GLC)	Per 1-SD change in subjects: 0.06, %	CRC	0.94 (0.88, 1.00)	9
								CC	0.94 (0.87, 1.02)	
								RC	0.94 (0.83, 1.06)	

PC, prospective cohort; NCC, nested case-control; CH, case cohort; CRC, colorectal cancer; CC, colon cancer; RC, rectal cancer; RR, risk ratio; CI, confidence interval; SD, standard deviation; FFQ, food frequency questionnaire; GLC, gas-liquid chromatography; GC-MS, gas chromatography-mass spectrometry; QS, quality scores; ATBCS, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; NCLS, The Netherlands Cohort Study; CPS, Cancer Prevention Study; SWHS, Shanghai Women's Health Study; JPHCS, Japan Public Health Center (JPHC)-Based Prospective Study; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; MCCS, Melbourne Collaborative Cohort Study; WLH, Swedish Women's Lifestyle and Health; SMHS, Shanghai Men's Health Study; JACC, Japan Collaborative Cohort; E3N, Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale; SCHS, Singapore Chinese Health Study.

delineated by the asymmetry of funnel plots and quantitatively evaluated by Egger's regression tests (25).

Dose-response meta-analyses were conducted to determine whether the levels of ALA in diet or circulating blood were dose-dependently associated with the risk of CRC. In brief, individual studies with three or more categories were included in the dose-response analysis and the median values of ALA levels in both diet and blood for each exposure category were assigned as previously described (26). A curvilinear trend was tested by using the methods previously described (27, 28). Specifically, restricted cubic splines with 3 knots (2 spline transformations) at fixed percentiles (25%, 50%, and 75%) were first created, and then a *P*-value for non-linearity was calculated to detect a potential departure from a simpler linear trend by testing the coefficient of the second spline equal to zero (29). In the presence of substantial linear trends (P for non-linearity > 0.05), a linear trend was estimated to achieve the association of per 1-g/day increment in dietary intake of ALA and per 0.1% increase in the levels of blood ALA with the risk of CRC by using a generalized least-squares regression (2-stage GLST in Stata) (27). Two-tailed $P < 0.05$ was considered statistically significant. Statistical analyses of all the data were performed by STATA version 15.1 (Stata CORP, College Station, TX).

RESULTS

The major result of the search strategy is presented in the PRISMA flow diagram (**Figure 1**). The initial search identified 20,196 records including 1 record through checking the reference list, from which 6,120 duplicates were removed. The remaining 14,075 records were screened for titles and abstracts. The preliminary screening left 119 potential articles, and subsequently, 104 articles were excluded for additional reasons after a full-text review (**Supplementary Table 1**). Finally, 15 prospective studies were eligible for the present meta-analysis, including 11 cohorts on dietary intake and 5 cohorts on biomarkers (four studies on blood and one study on adipose tissue).

Baseline Characteristics

The characteristics of 15 independent prospective studies are presented in **Table 1**. During the follow-up duration of a 9.3-year median ranging from 1 to 28 years, 12,239 CRC cases were identified among 861,725 participants from the 15 prospective studies. Eleven cohorts of dietary intake of ALA were included, involving 10,583 cases and 854,818 participants in 9 prospective cohort studies (10, 30, 31, 33–36, 38, 39) and 2 case-cohort studies (32, 37), and dietary measurements were evaluated by food frequency questionnaires. For biomarkers of ALA, 5 prospective studies were included, involving 2,051 cases and 48,421 participants in 2 studies based on plasma (17, 37), 2 on erythrocyte (18, 40), and 1 on AT (16). ALA levels in different biospecimens were quantified by gas-liquid chromatography (GLC) and the measurement unit was set as a percentage, except for one study (μmol/L) (17). Both male and female were reported in five articles (17, 32, 37, 39, 40), only male in two articles (10, 30), only female in four articles (18, 31, 34, 38), and 4 articles separately reported male and female (16, 33, 35, 36). As for CRC locations, 13 articles reported total CRC (10, 16–18, 30, 31, 33–39), whereas 2 articles only separately reported colon cancer and rectal cancer (32, 40). Among all of the included studies, quality scores assessed by the 9-star NOS ranged from 7 to 9, with a median quality ($\leq$ 7 stars) in 2 studies (36, 39) and high quality ($\geq$ 8 stars) in 13 studies.

The Top Quartiles Vs. Bottom Analyses

The pooled association comparing the top with the bottom quartiles of dietary intake and biomarkers of ALA were presented in **Figures 2**, **3**. The SRR for ALA in diet was 1.03 (95% CI: 0.97, 1.10), with no between-study heterogeneity ($I^2 = 0.00\%$). An inverse association was found between ALA biomarker and CRC (SRR = 0.83, 95%CI: 0.69, 0.99), with no between-study heterogeneity ($I^2 = 0.00\%$).

In the stratified analysis of dietary ALA intake concerning CRC (**Supplementary Table 4**), there was no evidence that the estimated summary RR differed significantly by living regions, age, gender, follow-up duration, cancer location, quality scores, study design, and multiple adjustments. In stratified analyses for the biomarker of ALA (**Supplementary Table 5**), increased levels of ALA in biospecimens were more pronounced with decreased risk of CRC in middle-aged persons (SRR = 0.83, 95%CI: 0.69, 0.99) but not in elderly persons (SRR = 0.88, 95%CI: 0.47, 1.65), while the difference between the two populations cannot be tested with a meta-regression. As for different types of biomarkers, although the pooled associations for circulating levels of ALA (SRR = 0.83, 95% CI: 0.69, 0.99) were found to be more apparent than that for AT (SRR = 0.88, 95% CI: 0.47, 1.65), results of meta-regression did not show a statistically significant difference between the two biomarkers.

In sensitivity analyses that exclude one study at a time and reanalyzed the remaining data, the exclusion of any individual study of ALA in the diet as interest exposure did not substantially change the summary result (**Supplementary Figure 1**). As for biomarkers, results of sensitivity analyses found that the overall summary associations were modestly changed when one study by Cottet V et al. (18) was omitted, with the SRR ranging from 0.83 (0.69, 0.98) to 0.86 (0.72, 1.02) (**Supplementary Figure 2**).

In publication bias analyses for either dietary intake or biomarker, no publication bias was indicated by Begg's funnel plot (P for bias of dietary intake = 0.06, P for bias of biomarker = 0.46) (**Supplementary Figures 3, 4**) or Egger's regression test (P for bias of dietary intake = 0.06, P for bias of dietary intake = 0.55) (**Supplementary Figures 5, 6**).

Dose-Response Analyses

Nine cohorts with dietary intake of ALA were available for the dose-response analyses (30–38). There was no significantly curvilinear relationship with the CRC risk (**Figure 4A**), and the association was not statistically significant in the linear model with per 1.0-g/d ALA increase (p for linearity = 0.22) (**Supplementary Figure 7**).

Four cohorts with blood levels of ALA were available for the dose-response analyses (17, 18, 37, 40), and there was no significantly curvilinear relationship with a test for non-linearity

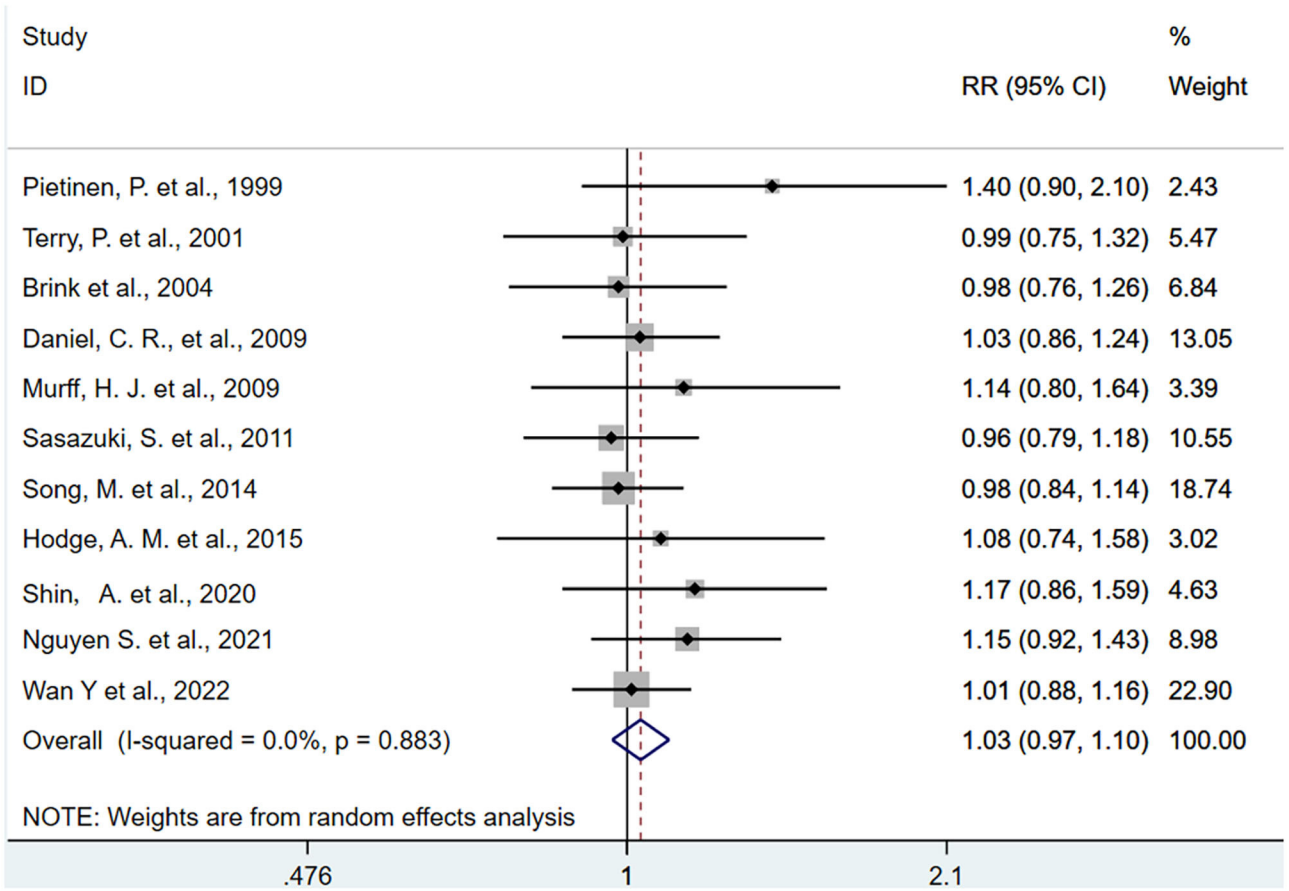


FIGURE 2 | Forest plot of the association between dietary alpha-linolenic acid and risk of colorectal cancer in the top quartile compared with the bottom. Gray square represents RR in each original study, with square size reflecting the study-specific weight and the 95% confidence interval (CI) represented by horizontal bars. RRs from the individual study were pooled by random-effects model. The summary RR (SRR) and corresponding 95% CI were represented by the diamond. The degree of heterogeneity between individual studies was indicated by the I square statistic.

(**Figure 4B**). The levels of ALA in blood had a linear dose-response association with CRC (p for linearity = 0.04), and each 0.1% increase in ALA levels resulted in a 10% reduction of risk of CRC (SRR= 0.90, 95%CI: 0.81, 0.99; I^2 = 35.9%) (**Supplementary Figure 8**).

DISCUSSION

To the best of our knowledge, this present study is the first meta-analysis that specially focused on the impacts of plant-based n-3 ALA (diet vs. biomarker) on the risk of CRC. Our pooled analysis of prospective cohorts suggested that blood levels of ALA were linearly and inversely associated with CRC risk, but no significant association was found for dietary intake of ALA. Such findings support that increased levels of ALA intake have potential benefits in preventing the development of CRC, which may further extend the previous meta-analyses with mixed n-3 PUFAs as interest exposure to highlight that the plant-derived ALA remains a protective nutrient for the incident CRC (9, 10, 15).

Results of our meta-analysis based on the prospective cohorts with the dietary estimation of AL showed a null association with CRC risk, and there was no significant difference by age, gender, geographical regions, cancer locations, duration of follow-up, or multiple adjustments. Compared with our present study, most of the previous studies especially focused on food n-3 PUFAs mixed plant- with marine-based sources (9, 10). The summary evidence for especially focusing on the association between ALA intake (plant n-3 fatty acids) and CRC risk was currently limited. Nevertheless, our observation of the null findings for dietary ALA intake was consistent with the previous results in three publications of meta-analytic reviews with mixed n-3 PUFA as interest exposures. Of note, in population-based food investigation, measurement error and bias always occurred during the performance of dietary assessment using the food frequency questionnaire, which may have changed the direction of the observed associations. Fatty acids are especially prone to this misclassification of dietary intake because similar foods may have different PUFA compositions that are difficult to be distinguished by using food descriptions in the questionnaire

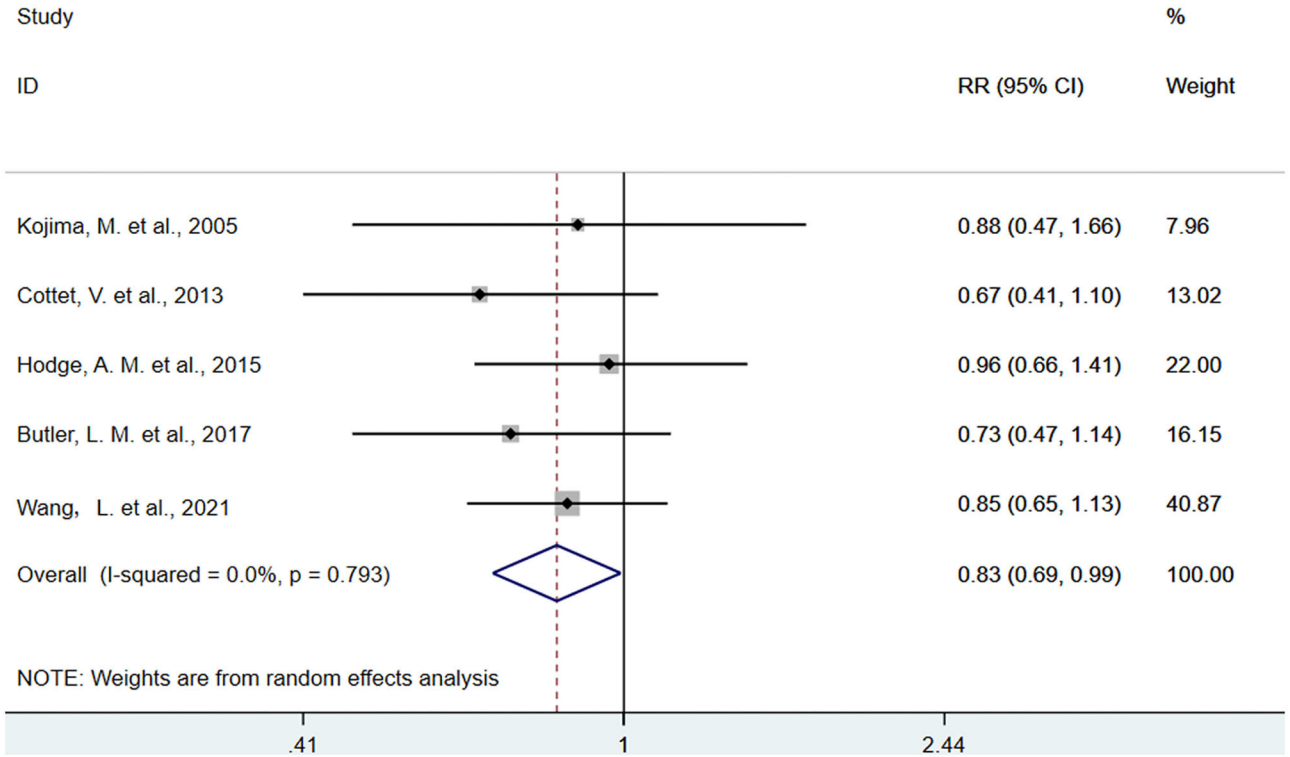


FIGURE 3 | Forest plot of the association between biomarkers of alpha-linolenic acid and risk of colorectal cancer in the top quartile compared with the bottom. Gray square represents RR in each original study, with square size reflecting the study-specific weight and the 95% confidence interval (CI) represented by horizontal bars. RRs from the individual study were pooled by random-effects model. The summary RR (SRR) and corresponding 95% CI were represented by the diamond. The degree of heterogeneity between individual studies was indicated by the I square statistic.

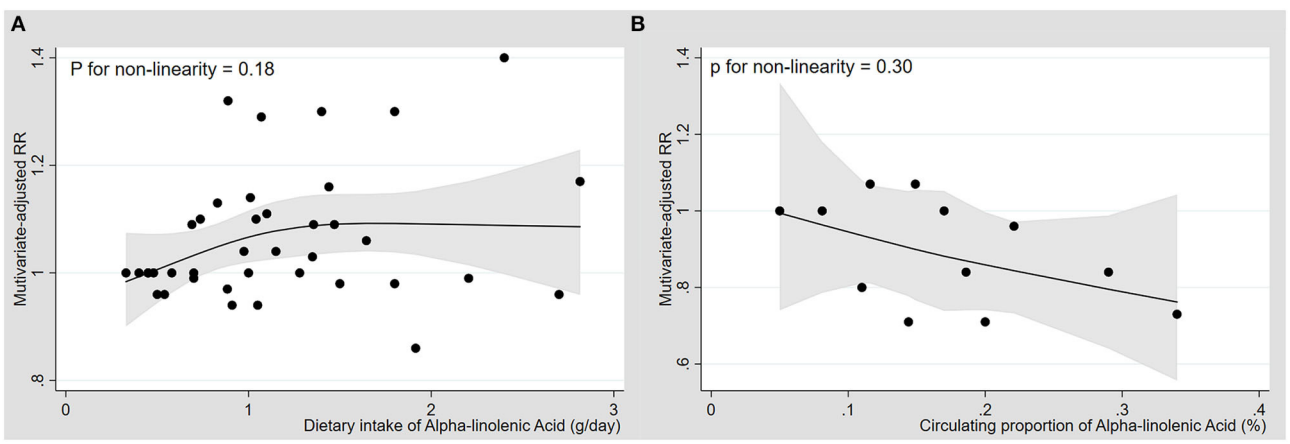


FIGURE 4 | Dose-response association between diet and blood levels of alpha-linolenic acid and risk of colorectal cancer. Multivariate-adjusted relative risks (RRs) from all categories of ALA levels in diet or blood in each original study were represented by the small black circle. The corresponding non-linear dose-response relationships of dietary **(A)** and blood levels of ALA **(B)** with the risk of colorectal cancer were assessed by a restricted cubic spline model with three fixed knots and represented by the black solid line, respectively.

tools. Another possibility was that measurement errors in assessing individual fatty acid intake may have attenuated the beneficial association with ALA intake toward a null. Third, although direct evidence in laboratory studies proved that the plant-derived ALA may suppress the development of CRC through downregulation of malignant in human and mouse colon cancer cells, the dosage of n-3 PUFA used in animal studies is much higher than the daily intake of ALA in humans (41).

Therefore, it is possible that in the normal range of the human diet, it cannot be concluded that there is a protective effect of dietary intake of plant-based n-3 PUFA on the development of CRC.

PUFA levels in human tissue (e.g., blood or AT) are currently regarded as a reasonable biological marker of habitual dietary fat intake, with sufficient evidence in the strong correlation between dietary fatty acid intake and circulating levels even if it is a single blood sample. Results of our meta-analysis based on five prospective cohorts revealed that increased levels of ALA in biospecimen (blood and AT) were significantly associated with a reduced risk of CRC. In support of these major findings, a similar inverse association with biomarker ALA was also observed in two publications of population-based epidemiological studies (16, 17). Nevertheless, the perfect associations did not reach a statistical significance in most of the previous prospective studies including a recent meta-analytic review (15, 18, 32, 37). One possible explanation was that the results of the prior meta-analysis could probably be affected by a limited number of included studies (only three cohorts with blood PUFAs), thereby perhaps leading to insufficient statistical power. Compared with the recent publication of meta-analysis, available data on different biomarkers of ALA (serum/plasm/erythrocyte/AT) from more comprehensive cohorts including recent literature of erythrocyte measurements in a larger number of 4,517 participants and another research on AT measurement were pooled in the present study (16, 40), which help enhance the statistical power to update the previous summary evidence. Moreover, multivariate-adjusted RR for the highest vs. the lowest category from each eligible study was transformed to involve comparisons between the highest and the lowest quartiles of baseline ALA levels, which may have greatly minimized statistical heterogeneity to achieve the reliability of our summary results. Finally, our findings based on dose-response meta-analyses with a test for linearity or non-linearity showed that decreased risk of CRC is linearly related to increased levels of ALA in blood, which may reinforce the robustness in association with biomarkers.

In the stratified analysis of biomarker ALA in relation to CRC, we found a beneficial association estimation in males rather than in females. Given that estrogen might have participated in the etiology of CRC (42, 43), losing adjustments for menopausal status and hormone therapy drugs might have lowered the ability to test the preferred effects in females. However, the results of the meta-analysis with interaction tests did not detect the gender-based difference. Moreover, a negative association was found to be more significant in elderly persons than in elderly persons, but the difference between the two populations cannot be tested with meta-regression analyses. It is noted that the elderly individuals seem to have more commodities with the obesity-related metabolic disorder such as dyslipidemia than young persons, which may have additionally increased the initiation and progression of CRC (44). When further stratified by biomarker types, we found that the lower risk of CRC was linearly associated with blood levels of ALA but not with AT-based biomarkers. Most of the observational studies measured fatty acid profiles in AT that can mostly represent triacylglycerol to mirror a relative long-term intake (over 2 years), this tissue does not seem to be a perfect biomarker of n-3 PUFA intake due to relatively low incorporation of ALA in AT (45). One cohort of AT measurement only was eligible for the current study, which could greatly minimize the possibility of generalizable results for all persons. Of note, erythrocyte levels mostly represent membrane PL to indicate a medium-term FA intake (several months) than blood lipids in plasma/serum indicating PUFAs' concentrations over recent days and cannot be easily affected by the postprandial status of the individual (46). Therefore, the summary estimates based on prospective cohorts with blood measurements are more reliable to diet-related evidence in elucidating the causal relationship with ALA. However, our observation of erythrocyte-based biomarkers had a marginally significant association with risk of CRC, which may have in part or at least attenuated our ultimate findings. Moreover, given that the limited number of eligible articles in each stratum analyzed might have diminished statistical power, such findings based on each subgroup need to be interpreted with caution and requires future confirmation in more large-scale cohorts at biomarker levels.

There are several biological mechanisms underlying the protective effect of ALA on the development of CRC. First, ALA is an essential precursor of long-chain n-3 fatty acids *in vivo*, which can be progressively transferred to eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and finally docosahexaenoic acid (DHA) (47), through an extremely low-conversion rate (48). ALA might have a potential inhibitive effect on the CRC development by the limited transformation to marine n-3 PUFAs (49). In addition, as a plant-derived member of food n-3 fatty acids, ALA could modulate the activity of cyclooxygenases (COX) and inhibit tumor growth by reducing n-6 PUFA-derived 2-series prostaglandin (PGE2) and promoting n-3 family derived 3-series prostaglandin (PGE3) (50, 51). Third, ALA dampened the inflammatory phenotype of M1-like macrophages, thereby reducing the expression levels of pro-inflammatory markers such as IL-6, IL-1β, TNF-α, and MCP-1 in human THP-1 cells (52). Finally, ALA might have individually regulated the apoptosis mechanism and NF-κB signaling pathway related with inflammatory response to control tumor proliferations, migrations, and invasions (41, 53, 54).

Several strengths are currently emphasized in our study. The eligible prospective cohort studies only were included, and therefore no recall and selection bias caused by retrospective studies would influence the summary result. Besides, stratified analyses with a meta-regression test indicated that the overall association estimations were not affected by the strata analyzed such as age, follow-up years, cancer location, and multiple adjustments, thereby increasing the potential possibility of the robust performance of final results. Third, compared with previous meta-analyses (10, 15), we included many published cohorts to update the previous summary evidence, which helps to enhance statistical power. Fourth, because of report bias in dietary measurements of fatty acids, we summarized the evidence on biomarker levels in the blood or AT, thereby increasing the stable generalizability of findings. Finally, no significant publication bias or between-study heterogeneity may have greatly enhanced the reliability of the summary result in the present study.

There are also several limitations in the present study. First, sensitivity analysis for biomarkers ALA indicated that exclusion of one study would potentially change the direction of the overall result toward a null (18). However, this study enrolled volunteers from a selected population of highly educated women, which was not representative of the general population. We therefore cannot rule out the possibility that selection bias might have seriously affected the association estimated in this study. Second, RRs (HRs) for various category levels in each original study were transformed with the top vs. bottom quartiles to provide a consistent approach to the meta-analysis, in which systematic error might have occurred during the data transformation. Third, though each original study controlled multiple confounding factors, there were still some residual confounders that might have changed the direction of the summary association. Fourth, though the beneficial association for the biomarker of ALA was found to be more significant in male and middle-aged populations, the results based on the subgroup analyses may not be popularized because of the limited number of included studies. Fifth, dietary changes or changes in food compositions may have occurred after blood collections and before the onset of CRC, perhaps leading to an underestimation of the pooled association. Sixth, misclassification in dietary estimations is inevitable, which was likely to bias the pooled association toward a null. Finally, we found a significant inverse association for ALA levels in the blood, but the results of AT measurement in relation to CRC risk are needed to be interpreted with more caution because of the limited number of published cohorts.

CONCLUSION

The current meta-analysis indicated that biomarkers of ALA were inversely associated with the incident CRC, and each 0.1% increase in circulating levels of ALA was associated with 10% reduction in CRC risk. Encouraging the consumption of foods rich in ALA to improve its levels in the blood may potentially decrease the risk of CRC. Nevertheless, well-designed and large-scale cohorts with biomarkers are still needed for better reconfirming the potential impacts of ALA intake in the primary prevention of CRC.

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.

AUTHOR CONTRIBUTIONS

BY conceived the idea and designed the study strategy and provided critical revisions of the manuscript for important intellectual content, administrative and funding support, and supervision. Z-BD, Y-LX, YT, and B-BH conducted a reference search. Z-BD summarized the data and conducted data acquisition and statistical analyses. Z-BD and X-LR drafted the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work is supported by the Programs Foundation of the Wenzhou Medical University of Zhejiang Province, China (89217015), the Scientific Technician Funding of Wenzhou Science and Technology Bureau (X20210055), the Basic Medicine Funding of Wenzhou Science and Technology Bureau (Y20180195), and by the 2017 Chinese Nutrition Society (CNS) Nutrition Research Foundation—DSM Research Fund (95017008). The funders have no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.948604/full#supplementary-material

REFERENCES

1. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet.* (2019) 394:1467–80. doi: 10.1016/S0140-6736(19)32319-0
2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut.* (2017) 66:683–91. doi: 10.1136/gutjnl-2015-310912
3. Yang J, Yu J. The association of diet, gut microbiota and colorectal cancer: what we eat may imply what we get. *Protein Cell.* (2018) 9:474–87. doi: 10.1007/s13238-018-0543-6
4. Mocellin MC, Camargo CQ, Nunes EA, Fiates GMR, Trindade E. A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer. *Clin Nutr.* (2016) 35:359–69. doi: 10.1016/j.clnu.2015.04.013
5. Yuan Q, Xie F, Huang W, Hu M, Yan Q, Chen Z, et al. The review of alpha-linolenic acid: Sources, metabolism, and pharmacology. *Phytother Res.* (2022) 36:164–88. doi: 10.1002/ptr.7295
6. Caligiuri SP, Aukema HM, Ravandi A, Guzman R, Dibrov E, Pierce GN. Flaxseed consumption reduces blood pressure in patients with hypertension by altering circulating oxylipins via an alpha-linolenic acid-induced inhibition of soluble epoxide hydrolase. *Hypertension.* (2014) 64:53–9. doi: 10.1161/HYPERTENSIONAHA.114.03179
7. Gomes PM, Hollanda-Miranda WR, Beraldo RA, Castro AV, Geloneze B, Foss MC, et al. Supplementation of alpha-linolenic acid improves serum adiponectin levels and insulin sensitivity in patients with type 2 diabetes. *Nutrition.* (2015) 31:853–7. doi: 10.1016/j.nut.2014.12.028
8. Schiessel DL, Yamazaki RK, Kryczyk M, Coelho I, Yamaguchi AA, Pequito DC, et al. alpha-linolenic fatty acid supplementation decreases tumor growth and cachexia parameters in walker 256 tumor-bearing rats. *Nutr Cancer.* (2015) 67:839–46. doi: 10.1080/01635581.2015.1043021
9. Liu J, Li X, Hou J, Sun J, Guo N, Wang Z. Dietary intake of N-3 and N-6 polyunsaturated fatty acids and risk of cancer: meta-analysis of data from 32 studies. *Nutr Cancer.* (2021) 73:901–13. doi: 10.1080/01635581.2020.1779321
10. Nguyen S, Li H, Yu D, Cai H, Gao J, Gao Y, et al. Dietary fatty acids and colorectal cancer risk in men: a report from the Shanghai Men's Health Study and a meta-analysis. *Int J Cancer.* (2021) 148:77–89. doi: 10.1002/ijc.33196

11. Bingham SA, Luben R, Welch A, Wareham N, Khaw KT, Day N. Are imprecise methods obscuring a relation between fat and breast cancer? *Lancet.* (2003) 362:212–4. doi: 10.1016/S0140-6736(03)13913-X
12. Hosomi K, Kiyono H, Kunisawa J. Fatty acid metabolism in the host and commensal bacteria for the control of intestinal immune responses and diseases. *Gut Microbes.* (2020) 11:276–84. doi: 10.1080/19490976.2019.1612662
13. Clay SL, Fonseca-Pereira D, Garrett WS. Colorectal cancer: the facts in the case of the microbiota. *J Clin Invest.* (2022) 132:e155101. doi: 10.1172/JCI155101
14. Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids.* (2006) 75:161–8. doi: 10.1016/j.plefa.2006.05.013
15. Kim Y, Kim J. Intake or blood levels of n-3 polyunsaturated fatty acids and risk of colorectal cancer: a systematic review and meta-analysis of prospective studies. *Cancer Epidemiol Biomarkers Prev.* (2020) 29:288–99. doi: 10.1158/1055-9965.EPI-19-0931
16. Kojima M, Wakai K, Tokudome S, Suzuki K, Tamakoshi K, Watanabe Y, et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. *Am J Epidemiol.* (2005) 161:462–71. doi: 10.1093/aje/kwi066
17. Butler LM, Yuan JM, Huang JY, Su J, Wang R, Koh WP, et al. Plasma fatty acids and risk of colon and rectal cancers in the Singapore Chinese Health Study. *NPJ precision oncology.* (2017) 1:38. doi: 10.1038/s41698-017-0040-z
18. Cottet V, Collin M, Gross AS, Boutron-Ruault MC, Morois S, Clavel-Chapelon F, et al. Erythrocyte membrane phospholipid fatty acid concentrations and risk of colorectal adenomas: a case-control nested in the french E3N-EPIC cohort study. *Cancer Epidemiol Biomarkers Prev.* (2013) 22:1417–27. doi: 10.1158/1055-9965.EPI-13-0168
19. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-Analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008
20. Wells GA, Shea B, O'Connell, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* (2011). Available online at: www.ohri.ca/programs/clinical_epidemiology/oxford.htm (accessed November 21, 2012).
21. Chene G, Thompson SG. Methods for summarizing the risk associations of quantitative variables in epidemiologic studies in a consistent form. *Am J Epidemiol.* (1996) 144:610–21. doi: 10.1093/oxfordjournals.aje.a008971
22. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA.* (1998) 279:1477–82. doi: 10.1001/jama.279.18.1477
23. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2
24. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629
26. Thesing CS, Lamers F, Bot M, Penninx B, Milaneschi Y. Response to "International Society for Nutritional Psychiatry Research Practice Guidelines for Omega-3 Fatty Acids in the Treatment of Major Depressive Disorder" by Guu et al. (2019). *Psychother Psychosom.* (2020) 89:48. doi: 10.1159/000504100
27. Orsini N BR, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. *Stata J.* (2006) 6:40–57. doi: 10.1177/1536867X0600600103
28. Jackson D, White IR, Thompson SG. Extending DerSimonian and Laird's methodology to perform multivariate random effects meta-analyses. *Stat Med.* (2010) 29:1282–97. doi: 10.1002/sim.3602
29. Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol.* (2012) 175:66–73. doi: 10.1093/aje/kwr265
30. Pietinen P, Malila N, Virtanen M, Hartman TJ, Tangrea JA, Albanes D, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. *CCC.* (1999) 10:387–96. doi: 10.1023/A:1008962219408
31. Terry P, Bergkvist L, Holmberg L, Wolk A. No association between fat and fatty acids intake and risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev.* (2001) 10:913–4. Available online at: http://cebp.aacrjournals.org/content/10/8/913
32. Brink M, Weijenberg MP, De Goeij AF, Schouten LJ, Koedijk FD, Roemen GM, et al. Fat and K-ras mutations in sporadic colorectal cancer in The Netherlands Cohort Study. *Carcinogenesis.* (2004) 25:1619–28. doi: 10.1093/carcin/bgh177
33. Daniel CR, McCullough ML, Patel RC, Jacobs EJ, Flanders WD, Thun MJ, et al. Dietary intake of ω-6 and ω-3 fatty acids and risk of colorectal cancer in a prospective cohort of U. S men and women. *Cancer Epidemiol Biomarkers Prev.* (2009) 18:516–25. doi: 10.1158/1055-9965.EPI-08-0750
34. Murff HJ, Shu XO Li H, Dai Q, Kallianpur A, Yang G, et al. A prospective study of dietary polyunsaturated fatty acids and colorectal cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev.* (2009) 18:2283–91. doi: 10.1158/1055-9965.EPI-08-1196
35. Sasazuki S, Inoue M, Iwasaki M, Sawada N, Shimazu T, Yamaji T, et al. Intake of n-3 and n-6 polyunsaturated fatty acids and development of colorectal cancer by subsite: Japan Public Health Center-based prospective study. *Int J Cancer.* (2011) 129:1718–29. doi: 10.1002/ijc.25802
36. Song M, Chan AT, Fuchs CS, Ogino S, Hu FB, Mozaffarian D, et al. Dietary intake of fish, ω-3 and ω-6 fatty acids and risk of colorectal cancer: A prospective study in U. S men and women. *Int J Cancer.* (2014) 135:2413–23. doi: 10.1002/ijc.28878
37. Hodge AM, Williamson EJ, Bassett JK, MacInnis RJ, Giles GG, English DR. Dietary and biomarker estimates of fatty acids and risk of colorectal cancer. *Int J Cancer.* (2015) 137:1224–34. doi: 10.1002/ijc.29479
38. Shin A, Cho S, Sandin S, Lof M, Oh MY, Weiderpass E. Omega-3 and−6 fatty acid intake and colorectal cancer risk in swedish women's lifestyle and health cohort. *Cancer Res Treatment.* (2020) 52:848–54. doi: 10.4143/crt.2019.550
39. Wan Y, Wu K, Wang L, Yin K, Song M, Giovannucci EL, et al. Dietary fat and fatty acids in relation to risk of colorectal cancer. *Eur J Nutr.* (2022). doi: 10.1007/s00394-021-02777-9
40. Wang L, Hang D, He X, Lo CH, Wu K, Chan AT, et al. A prospective study of erythrocyte polyunsaturated fatty acids and risk of colorectal serrated polyps and conventional adenomas. *Int J Cancer.* (2021) 148:57–66. doi: 10.1002/ijc.33190
41. Chamberland JP, Moon HS. Down-regulation of malignant potential by alpha linolenic acid in human and mouse colon cancer cells. *Fam Cancer.* (2015) 14:25–30. doi: 10.1007/s10689-014-9762-z
42. Tamakoshi K, Wakai K, Kojima M, Watanabe Y, Hayakawa N, Toyoshima H, et al. A prospective study on the possible association between having children and colon cancer risk: findings from the JACC Study. *Cancer Sci.* (2004) 95:243–7. doi: 10.1111/j.1349-7006.2004.tb02210.x
43. Mason JK, Kharotia S, Wiggins AK, Kitson AP, Chen J, Bazinet RP, et al. 17beta-estradiol increases liver and serum docosahexaenoic acid in mice fed varying levels of alpha-linolenic acid. *Lipids.* (2014) 49:745–56. doi: 10.1007/s11745-014-3913-8
44. Yarla N, Madka V, Rao C. Targeting triglyceride metabolism for colorectal cancer prevention and therapy. *Curr Drug Targets.* (2021). doi: 10.2174/1389450122666210824150012
45. Giuliani A, Ferrara F, Scimo M, Angelico F, Olivieri L, Basso L. Adipose tissue fatty acid composition and colon cancer: a case-control study. *Eur J Nutr.* (2014) 53:1029–37. doi: 10.1007/s00394-013-0605-8
46. Arab L, Akbar J. Biomarkers and the measurement of fatty acids. *Public Health Nutr.* (2002) 5(6A):865–71. doi: 10.1079/PHN2002391
47. Barcelo-Coblijn G, Murphy EJ. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Prog Lipid Res.* (2009) 48:355–74. doi: 10.1016/j.plipres.2009.07.002
48. Burns-Whitmore B, Froyen E, Heskey C, Parker T, San Pablo G. Alpha-Linolenic and linoleic fatty acids in the vegan diet: do they require dietary reference intake/adequate intake special consideration? *Nutrients.* (2019) 11:2365. doi: 10.3390/nu11102365

49. Yang B, Wang FL, Ren XL Li D. Biospecimen long-chain N-3 PUFA and risk of colorectal cancer: a meta-analysis of data from 60,627 individuals. *PLoS ONE.* (2014) 9:e110574. doi: 10.1371/journal.pone.0110574
50. Hopkins GJ, Kennedy TG, Carroll KK. Polyunsaturated fatty acids as promoters of mammary carcinogenesis induced in Sprague-Dawley rats by 7,12-dimethylbenz[a]anthracene. *J Natl Cancer Inst.* (1981) 66:517–22.
51. Poole EM, Bigler J, Whitton J, Sibert JG, Kulmacz RJ, Potter JD, et al. Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps. *Carcinogenesis.* (2007) 28:1259–63. doi: 10.1093/carcin/bgm026
52. Pauls SD, Rodway LA, Winter T, Taylor CG, Zahradka P, Aukema HM. Anti-inflammatory effects of alpha-linolenic acid in M1-like macrophages are associated with enhanced production of oxylipins from alpha-linolenic and linoleic acid. *J Nutr Biochem.* (2018) 57:121–9. doi: 10.1016/j.jnutbio.2018.03.020
53. Hassan A, Ibrahim A, Mbodji K, Coeffier M, Ziegler F, Bounoure F, et al. An alpha-linolenic acid-rich formula reduces oxidative stress and inflammation by regulating NF-kappaB in rats with TNBS-induced colitis. *J Nutr.* (2010) 140:1714–21. doi: 10.3945/jn.109.119768
54. Gonzalez-Fernandez MJ, Ortea I, Guil-Guerrero JL. alpha-Linolenic and gamma-linolenic acids exercise differential antitumor effects on HT-29 human colorectal cancer cells. *Toxicol Res (Camb).* (2020) 9:474–83. doi: 10.1093/toxres/tfaa046

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.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 12 July 2022
doi: 10.3389/fnut.2022.905162

Habitual Fish Oil Supplementation and Risk of Incident Inflammatory Bowel Diseases: A Prospective Population-Based Study

Xiaoxu Huang[1†], Yin Li[2†], Pan Zhuang[3], Xiaohui Liu[2], Yu Zhang[3], Pianhong Zhang[1] and Jingjing Jiao[1,2*]*

[1] Department of Clinical Nutrition, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Department of Nutrition, School of Public Health, Department of Clinical Nutrition, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [3] Department of Food Science and Nutrition, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, China

OPEN ACCESS

Edited by:
Li Cai,
Sun Yat-sen University, China

Reviewed by:
Wanshui Yang,
Anhui Medical University, China
Xin Geng,
Eastern Hepatobiliary Surgery Hospital, China

***Correspondence:**
Pianhong Zhang
zrlcyyzx@zju.edu.cn
Jingjing Jiao
jingjingjiao@zju.edu.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *26 March 2022*
Accepted: *13 June 2022*
Published: *12 July 2022*

Citation:
Huang X, Li Y, Zhuang P, Liu X, Zhang Y, Zhang P and Jiao J (2022) Habitual Fish Oil Supplementation and Risk of Incident Inflammatory Bowel Diseases: A Prospective Population-Based Study. Front. Nutr. 9:905162. doi: 10.3389/fnut.2022.905162

Background: Inflammatory Bowel Diseases (IBDs) have been emerging in recent years with the advance of global industrialization and diet pattern transformation. Marine n-3 polyunsaturated fatty acids (n-3 PUFAs), enriched in fish oils, have well-known human health promotion. Evidence on the association of fish oil supplementation with the risk of developing IBDs was scarce. This study aimed to examine the association between the use of fish oil supplements and the risk of developing inflammatory bowel diseases (IBDs) among the general population.

Methods: We conducted a prospective cohort study of 447,890 participants aged 40–69 years from the UK Biobank. A touch screen questionnaire was used to get the data about fish oil intake at baseline. Incident diagnoses of IBDs were ascertained by the International Classification of Diseases (ICD-9 and ICD-10) or self-report. Cox proportional hazards model was applied to calculate hazard ratios (*HRs*) and 95% confidence intervals (*CIs*) of developing IBDs and their subtypes.

Results: We documented 1,646 incident cases of IBDs, including 533 incident cases of Crohn's disease (CD) and 1,185 incident cases of ulcerative colitis (UC) during an average of 8 years of follow-up. After multivariate adjustment, the use of fish oil was associated with a 12% lower risk of IBDs (*HR*: 0.88, 95% *CI*: 0.78–0.99, $p = 0.03$) compared with non-consumers. For subtypes of IBDs, fish oil supplementation was inversely associated with a 15% lower risk of UC (*HR*: 0.85, 95% *CI*: 0.75–0.99, $p = 0.02$) but was not correlated with the risk of CD ($p = 0.22$). Besides, fish oil supplementation showed a significant inverse correlation with baseline CRP levels ($\beta = -0.021$, $p < 0.001$) and a positive association with baseline albumin levels ($\beta = 0.135$, $p < 0.001$) after adjustment for multiple variates.

Conclusion: Habitual intake of fish oil supplements was associated with a lower risk of IBDs and UC. Fish oil users tended to have lower baseline C-reactive protein levels and higher baseline albumin levels compared with non-users. It was concluded that fish oil supplement use may be recommended for the prevention and control of IBDs.

Keywords: Crohn's disease, fish oil supplementation, inflammatory bowel diseases, ulcerative colitis, UK Biobank, C-reactive protein, album

INTRODUCTION

Inflammatory bowel diseases (IBDs) are non-specific chronic gastrointestinal tract inflammatory disorders, mainly including Crohn's disease (CD) and ulcerative colitis (UC). The Global Burden of Diseases, Injuries, and Risk Factors Study reported that approximately 6.8 million people around the world had suffered from IBDs in 2017 (1–3). The population of IBDs has been rising in recent years with the advance of global industrialization and diet pattern transformation (3–5), which brings a heavy financial burden to patients' families and society. It is urgent to formulate beneficial roles of social and dietary factors in controlling the prevalent trend of epidemic IBDs. Although the pathogenesis of IBDs is still unclear, genetic characteristics, environmental or microbial factors, and immune responses were involved in the etiology of IBDs (3). Diet has been reported to play an important role in IBDs by influencing the composition and functionality of the microbiome. Various dietary therapies have also become a potent tool for the remission of IBDs in some clinical studies (6). Marine n-3 polyunsaturated fatty acids (n-3 PUFAs), which are enriched in fish oils, have well-known anti-inflammatory, antiplatelet aggregatory, vasodilation, vasoconstriction, and ameliorating immune response effects for human health promotion (7–9). Interleukin-10 (IL-10) which is produced by resolvin E1 (RvE1) is regarded as the predominant anti-inflammatory cytokine in the intestine (10). RvE1, derived from n-3 PUFAs, also promotes intestinal mucosal repair (11). In addition, fish oil supplementation may weaken cellular immune responses, contributing to lower expression of Th17 cell type cytokine genes which play a part in the etiology of IBDs (12). Overall, n-3 PUFAs may be beneficial for preventing the occurrence of IBDs by regulating inflammatory mediators and ameliorating immune responses (13–17).

In humans, previous epidemiology studies tried to shed light on the association between dietary intake of n-3 PUFAs and the risk of IBDs, but the results were inconsistent. A case-control study found no significant association between dietary n-3 PUFA intake and the risk of UC in Caucasians (18), whereas a Japanese multicenter case-control study revealed that dietary n-3 fatty acid intake was correlated with the increase in CD risk (19). Another large prospective study reported that a long-term dietary intake of n-3 PUFAs was associated with a lower risk of UC in 170,805 women in the United States Nurses' Health Study (20). Similarly, intake of docosahexaenoic acid (DHA), a major ingredient of marine n-3 PUFAs and fish oils, was inversely correlated with the incidence of IBDs in several studies (21–23). A meta-analysis of observational studies suggested no significant association between dietary n-3 PUFA intake and the risk of IBDs but indicated a significant inverse association for the risk of UC (pooled effect size: 0.75, 95% *CI*: 0.57–0.98, $p = 0.03$) (24). However, another meta-analysis did not observe an association between dietary fat intake including PUFA intake and the incidence of UC (18, 25).

Therefore, whether n-3 PUFA supplementation is beneficial for a lower incidence of IBDs and its subtypes remains controversial due to limited participants and IBDs cases in most studies. In the current study, we aim to conduct a large-scale population-based cohort study among participants aged 40–69 years and investigate the association between fish oil use and the incidence of IBDs and their subtypes in the UK Biobank.

MATERIALS AND METHODS

Study Design and Participants

The UK Biobank is a prospective population-based cohort study that included a total of 502,505 participants, including 208,434 men and 239,456 women aged 40–69 years who were recruited between 2006 and 2017 in the United Kingdom (26). They were invited to complete a series of touch screen questionnaires, provide biological samples, and take various physical examinations at baseline. Informed consent was provided by each participant, and the UK Biobank's study protocol was approved by the United Kingdom North West Multi-centre Research Ethics Committee. After excluding participants' withdrawing on subsequent follow-up ($n = 11$), patients with IBDs ($n = 5{,}410$) and cancer ($n = 43{,}459$) at baseline, and those lacking data on the fish oil use ($n = 5{,}735$), the remaining 447,890 participants were included in the final analysis (**Figure 1**).

Assessment of Fish Oil Use and Covariates

Participants were required to complete some questions from a series of touch screen questionnaires, including the question that "Do you regularly take any of the following?" in their own assessment centers and they could answer this question with a list of supplements including fish oils. To assess the reproducibility and effectiveness of the use of fish oil, two repeated surveys were conducted among 18,093 and 44,366 participants, respectively. In the first repeated survey completed from 2012 to 2013, 72.2% of fish oil consumers at baseline were reported to continue to use fish oil supplements (Spearman $r = 0.61$), while in the second repeated survey conducted in 2014 and later, 55.2% of

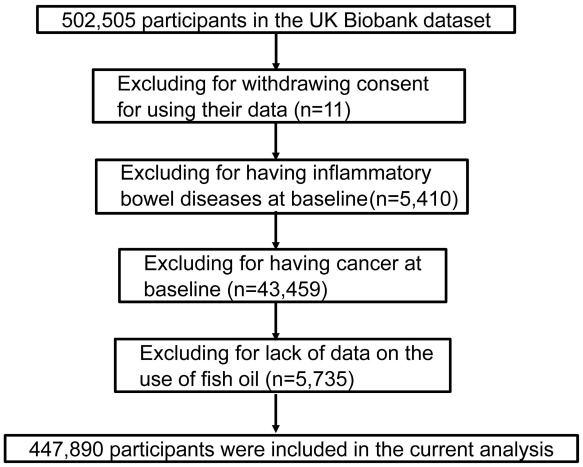


FIGURE 1 | Flow chart of eligible participants in this study.

fish oil users at baseline continued to take fish oil supplements (Spearman $r = 0.47$) (**Supplementary Table 1**).

Covariates included age, sex, race, assessment centers, body mass index (BMI), education, Townsend deprivation index (TDI) (27), household income, smoking status, alcohol consumption, physical activity, other dietary supplementation use, medication, and dietary intake. BMI was defined as the ratio of weight to squared height (kg/m^2). The metabolic equivalent of task (MET) was computed according to the International Physical Activity Questionnaire short form (28). The use of aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), and/or hormones was also assessed at baseline. Participants were required to answer 29 questions about the frequency of food intake and 18 questions on alcohol consumption by the touch screen questionnaires after the entry in the current study. We also referred to the definition that described the ideal intake of dietary components for cardiometabolic health and made a healthy diet score for each participant (**Supplementary Table 2**) (29). The healthy diet score was calculated by 10 kinds of dietary components (fruits, vegetables, whole grains, fish, dairy products, vegetable oil, refined grains, processed meat, unprocessed meat, and sugar-sweetened beverages) and each food had its own intake goal for keeping cardiometabolic health. Once the intake goal was met (**Supplementary Table 2**), one point was given for each favorable diet factor. The total healthy diet score ranged from 0 to 10. A healthier diet was related to a higher diet score.

The levels of C-reactive protein (CRP) and albumin at baseline were also measured (30). Due to the right-skewed distribution of CRP values, log (units + 1) of CRP values were used in our analyses (31). More detailed information about the study can be achieved online at https://biobank.ctsu.ox.ac.uk/showcase.

Outcome Ascertainment

Definitions of IBDs are presented in **Supplementary Table 3**. A case was considered eligible if the participant had either a relevant inpatient International Classification of Diseases (ICD) code or self-reported illness (32). K51 and K50 were considered as the codes of UC and CD in ICD version 10, respectively. Alternatively, codes 556 and 555 could also be used for the judgment of UC and CD in ICD version 9, respectively. Self-reported IBD cases were documented during the assessment interview. If a participant had recorded diagnoses for both UC and CD, then the individual was only defined as one case of IBDs.

Statistical Analysis

The follow-up duration of participants was from the date of attending baseline assessment until the time of IBD diagnosis, lost to follow-up, death, or the end of follow-up (March 31, 2017), whichever came first. We used Cox proportional hazards models to estimate the hazard ratios (*HRs*) and 95% confidence intervals (*CIs*) of IBDs in accordance with the use or non-use of fish oil supplements after adjustment for potential confounders in a group of stepwise covariate-adjusted models. Model 1 was adjusted for age, sex, race, assessment centers, BMI, education, TDI, household income, smoking status, alcohol consumption, physical activity, vitamin supplement use, mineral supplement use, aspirin use, hormone use, and NSAID drug use. Model 2 was additionally adjusted for the intake of oily fish, processed red meat, vegetables, fruits, whole grains, and cheeses. Model 3 was further adjusted for a healthy diet score based on model 1 given the potential interactions between different dietary components. We also assessed the associations of fish oil supplementation with baseline albumin levels and CRP levels in secondary analyses. Then, we performed subgroup analyses to evaluate the associations stratified by potential effect modifiers, including age, BMI, TDI, smoking status, alcohol consumption, physical activity, healthy diet score, healthy lifestyle score, vitamin supplement use, mineral supplement use, aspirin use, NSAID use, oily fish intake, and non-oily fish intake. The *p*-value for interaction was reckoned by adding the cross-product term of fish oil use with either of the above stratifying variables into the model. Moreover, an overall healthy lifestyle score based on BMI (<30 kg/m^2), smoking (never), physical activity ($\geq$600 MET min/week), and healthy diet (yes) were also considered in subgroup analyses (33). When participants satisfied any of the scoring criteria, they could get one point. The healthy lifestyle score was the sum of five component scores and ranged from 0 to 5. Sensitivity analyses were conducted by further adjusting for coffee intake, contraceptive use, depression, or further excluding extreme BMI values. Besides, we further adjusted CRP and albumin levels to test whether the association was weakened. Participants were grouped into three categories according to CRP concentration (<5 mg/l, 5–10 mg/l, ≥ 10 mg/l) (34). For albumin, those with 35–50 g/L albumin were seen as the reference group and the rest of the participants were classified into two categories (<35 g/l, ≥ 50 g/l) (35). To minimize the possibility of reverse causation, we also further excluded incident IBD cases that occurred within 2 years in a sensitivity analysis. All the statistical analyses were conducted by SAS version 9.4 (SAS Institute, Cary, NC, United States). Two-sided *p*-values less than 0.05 indicated statistical significance.

RESULTS

Baseline Characteristics

Our study followed 447,890 participants with 3,606,243 person-years in the UK Biobank study. During an average of 8 years of follow-up, we confirmed 1,646 IBD cases (incidence 46 per 100,000 person-years), including 533 CD cases (incidence 15 per 100,000 person-years) and 1,185 UC cases (incidence 33 per 100,000 person-years). **Table 1** shows the baseline characteristics of participants according to whether they used fish oil supplements. Compared with non-users, fish oil users were generally older and more often female, and had lower household income and TDI. They also tended to be more physically active, not current smokers, have lower BMI, and were more likely to drink alcohol and use aspirin, NSAID drugs, vitamins, and minerals. They preferred oily fish, non-oily fish, vegetables, fruits, and whole grains, whereas they were less likely to consume processed meat, refined grains, cheeses, and sugar-sweetened beverages (SSB). Moreover, the dietary pattern of fish oil users was healthier than non-users.

Fish Oil Supplementation and Incident Inflammatory Bowel Disease Risk

In our age- and sex-adjusted model, we did not observe any significant association between fish oil use and the risk of incident IBDs (**Table 2**). After adjustment for other demographic characteristics and the use of other supplements and medications (model 1), the use of fish oil supplements was inversely associated with a 13% lower risk of IBDs (*HR*: 0.87, 95% *CI*: 0.78–0.98; $p = 0.02$). The results also did not remarkably change after further adjustment for potentially related dietary factors, such as oily fish, processed red meat, vegetables, fruits, whole grains, and cheeses (model 2). Finally, fish oil use was also correlated with the risk of IBDs after adjustment for a healthy diet score (model 3). The *HR* (95% *CI*) of IBD risk associated with fish oil use was 0.88 (0.78–0.99) ($p = 0.03$) in this model. Then, we separately analyzed the associations with the risk of UC and CD and found different results. The fish oil intake was inversely associated with a 15% lower risk of UC (*HR*: 0.85, 95% *CI*: 0.75–0.99, $p = 0.02$) (**Table 3**) after the adjustment for multiple variates, but was not significantly associated with the risk of the CD ($p = 0.22$).

The Correlation Between Fish Oil Supplementation and Blood Biomarkers

At baseline, fish oil supplementation showed a significant inverse correlation with CRP levels ($\beta = -0.021$, $p < 0.001$) (**Table 4**) and a positive association with albumin levels ($\beta = 0.135$, $p < 0.001$) after adjustment for multiple variates.

Subgroup and Sensitivity Analyses

We did not find a significant interaction between fish oil use and risk of all-cause IBDs, when the analyses were stratified by sex, age, BMI, TDI, smoking, alcohol consumption, physical activity, healthy diet score, healthy lifestyle score, vitamin supplement use, mineral supplement use, aspirin use, NSAID drug use, oily fish intake, or non-oily fish intake (**Figure 2**). Sensitivity analyses

TABLE 1 | Basic characteristics of participants by use of fish oil in the UK Biobank cohort.

Characteristics	Overall (*n* = 447,890)	Fish oil non-users (*n* = 308,111)	Fish oil users (*n* = 139,779)
Male, %	46.5	47.6	44.2
Age, years	56.2 (8.1)	55.2 (8.2)	58.4 (7.5)
Race, %			
White	94.1	93.8	94.7
Asian	2.4	2.6	1.8
Black	1.7	1.6	1.8
Mixed	0.6	0.6	0.6
Others	0.9	1.0	0.8
BMI, kg/m^2	27.4 (4.8)	27.5 (4.9)	27.2 (4.6)
Household income (£), %			
<18,000[a]	19.0	18.5	20.3
18,000 to 30,999	21.6	20.6	23.8
31,000 to 51,999	22.6	23.0	21.7
52,000 to 100,000	17.8	19.0	15.1
>100,000	4.7	5.3	3.6
Townsend deprivation index	−1.3 (3.1)	−1.2 (3.1)	−1.5 (3.0)
Education, %			
College or University degree	32.6	33.7	30.2
Vocational qualifications	11.7	11.3	12.6
Optional national exams at ages 17–18 years	26.7	26.7	26.7
National exams at age 16 years	16.8	16.1	18.3
Others	1.0	1.0	1.1
Physical activity, MET-h/wk	44.3 (45.3)	42.7 (44.8)	47.8 (46.4)
Smoking status, %			
Never	55.0	55.5	54.1
Previous	34.0	32.5	37.3
Current	10.6	11.6	8.2
Alcohol consumption, %			
Never or special occasions only	19.3	19.8	18.3
1 to 3 times/month	11.2	11.4	10.7
1 or 2 times/week	25.9	25.8	26.0
3 or 4 times/week	23.2	22.9	24.1
Daily or almost daily	20.3	20.1	20.8
NSAIDs use, %	37.6	36.1	41.1
Aspirin use, %	13.9	12.7	16.5
Hormone, %	3.9	3.6	4.6
Vitamin supplementation, %	31.4	20.1	56.3
Mineral supplementation, %	12.1	8.1	20.9
Dietary consumption, %			
Oily fish, times/week			
<1	44.2	47.9	36.1
1	37.4	35.8	41.1

(Continued)

TABLE 1 | (Continued)

Characteristics	Overall (*n* = 447,890)	Fish oil non-users (*n* = 308,111)	Fish oil users (*n* = 139,779)
≥2	17.8	15.7	22.4
Non-oily fish, times/week			
<1	33.8	35.8	29.4
1	49.4	48.2	52.2
≥2	16.2	15.4	18.0
Poultry, times/week			
<2	51.6	51.7	51.4
2–4	45.9	45.7	46.2
>4	2.3	2.4	2.2
Processed meat, times/week			
<1	39.5	38.5	41.8
1	29.1	28.9	29.6
≥2	31.2	32.4	28.5
Unprocessed red meat, times/week			
<2.0	49.8	49.6	50.2
2.0–4.0	41.8	41.8	41.9
>4.0	8.2	8.4	7.8
Vegetable, servings/day			
<1.0	18.0	19.6	14.6
1.0–2.9	72.6	71.4	75.3
≥3.0	8.7	8.3	9.5
Fruit, servings/day			
<2.0	35.2	38.5	27.9
2.0–3.9	47.5	45.8	51.1
≥4.0	17.1	15.4	20.8
Whole grain, servings/day			
<1.0	42.1	45.2	35.3
1.0–2.9	43.4	41.0	48.8
≥3.0	13.3	12.6	14.9
Refined grain, servings/day			
<1.0	58.2	55.9	63.3
1.0–2.9	31.3	32.8	28.1
≥3.0	9.3	10.1	7.6
Cheese, times/week			
<2	40.5	39.9	41.8
2–4	44.1	44.2	43.9
>4	12.9	13.5	11.5
Coffee, cups/day			
<1	29.3	29.7	28.5
1–2	38.6	37.0	42.1
≥3	31.8	33.0	29.2
Sugar-sweetened beverages consumer, %	81.7	83.0	78.8
Healthy diet score	3.0 (1.4)	2.9 (1.4)	3.3 (1.4)

BMI, body mass index. Values are means (SD) or percentages unless stated otherwise. [a]£1.00 = $1.30, €1.20. NSAIDs, non-steroidal anti-inflammatory drugs. Hormone, hormone replacement therapy.

showed that the documented significant association between fish oil supplementation and the incidence of IBDs did not change substantially after further adjustment for coffee intake, depression, and contraceptive use (**Supplementary Table 4**). The relationship was still significant after further adjustment for CRP (*HR*: 0.89, 95% *CI*: 0.79–0.99, $p = 0.04$) and albumin (*HR*: 0.88, 95% *CI*: 0.79–0.99, $p = 0.03$). Meanwhile, the association was also robust after excluding the incident IBD cases that occurred within 2 years or participants who had extreme BMI values (<18.5 or > 40 kg/m^2).

DISCUSSION

In this study, we found that habitual fish oil use was correlated with a 12% lower risk of developing IBDs after adjustment for potential confounders. For IBD subtypes, fish oil supplementation was significantly associated with a lower risk of UC but not CD. We also assessed inverse associations of fish oil supplementation with baseline albumin levels and CRP levels.

To our knowledge, our study is the most extensive prospective cohort study to investigate the association between habitual marine n-3 PUFA intake and the risk of incident IBDs. We showed that the incidence of UC (33 per 100,000 person-years) and CD (15 per 100,000 person-years) was both higher than those that were previously reported in Europe (36). The increased incidence of UC and CD in the current study was in line with the elevating global trend (1). Some pieces of evidence from the previous epidemiological studies have supported our findings. Two case-control studies acknowledged

TABLE 2 | HRs (95% CIs) of inflammatory bowel diseases according to fish oil use in the UK Biobank.

	Fish oil non-users (*n* = 308,111)	Fish oil users (*n* = 139,779)	*P*-value
Number of cases	1,143	503	
Person-years	2,477,757	1,128,486	
Age- and sex-adjusted HR (95% CI)	1 [Ref]	0.93 (0.83–1.03)	0.16
Model 1[a]	1 [Ref]	0.87 (0.78–0.98)	**0.02**
Model 2[b]	1 [Ref]	0.88 (0.79–0.99)	**0.04**
Model 3[c]	1 [Ref]	0.88 (0.78–0.99)	**0.03**

CI, confidence interval; HR, hazard ratio. [a]Adjusted for age, sex, race (White, Asian, Black, mixed, or other ethnic group), assessment centers (22 categories), BMI (in kg/m^2; <18.5, 18.5 to 25, 25 to 30, 30 to 35, ≥35, or missing), education (college or university degree, vocational qualifications, optional national exams at ages 17–18 years, national exams at age 16 years, others, or missing), Townsend deprivation index (quintiles), household income (<£18,000, £18,000-£30,999, £31,000-£51,999, £52,000-£100,000, >£100,000, or missing), smoking status (never, former, current, or missing), alcohol consumption (never, special occasions only, 1–3 times/month, 1 or 2 times/week, 3 or 4 times/week, or daily/almost daily), physical activity (in MET-h/wk; quintiles), vitamin supplement use (yes or no), mineral supplement use (yes or no), aspirin use (yes or no), hormone use (yes or no), and non-steroidal anti-inflammatory drugs use (yes or no). [b]Additionally adjusted for oily fish (<1, 1, or ≥2 times/week), processed red meat (<1, 1, or ≥2 times/week), vegetables (<1, 1–3, or ≥3 servings/day), fruits (<2.0, 2.0–3.9, ≥4.0 servings/day), whole grains (<1.0, 1.0–2.9, ≥3.0 servings/day), and cheeses (<2, 2–4, >4 times/week) based on model 1. [c]Further adjusted for healthy diet score (quintiles) based on model 1. Bold value indicates statistical significance.

TABLE 3 | HRs (95% CIs) of ulcerative colitis and Crohn's disease according to fish oil use in the UK Biobank.

	Fish oil non-users (*n* = 308,111)	Fish oil users (*n* = 139,779)	*P*-value
Ulcerative colitis			
Number of cases	826	359	
Person-years	2,478,885	1,128,999	
Age- and sex-adjusted HR (95% CI)	1 [Ref]	0.91 (0.81–1.04)	0.16
MV-adjusted HR (95% CI)[a]	1 [Ref]	0.85 (0.75–0.99)	**0.02**
Crohn's disease			
Number of cases	376	157	
Person-years	2,480,599	1,129,753	
Age- and sex-adjusted HR (95% CI)	1 [Ref]	0.88 (0.73–1.06)	0.19
MV-adjusted HR (95% CI)[a]	1 [Ref]	0.88 (0.72–1.08)	0.22

CI, confidence interval; HR, hazard ratio; MV, multivariable. [a]MV-adjusted model was adjusted for age, sex, race (White, Asian, Black, mixed, or other ethnic group), assessment centers (22 categories), BMI (in kg/m^2; <18.5, 18.5 to 25, 25 to 30, 30 to 35, ≥35, or missing), education (college or university degree, vocational qualifications, optional national exams at ages 17–18 years, national exams at age 16 years, others, or missing), Townsend deprivation index (quintiles), household income (<£18,000, £18,000-£30,999, £31,000-£51,999, £52,000-£100,000, >£100,000, or missing), smoking status (never, former, current, or missing), alcohol consumption (never, special occasions only, 1–3 times/month, 1 or 2 times/week, 3 or 4 times/week, or daily/almost daily), physical activity (in MET-h/wk; quintiles), vitamin supplement use (yes or no), mineral supplement use (yes or no), aspirin use (yes or no), hormone use (yes or no), non-steroidal anti-inflammatory drugs use (yes or no), and healthy diet score (quintiles). Bold value indicates statistical significance.

TABLE 4 | β coefficients of fish oil use for blood indicators from general linear regression analysis in the UK Biobank.

	β	SE	*P*-value
Albumin	0.135	0.010	**<0.001**
CRP[a]	−0.021	0.002	**<0.001**

[a]Log (units + 1) of CRP values were used. Model was adjusted for age, sex, race, assessment centers, BMI, education, Townsend deprivation index, household income, smoking status, alcohol consumption, physical activity, vitamin supplement use, mineral supplement use, aspirin use, hormone use, non-steroidal anti-inflammatory drugs use, and healthy diet score. Bold value indicates statistical significance.

that fish consumption had a protective role in the incidence of IBDs (37, 38). A nested case-control study in the European Prospective Investigation into Cancer and Nutrition (EPIC) agreed that a higher intake of n-3 PUFAs and DHA from the diet could be beneficial for a lower risk of developing UC (39). A United Kingdom prospective cohort study found similar results and reported a significant association of DHA intake with UC and a similar borderline significant association for total n-3 PUFA and eicosapentaenoic acid (EPA) intake (40). A meta-analysis including 282,610 participants and 2,002 IBD cases also observed that dietary long-chain n-3 PUFA intake was associated with a lower risk of UC (pooled effect size: 0.75, 95% *CI*: 0.57–0.98, $p = 0.03$) (24). However, fish consumption showed no significant association with UC and CD in two case-control studies (41, 42).

Dietary n-3 PUFA intake may be beneficial to the control of UC, but the inverse association was not significant in most studies due to other confounding dietary factors. Higher dietary intake of long-chain n-3 PUFAs was associated with a trend toward a 27% lower risk of developing UC in the EPIC cohort (20). Another meta-analysis revealed that the intake of n-3 PUFAs was not associated with the risk of UC, and only DHA showed a potential protective effect in the development of UC (25). The intake of n-3 PUFAs from the diet may be influenced by other food nutrients including fatty acids, such as n-6 PUFAs. Data from a European perspective cohort study supported that linoleic acid intake was positively correlated with the risk of UC (39), indicating the protective role of n-3 PUFAs from the diet may be weakened by other types of dietary fatty acids. Most studies reported that the intake of DHA played an important role in the development of UC (25, 39, 40). Given high amounts of marine n-3 PUFAs, the habitual use of fish oil supplements may elevate the daily intake level of n-3 PUFAs from the diet, which contributed to the inverse association with the occurrence of IBDs and UC. However, current research on the relationship between the use of fish oil supplements and the incidence of IBDs is still scarce. On the other hand, the outcomes from existing studies regarding dietary n-3 PUFA intake remain inconsistent due to small-scale participants, a small number of cases, and heterogeneity of diet assessment. In the current study, we found significant inverse associations and filled the gap of direct evidence on the protective role of fish oil supplementation in the risk of all-cause IBDs and UC since fish oil supplements contain much higher amounts of marine n-3 PUFAs than the dietary intake level of n-3 PUFAs from foods.

For another subtype of IBDs, conflicting results also existed for the association between n-3 PUFA intake and the risk of CD. A multicenter case-control study in Japan found a positive association between dietary n-3 PUFA intake and CD risk (19), whereas another Canadian population-based case-control study demonstrated an inverse association (43).

A recent meta-analysis revealed that dietary long-chain n-3 PUFA intake was not correlated with incident CD (pooled effect size in fixed model: 0.85, 95% *CI*: 0.59–1.23, $p = 0.37$) (24). Similarly, we found no association between fish oil use and incident CD in the current study. Although UC and CD present similar clinical symptoms, their pathogenesis and the course of developing diseases are obviously different (44), resulting in their possible different responses to fish oil supplementation. A further organ culture experiment investigated differential effects of fish oil-enriched enteral diet on UC and CD in tissue by showing an increase in IL-1ra/IL-1β cytokine ratios and thus improving inflammatory status, and proved that this modification effect was significantly more marked in UC compared with CD (45). The above study provided insights into the current finding that fish oil supplementation was conducive to the prevention of UC rather than CD.

The finding that fish oil use was correlated with a lower risk of IBDs in our study was supported by several plausible biological mechanisms. EPA as one of the key ingredients in fish oil is metabolized to prostaglandin E3 and leukotriene B5, which have anti-inflammatory properties (46). Besides, n-3 PUFAs can promote the release of phospholipases D from cell membranes

Subgroup	No of participants	Hazard ratio (95% CI)	P value for interaction
Sex			
Women	239,456	0.90 (0.76–1.06)	0.84
Men	208,434	0.86 (0.73–1.01)	
Age(years)			
<65	367,022	0.86 (0.75–0.99)	0.57
≥65	80,868	0.93 (0.74–1.18)	
BMI(kg/m^2)			
<30	336,504	0.92 (0.80–1.05)	0.34
≥30	109,042	0.76 (0.61–0.96)	
Townsend deprivation index			
Below median	223,669	0.84 (0.71–0.99)	0.55
Above median	223,667	0.92 (0.78–1.08)	
Smoking status			
Never	246,529	0.95 (0.80–1.13)	0.12
Previous	152,352	0.81 (0.68–0.96)	
Current	47,360	0.88 (0.63–1.22)	
Alcohol consumption			
Never or special occasions only	86,623	0.75 (0.59–0.96)	0.33
≥1 time/month	360,875	0.92 (0.81–1.05)	
Physical activity (MET−h/wk)			
Below median	181,288	0.81 (0.68–0.98)	0.73
Above median	181,783	0.90 (0.75–1.08)	
Healthy diet score			
Below median	163,362	0.95 (0.79–1.15)	0.07
Above median	284,515	0.84 (0.73–0.98)	
Healthy lifestyle score			
≤2	288,569	0.86 (0.75–0.98)	0.19
>2	159,321	0.94 (0.76–1.16)	
Vitamin supplementation			
No	307,272	0.84 (0.72–0.99)	0.56
Yes	140,618	0.92 (0.77–1.09)	
Mineral supplementation			
No	393,686	0.91 (0.80–1.03)	0.16
Yes	54,204	0.72 (0.56–0.95)	
Aspirin use			
No	385,825	0.88 (0.78–1.00)	0.84
Yes	62,065	0.86 (0.66–1.12)	
Non steroidal anti−inflammatory drugs use			
No	274,590	0.94 (0.80–1.10)	0.56
Yes	168,463	0.82 (0.69–0.97)	
Oily fish (times/week)			
<1	198,077	0.85 (0.71–1.01)	0.45
≥1	247,127	0.89 (0.77–1.04)	
Non−oily fish (times/week)			
<1	151,356	0.84 (0.68–1.03)	0.58
≥1	294,196	0.90 (0.79–1.04)	

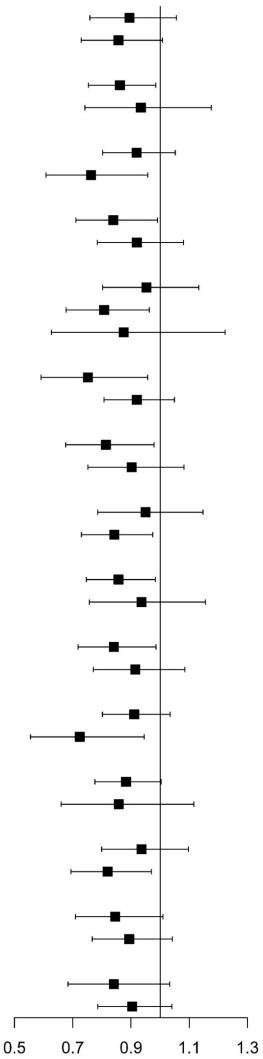


FIGURE 2 | The association of fish oil supplementation with inflammatory bowel disease risk in subgroup analyses. BMI, body mass index; MET, metabolic equivalent of task. Forest plots show the multivariable HRs of IBDs associated with fish oil use in subgroups. Model was adjusted for age, sex, race, assessment centers, BMI, education, Townsend deprivation index, household income, smoking status, alcohol consumption, physical activity, vitamin supplement use, mineral supplement aspirin use, hormone use, non-steroidal anti-inflammatory drugs use, and healthy diet score. Horizontal line represents 95% CIs.

and thus activate its anti-proliferative effects in lymphoid cells (47). The n-3 PUFAs also can decrease levels of secondary messengers involved in inflammation by inhibiting protein kinase C (PKC) activity (48). In addition, fish oil supplements may suppress cell-mediated immune responses by diminishing the expression of major histocompatibility complex class II molecules in peripheral blood monocytes (49). Furthermore, n-3 PUFAs reduced IL-1β and IL-18 gene expression in human adipocytes and macrophages (50). By upregulating gene expression of peroxisome proliferator-activated receptors, marine n-3 PUFAs could inhibit the activation of nuclear factor κB, which plays a key role in initiating genes encoding for inflammatory factors involving tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) (12). Last, n-3 PUFAs ameliorated gut microbiota composition and influenced the gut–brain axis, which increased the production of anti-inflammatory compounds, such as short-chain fatty acids, for the improvement of IBDs (15). Therefore, the interaction between n-3 PUFAs, immunity, and gut microbiota could be in favor of maintaining the intestinal wall integrity and communicating with host immune cells and alleviating the pathogenesis of IBDs. On the other hand, serum levels of CRP and albumin were all closely related to the occurrence of IBDs and are considered to be possible biomarkers for auxiliary diagnosis (30). Meta-analyses concluded that the intake of n-3 PUFA could significantly reduce the levels of inflammation markers, such as CRP, IL-6, and TNF-α (51), and elevated the serum level of albumin (52). Consistently, we showed that fish oil users had significantly lower levels of CRP and

higher serum levels of albumin, which contributed to a lower risk of IBDs. Besides, a slight association was found after further adjustment for CRP, which may be explained that n-3 PUFA supplementation could reduce the risk of IBDs by alleviating inflammatory response.

There are several advantages. The current study investigated the associations with large numbers of participants and cases and the long duration of an average of 8-year follow-up using a prospective design, which maximally reduces the possibility of reverse causality and allows us to further enable the IBD subtypes and various subgroup analyses. Besides, we focused on the incident IBDs associated with the habitual use of fish oil supplements, indicating a purer and higher intake of n-3 PUFAs than dietary n-3 PUFA intake, which also fills the gap of n-3 PUFA supplementation in relation to the risk of IBDs. Additionally, we have a wealth of information on socioeconomic characteristics, lifestyle and dietary factors, and other covariates, which largely eliminated the interference from confounding factors. Sensitivity analyses also demonstrated the robustness of our findings.

Several limitations should be noted in this study. First, we lacked detailed information about fish oil use, such as intake dosage, intake duration, and the ratio of EPA to DHA in fish oils, which might help us deeply analyze the associations, such as dose–response analysis and the long-term role of supplementation in the risk of IBDs. Although regularly taking fish oil supplements was registered, the frequency of fish oil use could not be acquired. Nonetheless, we conducted repeated surveys to assess the reproducibility and validity of fish oil use and found that more than half of fish oil users at baseline continuously intake the supplements all the time. Second, we only measured CRP and albumin levels at baseline, which might not reflect a long-term inflammation status among participants. Thus, our findings could not reveal the causal effect of fish oil use on the levels of CRP and albumin. Third, our association outcomes might be biased by residual confounding that was unable to be corrected, such as the family history of IBDs. Finally, due to the nature of the observational study design, the causal relationship between fish oil use and the risk of IBDs could not be established.

CONCLUSION

In conclusion, the habitual use of fish oil supplements was significantly associated with a lower incidence of IBDs and its subtype UC but not CD, which might partly be ascribed to the anti-inflammatory effect of marine n-3 PUFAs. Further studies should understand the pathogenesis of IBDs and the role of inflammatory improvement by various dietary components or supplements in the treatment of IBDs. Meanwhile, more trials with a large sample size and a long duration of follow-up are warranted to formulate the recommendations about intake of n-3 PUFA supplements to prevent the incident IBDs in the future.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data that support the findings of this study are available from UK Biobank project site, subject to successful registration and application process. Requests to access the datasets should be directed to https://biobank.ctsu.ox.ac.uk/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the United Kingdom North West Multi-centre Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JJ and PiZ conceived and designed the study and the guarantors and responsible for the authenticity and accuracy of the study. XH, YL, PaZ, and XL cleaned, analyzed, and interpreted the data. XH and YL completed the manuscript. PaZ and XL provided expertise and assistance with the statistic. YZ, PaZ, and XL revised the manuscript. All authors finally approved the draft, contributed to the article, and approved the submitted version.

FUNDING

There were some institutes supporting our study, including the Fundamental Research Funds for the Central Universities (grant no. 2021QNA7008) and the China National Program for Support of Top-notch Young Professionals. Funders were not involved in any work of the research.

ACKNOWLEDGMENTS

The authors appreciated the participants and staff of the UK Biobank for their contributions and took full responsibility for the analyses and interpretation of these data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.905162/full#supplementary-material

REFERENCES

1. GBD 2017 Inflammatory Bowel Disease Collaborators. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol.* (2020) 5:17–30. doi: 10.1016/s2468-1253(19)30333-4

2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature.* (2007) 448:427–34. doi: 10.1038/nature06005
3. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. *World J Gastroenterol.* (2014) 20:91–9. doi: 10.3748/wjg.v20.i1.91
4. Bernstein CN, Eliakim A, Fedail S, Fried M, Gearry R, Goh KL, et al. World gastroenterology organisation global guidelines inflammatory bowel disease: update august 2015. *J Clin Gastroenterol.* (2016) 50:803–18. doi: 10.1097/mcg.0000000000000660
5. Taleban S, Colombel JF, Mohler MJ, Fain MJ. Inflammatory bowel disease and the elderly: a review. *J Crohns Colitis.* (2015) 9:507–15. doi: 10.1093/ecco-jcc/jjv059
6. Levine A, Sigall Boneh R, Wine E. Evolving role of diet in the pathogenesis and treatment of inflammatory bowel diseases. *Gut.* (2018) 67:1726–38. doi: 10.1136/gutjnl-2017-315866
7. Barden AE, Mas E, Mori TA. N-3 Fatty acid supplementation and proresolving mediators of inflammation. *Curr Opin Lipidol.* (2016) 27:26–32. doi: 10.1097/mol.0000000000000262
8. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans.* (2017) 45:1105–15. doi: 10.1042/bst20160474
9. Shahidi F, Ambigaipalan P. Omega-3 polyunsaturated Fatty acids and their health benefits. *Annu Rev Food Sci Technol.* (2018) 9:345–81. doi: 10.1146/annurev-food-111317-095850
10. Xiao P, Zhang H, Zhang Y, Zheng M, Liu R, Zhao Y, et al. Phosphatase Shp2 exacerbates intestinal inflammation by disrupting macrophage responsiveness to interleukin-10. *J Exp Med.* (2019) 216:337–49. doi: 10.1084/jem.20181198
11. Quiros M, Feier D, Birkl D, Agarwal R, Zhou DW, García AJ, et al. Resolvin E1 is a pro-repair molecule that promotes intestinal epithelial wound healing. *Proc Natl Acad Sci U S A.* (2020) 117:9477–82. doi: 10.1073/pnas.1921335117
12. Calder PC. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochim Biophys Acta.* (2015) 1851:469–84. doi: 10.1016/j.bbalip.2014.08.010
13. Yao J, Lu Y, Zhi M, Hu P, Wu W, Gao X. Dietary n-3 polyunsaturated fatty acids ameliorate Crohn's disease in rats by modulating the expression of PPAR−γ/NFAT. *Mol Med Rep.* (2017) 16:8315–22. doi: 10.3892/mmr.2017.7673
14. Marton LT, Goulart RA, Carvalho ACA, Barbalho SM. Omega fatty acids and inflammatory bowel diseases: an overview. *Int J Mol Sci.* (2019) 20:4851. doi: 10.3390/ijms20194851
15. Costantini L, Molinari R, Farinon B, Merendino N. Impact of omega-3 fatty acids on the gut microbiota. *Int J Mol Sci.* (2017) 18:2645. doi: 10.3390/ijms18122645
16. Lorente-Cebrián S, Costa AG, Navas-Carretero S, Zabala M, Laiglesia LM, Martínez JA, et al. An update on the role of omega-3 fatty acids on inflammatory and degenerative diseases. *J Physiol Biochem.* (2015) 71:341–9. doi: 10.1007/s13105-015-0395-y
17. Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev.* (2010) 68:280–9. doi: 10.1111/j.1753-4887.2010.00287.x
18. Rashvand S, Somi MH, Rashidkhani B, Hekmatdoost A. Dietary fatty acid intakes are related to the risk of ulcerative colitis: a case-control study. *Int J Colorectal Dis.* (2015) 30:1255–60. doi: 10.1007/s00384-015-2232-8
19. Sakamoto N, Kono S, Wakai K, Fukuda Y, Satomi M, Shimoyama T, et al. Dietary risk factors for inflammatory bowel disease: a multicenter case-control study in Japan. *Inflamm Bowel Dis.* (2005) 11:154–63. doi: 10.1097/00054725-200502000-00009
20. Ananthakrishnan AN, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Fuchs CS, et al. Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. *Gut.* (2014) 63:776–84. doi: 10.1136/gutjnl-2013-305304
21. Chan SS, Luben R, Olsen A, Tjonneland A, Kaaks R, Lindgren S, et al. Association between high dietary intake of the n-3 polyunsaturated fatty acid docosahexaenoic acid and reduced risk of Crohn's disease. *Aliment Pharmacol Ther.* (2014) 39:834–42. doi: 10.1111/apt.12670
22. Malinowski B, Wiciński M, Sokołowska MM, Hill NA, Szambelan M. The rundown of dietary supplements and their effects on inflammatory bowel disease-a review. *Nutrients.* (2020) 12:1423. doi: 10.3390/nu12051423
23. Tabbaa M, Golubic M, Roizen MF, Bernstein AM. Docosahexaenoic acid, inflammation, and bacterial dysbiosis in relation to periodontal disease, inflammatory bowel disease, and the metabolic syndrome. *Nutrients.* (2013) 5:3299–310. doi: 10.3390/nu5083299
24. Mozaffari H, Daneshzad E, Larijani B, Bellissimo N, Azadbakht L. Dietary intake of fish, n-3 polyunsaturated fatty acids, and risk of inflammatory bowel disease: a systematic review and meta-analysis of observational studies. *Eur J Nutr.* (2020) 59:1–17. doi: 10.1007/s00394-019-01901-0
25. Wang F, Lin X, Zhao Q, Li J. Fat intake and risk of ulcerative colitis: systematic review and dose-response meta-analysis of epidemiological studies. *J Gastroenterol Hepatol.* (2017) 32:19–27. doi: 10.1111/jgh.13416
26. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* (2015) 12:e1001779. doi: 10.1371/journal.pmed.1001779
27. Tyrrell J, Jones SE, Beaumont R, Astley CM, Lovell R, Yaghootkar H, et al. Height, body mass index, and socioeconomic status: mendelian randomisation study in UK Biobank. *BMJ.* (2016) 352:i582. doi: 10.1136/bmj.i582
28. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med.* (2020) 54:1451–62. doi: 10.1136/bjsports-2020-102955
29. Said MA, Verweij N, van der Harst P. Associations of combined genetic and lifestyle risks with incident cardiovascular disease and diabetes in the UK Biobank Study. *JAMA Cardiol.* (2018) 3:693–702. doi: 10.1001/jamacardio.2018.1717
30. Sands BE. Biomarkers of inflammation in inflammatory bowel disease. *Gastroenterology.* (2015) 149:1275–85.e2. doi: 10.1053/j.gastro.2015.07.003
31. Raison CL, Pikalov A, Siu C, Tsai J, Koblan K, Loebel A. C-reactive protein and response to lurasidone in patients with bipolar depression. *Brain Behav Immun.* (2018) 73:717–24. doi: 10.1016/j.bbi.2018.08.009
32. Meyers TJ, Weiner AB, Graff RE, Desai AS, Cooley LF, Catalona WJ, et al. Association between inflammatory bowel disease and prostate cancer: a large-scale, prospective, population-based study. *Int J Cancer.* (2020) 147:2735–42. doi: 10.1002/ijc.33048
33. Ma H, Li X, Zhou T, Sun D, Liang Z, Li Y, et al. Glucosamine use, inflammation, and genetic susceptibility, and incidence of type 2 diabetes: a prospective study in UK Biobank. *Diabetes Care.* (2020) 43:719–25. doi: 10.2337/dc19-1836
34. Menees SB, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. *Am J Gastroenterol.* (2015) 110:444–54. doi: 10.1038/ajg.2015.6
35. Weaving G, Batstone GF, Jones RG. Age and sex variation in serum albumin concentration: an observational study. *Ann Clin Biochem.* (2016) 53(Pt 1):106–11. doi: 10.1177/0004563215593561
36. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology.* (2012) 142:46–54. doi: 10.1053/j.gastro.2011.10.001
37. Maconi G, Ardizzone S, Cucino C, Bezzio C, Russo AG, Bianchi Porro G. Pre-illness changes in dietary habits and diet as a risk factor for inflammatory bowel disease: a case-control study. *World J Gastroenterol.* (2010) 16:4297–304. doi: 10.3748/wjg.v16.i34.4297
38. Pugazhendhi S, Sahu MK, Subramanian V, Pulimood A, Ramakrishna BS. Environmental factors associated with Crohn's disease in India. *Indian J Gastroenterol.* (2011) 30:264–9. doi: 10.1007/s12664-011-0145-1
39. Tjonneland A, Overvad K, Bergmann MM, Nagel G, Linseisen J, Hallmans G, et al. Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. *Gut.* (2009) 58:1606–11. doi: 10.1136/gut.2008.169078
40. John S, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. *Eur J Gastroenterol Hepatol.* (2010) 22:602–6. doi: 10.1097/MEG.0b013e3283352d05
41. Abubakar I, Myhill DJ, Hart AR, Lake IR, Harvey I, Rhodes JM, et al. A case-control study of drinking water and dairy products in Crohn's disease–further investigation of the possible role of mycobacterium avium paratuberculosis. *Am J Epidemiol.* (2007) 165:776–83. doi: 10.1093/aje/kwk067
42. Rashvand S, Somi MH, Rashidkhani B, Hekmatdoost A. Dietary protein intakes and risk of ulcerative colitis. *Med J Islam Repub Iran.* (2015) 29:253.

43. Amre DK, D'Souza S, Morgan K, Seidman G, Lambrette P, Grimard G, et al. Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children. *Am J Gastroenterol.* (2007) 102:2016–25. doi: 10.1111/j.1572-0241.2007.01411.x
44. Starr AE, Deeke SA, Ning Z, Chiang CK, Zhang X, Mottawea W, et al. Proteomic analysis of ascending colon biopsies from a paediatric inflammatory bowel disease inception cohort identifies protein biomarkers that differentiate Crohn's disease from UC. *Gut.* (2017) 66:1573–83. doi: 10.1136/gutjnl-2015-310705
45. Meister D, Ghosh S. Effect of fish oil enriched enteral diet on inflammatory bowel disease tissues in organ culture: differential effects on ulcerative colitis and Crohn's disease. *World J Gastroenterol.* (2005) 11:7466–72. doi: 10.3748/wjg.v11.i47.7466
46. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr.* (2000) 71:343s–8s. doi: 10.1093/ajcn/71.1.343s
47. Basiouni S, Fuhrmann H, Schumann J. The influence of polyunsaturated fatty acids on the phospholipase D isoforms trafficking and activity in mast cells. *Int J Mol Sci.* (2013) 14:9005–17. doi: 10.3390/ijms14059005
48. Russell FD, Bürgin-Maunder CS. Distinguishing health benefits of eicosapentaenoic and docosahexaenoic acids. *Mar Drugs.* (2012) 10:2535–59. doi: 10.3390/md10112535
49. Gan RW, Demoruelle MK, Deane KD, Weisman MH, Buckner JH, Gregersen PK, et al. Omega-3 fatty acids are associated with a lower prevalence of autoantibodies in shared epitope-positive subjects at risk for rheumatoid arthritis. *Ann Rheum Dis.* (2017) 76:147–52. doi: 10.1136/annrheumdis-2016-209154
50. Lee KR, Midgette Y, Shah R. Fish oil derived omega 3 fatty acids suppress adipose NLRP3 inflammasome signaling in human obesity. *J Endocr Soc.* (2019) 3:504–15. doi: 10.1210/js.2018-00220
51. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor α: a meta-analysis. *PLoS One.* (2014) 9:e88103. doi: 10.1371/journal.pone.0088103
52. Mocellin MC, Fernandes R, Chagas TR, Trindade E. A meta-analysis of n-3 polyunsaturated fatty acids effects on circulating acute-phase protein and cytokines in gastric cancer. *Clin Nutr.* (2018) 37:840–50. doi: 10.1016/j.clnu.2017.05.008

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.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 26 July 2022
DOI 10.3389/fnut.2022.952451

OPEN ACCESS

EDITED BY
Ioannis Zabetakis,
University of Limerick, Ireland

REVIEWED BY
Lu Zhao Liang,
Jinan University, China
Ziyi Li,
Guangdong Second Provincial General Hospital, China

*CORRESPONDENCE
Hui-Lian Zhu
zhuhl@mail.sysu.edu.cn
Zhao-Yan Liu
liuzhy235@mail.sysu.edu.cn

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 25 May 2022
ACCEPTED 05 July 2022
PUBLISHED 26 July 2022

CITATION
Lu X-T, Wang Y-D, Zhu T-T, Zhu H-L and Liu Z-Y (2022) Dietary fatty acids and risk of non-alcoholic steatohepatitis: A national study in the United States. *Front. Nutr.* 9:952451. doi: 10.3389/fnut.2022.952451

Dietary fatty acids and risk of non-alcoholic steatohepatitis: A national study in the United States

Xiao-Ting Lu[1,2], Yong-Dong Wang[3], Ting-Ting Zhu[4], Hui-Lian Zhu[1,2]* and Zhao-Yan Liu[1,2]*

[1]Department of Nutrition, School of Public Health, Sun Yat-sen University, Guangzhou, China, [2]Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou, China, [3]Department of Internal Medicine, Shaoguan First People's Hospital, Shaoguan, China, [4]Department of Food Science and Engineering, School of Food Science and Engineering, Hainan Tropical Ocean University, Sanya, China

Background: Non-alcoholic steatohepatitis (NASH), the early invertible stage of non-alcoholic fatty liver disease, has become a public health challenge due to the great burden and lack of effective treatment. Dietary nutrients are one of the modifiable factors to prevent and slow down disease progression. However, evidence linking dietary fatty acids intake and risk of NASH is lacking.

Objectives: This study aimed to examine the association between dietary total saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), their subtypes, the ratio of unsaturated (UFAs) to SFAs, and the risk of NASH among a nationwide population in the United States.

Methods: This cross-sectional study was conducted among 4,161 adults in the national health and nutrition examination survey in 2017–2018 cycle. Moreover, NASH was defined by transient elastography. Dietary fatty acids were assessed using a validated 24-h food recall method. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs).

Results: A total of 2,089 (50.2%) participants with NASH were identified. Compared with participants in the bottom tercile of dietary intakes of total PUFAs, those in the highest tercile had lower risk of NASH, with an adjusted OR of 0.67 (95% CI: 0.46–0.97). Similar associations were found between the subtype of PUFA 18:3 and NASH, while the fully adjusted OR in the highest tercile was 0.67 (95% CI: 0.47–0.96). Interactions of dietary PUFAs and body mass index (BMI) could be found influencing NASH risk. Stronger associations of dietary total PUFAs intakes with NASH risk were found in obese participants (OR, 95% CI: 0.41, 0.22–0.75) than in the non-obese participants (OR, 95% CI: 1.00, 0.70–1.43; p-interaction = 0.006). Similar effects on risk of NASH were also observed between BMI and dietary intakes of PUFA 18:3. However, no

significant associations were observed between NASH risk and dietary total SFAs, MUFAs, their subtypes as well as the ratio of UFAs to SFAs.

Conclusion: Dietary intakes of total PUFAs, as well as its subtype of PUFA 18:3, were inversely associated with risk of NASH. The further large prospective studies need to be conducted to confirm the findings of this study.

KEYWORDS

polyunsaturated fatty acids, non-alcoholic steatohepatitis, subtypes of fatty acids, dietary fatty acids, national study, national health and nutrition examination survey

Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the most common causes of chronic liver disease, as well as the most quickly growing promoters to liver morbidity and mortality in the United States (1). The prevalence of NAFLD has increased rapidly over the past three decades (1, 2) and the number of people suffering from NAFLD are expected to be more than 100 million by 2030 (3). Usually, parallels to the prevalence of obesity, type 2 diabetes and cardiovascular disease, NAFLD decreases life expectancy and increases requirements of liver transplantation (4, 5). Moreover, NAFLD progresses from steatosis, non-alcoholic steatohepatitis (NASH), to liver fibrosis and even ultimately to hepatocellular carcinoma (4), while approximately 20–30% of individuals may progress to NASH (6). It is estimated that NASH prevalence will increase up to 56% by 2030 in the United States at a greater rate than the other European and Asian countries (3). Due to the persistent cellular damage and excess fat deposition, patients with NASH are more likely to progress to the irreversible advanced fibrosis (4). Given the great burden as well as the lack of effective treatment, it is important to prevent and slow down disease progression at the early stage. The dietary nutrients are considered to be one of the effective and modifiable factors (7).

Fatty acids are composed of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs), the latter includes monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). They can also be subdivided into a number of subtypes according to the position and numbers of carbon atoms and double bonds. Fatty acids play an important and indispensable role in human diet and body metabolism, which not only are sources of essential fatty acids and precursors of bioactive substances but also involve in bio-membrane structure formation, signal transmission, and lipid transportation. Liver is the main metabolic organ of fatty acids. Improper dietary fatty acids consumption or *de novo* lipogenesis beyond liver capacity has the consequences of abnormal cellular lipid composition and toxic lipid accumulation, leading to organelle dysfunction, cellular damage, inflammation, and occurrence of diseases (8). However, the results from epidemiological studies linking the relationships between dietary fatty acids and NAFLD are sparse and contradictory. For example, a systematic review reported that patients with NAFLD had disturbed fatty acids metabolisms compared to healthy controls (6). Evidences from the previous studies demonstrated that people with NASH possessed elevated total SFAs, while total MUFAs and PUFAs may be protective (9). However, a non-linear association between total PUFAs intakes and NAFLD was found among Chinese Han adults, the total PUFAs was positively associated with risk of NAFLD at certain dose of total PUFAs consumption (10). The reasons for the discrepancies could be ascribed to differences of sample sizes, population, and different detect methods of NAFLD.

Liver biopsy, the gold standard of NAFLD assessments, is unsuitable for population screening due to its invasion. Other methods of detection such as hepatic steatosis index (11), fatty liver index (12), NAFLD liver fat score (13), SteatoTest (14), which are calculated by individual conditions and blood biomarkers, have several shortages such as limited sensitivity, specificity, popularization or expensive price. A non-invasive and effective approach to distinguish NAFLD as well as its stages is urgently needed. Compared with liver biopsy, transient elastography is a non-invasive, convenient and fast method to assess NAFLD from NASH to liver fibrosis, but few studies apply it to diagnosis of NAFLD in a large population.

Furthermore, different fatty acids subtypes have different effects on liver health. For example, as for hepatic fatty acids compositions, a decrease in the ratio of SFA 18:0 to SFA 16:0 was associated with the steatosis score and insulin resistance, while higher ratio of MUFA 16:1 to SFA 16:0 was associated

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; NHANES, national health and nutrition examination survey; MEC, mobile examination center; CAP, controlled attenuation parameter; HEI-2015, healthy eating index-2015; BMI, body mass index; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; SE, standard error; OR, odds ratio; CI, confidence interval; SREBP-1c, sterol regulatory element-binding protein-1c; PPAR-α, peroxisome proliferator-activated receptor-alpha; TNF-α, tumor necrosis factor-alpha; NLRP3, NOD-like receptor protein 3.

with lobular inflammation and hepatocellular ballooning in patients with NASH (15). However, none of the existing studies have investigated the association between different subtypes of dietary fatty acids and the risk of NAFLD. Therefore, in this study, we aimed to examine the association between dietary total SFAs, MUFAs, PUFAs, their subtypes, the ratio of UFAs to SFAs, and the risk of NASH (the early invertible stage of NAFLD), which was determined by liver ultrasound transient elastography among a nationwide population in the United States.

Materials and methods

Study population

The analysis of this study was conducted based on data from national health and nutrition examination survey (NHANES), which was carried out by the Center for Disease Control and Prevention of the United States. Detailed information could be found from the official website[1]. In this study, we included participants in the 2017–2018 NHANES cycle (n = 9,254). Participants were excluded if they were underage (<18 years, n = 3,398), had unavailable data on transient elastography (n = 737) or dietary assessment (n = 445), were hepatitis C virus (n = 44) or hepatitis B virus (n = 18) infected, or had heavy alcohol consumption (>30 g/day for men and >20 g/day for women, n = 451). Finally, 4,161 participants were included (**Figure 1**). Due to the discrepancies between adults and minors in many aspects, we only compare the characteristics of adults (≥18 years) in the included and the excluded participants. The included participants did not differ by most of the basic characteristics from adults excluded from this analysis (n = 1,695, **Supplementary Table 1**). The institutional review board approval of the National Center for Health Statistics and informed consent was obtained from all participants before data collection.

Assessment and definition of non-alcoholic steatohepatitis

The liver ultrasound transient elastography was first used to provide objective measures for hepatic steatosis in the NHANES Mobile Examination Center (MEC) in the 2017–2018 cycle (16). The participants who were aged ≥12 years, were able to lie down, were not pregnant, had no implanted electronic medical device, and had no lesions where measurements would be taken were eligible to test. Using the FibroScan model 502 V2 Touch equipped with a medium or extra-large probe, controlled attenuation parameter (CAP) was measured and recorded as the indicator for hepatic steatosis according to the fatness in the liver. The elastography exam was performed by well-trained NHANES health technicians according to the manufacture guidelines. The inter-rater reliability between health technician and reference examiners was 0.94 (mean differences 4.5 ± 19.8 dB/m), and variances within and between the device machines and the probes over time were under control (intra-machine coefficient of variation was 1.2–3.2%; inter-machine intra class correlation was 2–22%) for CAP. High accuracy of the CAP measurements for the detection of steatosis compared to biopsy has been reported in the previous studies (17–19).

In this study, those CAP scores equal or greater than 263 dB/m (had a 96% positive predictive value) were defined as cases of NASH (20), others as controls (non-NASH). The cut-off point was determined before statistical analysis.

Assessment of dietary fatty acids

Assessment of dietary intakes was undertaken by trained interviewers using a validated 24-h food recall method in the MEC, and was repeated by telephone 3–10 days later. Values of dietary intakes in these two discrete days were averaged to represent their dietary status, while values of the first time were used for participants with a lack of dietary data of the second time (12.2% in the current analysis). The intakes of total energy, SFAs, MUFAs, PUFAs, and their subtypes were estimated according to the United States Department of Agriculture's Food and Nutrient Database for Dietary Studies. The healthy eating index-2015 (HEI-2015) was calculated according to the MyPyramid Equivalents Database 2.0 for United States Department of Agriculture Survey Foods to reflect the quality of diet comprehensively (**Supplementary Table 2**). A higher score between 0–100 indicated a better dietary quality. The ratio of UFAs to SFAs was calculated as (PUFAs + MUFAs)/SFAs, and therefore divided into three levels according to the scoring standards of HEI-2015.

Covariates collection

Sociodemographic information including age, sex (men and women), ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and others), marital status (married/living with partner, separated/divorced/widowed, and never married), education levels (less than high school, high school or equivalent, and college or above), and family income-to-poverty ratio (<1.3, 1.3–3.5, and >3.5) were collected. Anthropometric measurements including height, weight, and waist circumference were measured. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). Never

1 https://www.cdc.gov/nchs/nhanes/

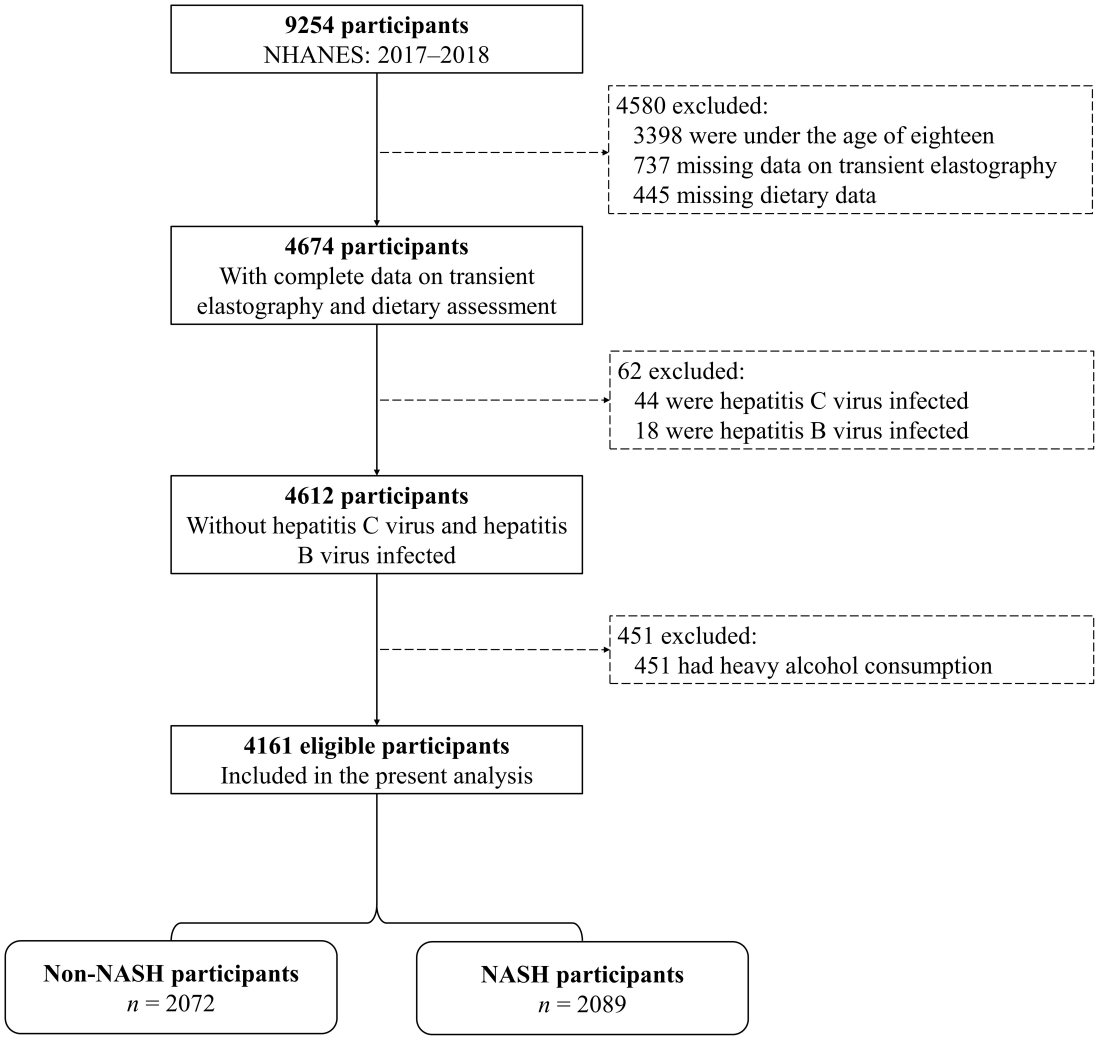


FIGURE 1
Flowchart of selection of participants from national health and nutrition examination survey (NHANES). Abbreviations: NASH, non-alcoholic steatohepatitis.

smokers were participants who smoked less than 100 cigarettes in their lifetime. Former smokers were those who had given up smoking before the interview, and current smokers were those who smoked more than 100 cigarettes in their whole life and kept the habit of smoking at the time of interview. The participants who had regular exercise were defined as those who reported that they had moderate- or vigorous-intensity physical activities at least 10 min in a typical week, in succession with small or large increases in heart rate or breathing. Those who were exposed to oral corticosteroid medication for more than 180 days were defined as the presence of use of oral corticosteroid. Hypertension was defined as elevated blood pressure (systolic/diastolic blood pressure equal or higher than 140/90 mm Hg), self-reported hypertension diagnosis by clinician or taking anti-hypertensive drugs. Diabetes mellitus was defined as fasting plasma glucose concentration $\geq$ 7.0 mmol/L, glycosylated hemoglobin level $\geq$ 6.5%, self-reported diabetes diagnosis, or use of diabetic pills (including insulin). Dyslipidemia was defined if any of the following status was matched: (1) Total cholesterol $\geq$ 200 mg/dl, (2) triglyceride $\geq$ 150 mg/dl, (3) low-density lipoprotein cholesterol $\geq$ 130 mg/dl, (4) high-density lipoprotein cholesterol $<$ 40 mg/dl or $<$50 mg/dl for men and women, respectively, (5) self-reported taking prescribed lipid-modifying medication. Cardiovascular disease was defined as self-reported diagnosis of angina, congestive heart failure, coronary heart disease, heart attack, or stroke. Cancer was defined as self-reported diagnosis of any kind of cancer by clinician during the whole lifetime.

Three consecutive blood pressure measurements were obtained after resting quietly in a seated position for 5 min in the MEC according to the physician examination procedures

manual (21). A fourth determination would be taken if a blood pressure measurement was interrupted or incomplete. The values of these three or four readings were averaged to represent their blood pressure status. Blood collection took place in the MEC under standardized conditions at each survey location including collecting, processing, storing, and shipping. Laboratory parameters including alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), glucose, glycosylated hemoglobin, total cholesterol, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were measured using corresponding methods described in the official website (22).

Statistical analysis

Given to the complex sampling design, appropriate sample weight was conducted according to NHANES analytic guidelines in the current analysis. Non-normally distributed data were natural logarithm transformed and estimates of fatty acids intakes were adjusted for energy intakes using the residuals method (23) before further analysis. The basic characteristics and dietary intakes of fatty acids of participants (overall, non-NASH, and NASH) were described by the weighted mean and standard error (SE) for continuous variables, as well as counts and weighted frequencies for categorical variables. The differences of basic characteristics between participants with and without NASH were compared by general linear models and Chi-squared test as appropriate. Analyses of covariance (ANCOVA) controlling for sex, age, and BMI were used for comparison of the mean differences in dietary intakes of energy and fatty acids.

Participants were divided into three groups according to terciles in the non-NASH group of dietary intakes of total SFAs, MUFAs, PUFAs and their subtypes. Logistic regression models were performed to examine the association between the dietary intakes of fatty acids and the risk of NASH. According to the previous studies (24) and the specialized knowledge, several covariates were selected for adjustments to minimize the residual confounding. Minimally adjusted models included age (continuous), sex (categorized), and BMI (continuous). Other potential risk factors, including ethnicity (categorized), marital status (categorized), education levels (categorized), family income-to-poverty ratio (categorized), waist circumference (continuous), smoking status (categorized), regular exercise (categorized), use of oral corticosteroid (categorized), HEI-2015 (continuous), ALT (continuous), ALP (continuous), AST (continuous), GGT (continuous), prevalence of hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, and cancer (categorized) were additionally adjusted in the fully adjusted models. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with the lowest terciles as the reference. Since only a few covariates were missing with a small portion, observations with missing data were automatically excluded from the corresponding adjusted models. Sensitivity analysis was conducted among participants without use of oral corticosteroid (n = 4,118). Stratified analysis was performed to examine whether the association between terciles of dietary fatty acids intakes and risk of NASH was different in participants with various characteristics. Interactions were estimated by including the multiplicative interaction terms.

The data were analyzed from January 2022 to May 2022. Statistical analyses were performed using the R software 4.1.0 (the "survey" package) and the SPSS, version 25.0 (IBM Corp., Armonk, NY, United States). All tests were two-sided and $p < 0.05$ was considered statistically significant.

Results

Basic characteristics and dietary intakes of fatty acids of study participants

Weighted distributions of sociodemographic information, lifestyle, laboratory parameters, and prevalence of several chronic diseases for overall population, participants with and without NASH were shown in **Table 1**. Of the 4,161 study participants, 48.6% were men and mean (SE) age was 47.5 (0.8) years. The participants in the current study tended to be obese with a mean BMI of 29.8 (0.3) kg/m^2, and a total of 2,089 (50.2%) participants with NASH were identified. Compared with non-NASH participants, those with NASH were more likely to be older, had a higher BMI, higher waist circumference, higher levels of ALT, ALP, AST, and GGT, higher prevalence of hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, and cancer, had lower education levels, and lower frequencies of regular exercise ($p < 0.05$). A significantly greater proportion of participants with NASH were men, were Mexican American, were married, and were former or current smokers ($p < 0.05$). No significant differences were observed in family income-to-poverty ratio and use of oral corticosteroid between participants with and without NASH ($p > 0.05$).

With regard to dietary intakes of overall participants, energy intakes controlling for sex, age, and BMI were 1,980.53 (12.21) kcal/day. Mean (SE) of dietary total SFAs, MUFAs and PUFAs adjusted for energy intakes, sex, age, and BMI were 23.21 (0.11) g/day, 24.97 (0.11) g/day, and 17.21 (0.09) g/day (**Table 2**). Compared with non-NASH participants, those with NASH had lower dietary intakes of total MUFAs, total PUFAs, MUFA 18:1, PUFA 18:2, PUFA 20:4, and lower score in HEI-2015 ($p < 0.05$). Other dietary intakes including energy intake, total SFAs, the ratio of UFAs and SFAs, and other 16 subtypes of fatty acids did not show significant difference between participants with and without NASH.

TABLE 1 Basic characteristics of participants of this study.

	Overall (n = 4,161)	Non-NASH (n = 2,072)	NASH (n = 2,089)	p
Age, years	47.5 ± 0.8	43.8 ± 0.9	51.3 ± 0.8	<0.001
Sex, n (%)				<0.001
Men	2,015 (48.6)	915 (43.9)	1,100 (53.7)	
Women	2,146 (51.4)	1,157 (56.1)	989 (46.3)	
Ethnicity, n (%)				<0.001
Non-Hispanic white	1,442 (62.6)	695 (63.0)	747 (62.1)	
Non-Hispanic black	971 (11.4)	571 (13.3)	400 (9.3)	
Mexican American	582 (9.1)	205 (6.3)	377 (12.0)	
Others	1,166 (17.0)	601 (17.4)	565 (16.6)	
Marital status, n (%)				<0.001
Married/living with partner	2,340 (60.0)	1,056 (53.1)	1,284 (67.4)	
Separated/divorced/widowed	885 (18.0)	424 (18.1)	461 (17.9)	
Never married	711 (18.1)	426 (22.9)	285 (12.9)	
Education levels, n (%)				0.002
Less than high school	753 (10.4)	350 (9.5)	403 (11.3)	
High school or equivalent	956 (27.0)	456 (25.1)	500 (29.0)	
College or above	2,225 (58.7)	1,099 (59.4)	1,126 (58.0)	
Family income-to-poverty ratio, n (%)				0.092
<1.3	1,039 (20.0)	543 (20.7)	496 (19.1)	
1.3–3.5	1,552 (37.6)	732 (34.7)	820 (40.7)	
>3.5	1,068 (42.4)	547 (44.6)	521 (40.1)	
BMI, kg/m^2	29.8 ± 0.3	26.3 ± 0.3	33.6 ± 0.3	<0.001
Waist circumference, cm	100.7 ± 0.8	91.3 ± 0.9	110.9 ± 0.8	<0.001
Smoking status, n (%)				0.001
Never smoker	2,517 (60.2)	1,303 (63.1)	1,214 (57.1)	
Former smoker	958 (24.1)	386 (19.7)	572 (28.7)	
Current smoker	686 (15.7)	383 (17.2)	303 (14.1)	
Regular exercise, n (%)	1,980 (53.8)	1,097 (60.7)	883 (46.5)	<0.001
Use of oral corticosteroid ≥ 180 days, n (%)	43 (0.9)	22 (0.9)	21 (0.9)	0.949
Laboratory parameters, IU/L				
ALT	22.5 ± 0.3	19.1 ± 0.3	26.1 ± 0.6	<0.001
ALP	77.4 ± 0.7	74.2 ± 1.2	80.8 ± 0.9	0.001
AST	21.5 ± 0.2	20.8 ± 0.3	22.4 ± 0.4	0.014
GGT	27.8 ± 0.6	22.5 ± 0.7	33.4 ± 0.9	<0.001
Prevalence of chronic diseases, n (%)				
Hypertension	1,854 (38.3)	722 (26.2)	1,132 (51.2)	<0.001
Diabetes mellitus	877 (15.4)	218 (5.6)	659 (25.9)	<0.001
Dyslipidemia	2,753 (65.0)	1,121 (53.2)	1,632 (77.7)	<0.001
Cardiovascular disease	442 (8.3)	166 (5.3)	276 (11.6)	<0.001
Cancer	415 (10.6)	177 (9.1)	238 (12.1)	<0.001

NASH, non-alcoholic steatohepatitis; BMI, body mass index; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; and GGT, gamma-glutamyl transferase.
Data were presented as weighted mean ± SE or counts (weighted frequencies).

Dietary intakes of fatty acids and risk of non-alcoholic steatohepatitis

Associations between terciles of dietary total SFAs, MUFAs, PUFAs, their subtypes, the ratio of UFAs to SFAs and NASH were presented in Table 3. An inverse association between dietary total PUFAs and NASH risk was found, with an OR of 0.67 (95% CI: 0.46–0.97) at the highest tercile in comparison with the bottom tercile after adjustments for potential risk factors. Similar associations were found between the subtype

TABLE 2 Dietary fatty acids intakes of participants of this study.

Dietary intakes	Overall (n = 4,161)	Non-NASH (n = 2,072)	NASH (n = 2,089)	p
Energy intake, kcal/day[a]	1,980.53 ± 12.21	1,974.64 ± 18.68	1,986.43 ± 18.52	0.674
Total SFAs, g/day[b]	23.21 ± 0.11	23.20 ± 0.16	23.23 ± 0.16	0.909
Total MUFAs, g/day[b]	24.97 ± 0.11	25.23 ± 0.16	24.72 ± 0.16	0.036
Total PUFAs, g/day[b]	17.21 ± 0.09	17.48 ± 0.14	16.95 ± 0.14	0.015
The ratio of UFAs to SFAs[c]				0.936
≤1.2	453 (12.0)	226 (11.8)	227 (12.2)	
1.2–2.5	3,070 (76.1)	1,508 (76.1)	1,562 (76.0)	
≥2.5	637 (12.0)	337 (12.1)	300 (11.8)	
Subtypes of fatty acids, g/day[b]				
SFA 4:0	0.394 ± 0.004	0.390 ± 0.007	0.397 ± 0.007	0.492
SFA 6:0	0.261 ± 0.003	0.261 ± 0.004	0.262 ± 0.004	0.819
SFA 8:0	0.223 ± 0.003	0.224 ± 0.004	0.222 ± 0.004	0.705
SFA 10:0	0.441 ± 0.004	0.441 ± 0.007	0.441 ± 0.007	0.988
SFA 12:0	0.805 ± 0.016	0.818 ± 0.025	0.793 ± 0.024	0.498
SFA 14:0	1.890 ± 0.016	1.880 ± 0.024	1.900 ± 0.024	0.591
SFA 16:0	12.875 ± 0.054	12.866 ± 0.083	12.884 ± 0.082	0.886
SFA 18:0	5.489 ± 0.028	5.480 ± 0.043	5.499 ± 0.042	0.763
MUFA 16:1	1.024 ± 0.008	1.035 ± 0.012	1.013 ± 0.012	0.207
MUFA 18:1	23.421 ± 0.099	23.658 ± 0.152	23.185 ± 0.151	0.038
MUFA 20:1	0.286 ± 0.003	0.290 ± 0.005	0.281 ± 0.005	0.198
MUFA 22:1	0.033 ± 0.002	0.034 ± 0.003	0.032 ± 0.003	0.536
PUFA 18:2	15.231 ± 0.085	15.469 ± 0.130	14.993 ± 0.129	0.015
PUFA 18:3	1.621 ± 0.012	1.641 ± 0.018	1.601 ± 0.018	0.140
PUFA 18:4	0.013 ± 0.001	0.012 ± 0.001	0.013 ± 0.001	0.653
PUFA 20:4	0.148 ± 0.002	0.152 ± 0.002	0.144 ± 0.002	0.034
PUFA 20:5	0.033 ± 0.001	0.032 ± 0.002	0.033 ± 0.002	0.744
PUFA 22:5	0.025 ± 0.001	0.026 ± 0.001	0.024 ± 0.001	0.368
PUFA 22:6	0.072 ± 0.003	0.071 ± 0.004	0.073 ± 0.004	0.764
HEI-2015	51.2 ± 0.8	52.2 ± 0.9	50.1 ± 0.7	0.008

NASH, non-alcoholic steatohepatitis; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; UFAs, unsaturated fatty acids; and HEI-2015, healthy eating index-2015.
Data were presented as mean ± SE or counts (weighted frequencies).
[a]Data were assessed with ANCOVA controlling for sex, age, and BMI.
[b]Dietary intakes of fatty acids were adjusted for energy intakes using the residuals method first, and then were assessed with ANCOVA controlling for sex, age, and BMI.
[c]The ratio of UFAs to SFAs was calculated as (PUFAs + MUFAs)/SFAs.

of PUFAs 18:3 and NASH, while the fully adjusted OR in the highest tercile was 0.67 (95% CI: 0.47–0.96). Participants who were in the second tercile of total MUFAs and MUFA 18:1, rather than in the highest tercile, had a lower risk of NASH compared with the first tercile after full adjustments. However, dietary intakes of total SFAs, other subtypes, as well as the ratio of UFAs to SFAs did not show significant associations with NASH risk after adjustment for potential risk factors in any tercile. Moreover, results remained largely unchanged in the sensitivity analysis when restricting participants to those who did not use oral corticosteroid (Supplementary Table 3).

Interactions and stratified analyses

Influences of dietary total PUFAs intakes on risk of NASH stratified by selected potential risk factors were shown in Table 4. Multiplicative interactions were statistically significant between terciles of dietary intakes of total PUFAs and BMI (<30 kg/m^2 or ≥30 kg/m^2) on associations with risk of NASH (p-interaction = 0.004). Stronger associations of dietary total PUFAs intakes with NASH risk were found in obese participants with a BMI ≥ 30 kg/m^2 (OR, 95% CI: 0.41, 0.22–0.75), while significant associations could not be observed in non-obese participants with a BMI < 30 kg/m^2 (OR, 95% CI: 1.00, 0.70–1.43). Null significant multiplicative interactions between other potential risk factors and terciles of dietary total PUFAs were identified on risk of NASH. Similar effects on risk of NASH were also observed between BMI and dietary intakes of PUFA 18:3 (p-interaction = 0.015, Figure 2). Moreover, inverse associations of dietary intakes of PUFA 18:3 and NASH risk were stronger among participants without presence of cardiovascular disease (OR, 95% CI: 0.66, 0.47–0.94), compared to their counterparts (OR, 95% CI: 0.64, 0.25–1.63, p-interaction = 0.042).

TABLE 3 Odds ratios (ORs) and 95% confidence intervals (CIs) of non-alcoholic steatohepatitis by terciles of dietary intakes of fatty acids among controls (n = 4,161).

Dietary intakes	NASH/non-NASH	Levels, g/day[b]	Model 1	Model 2	Model 3
Total SFAs					
T1	632/690	≤19.78	1.00	1.00	1.00
T2	745/691	19.78–25.56	1.08 (0.84, 1.39)	1.04 (0.78, 1.39)	0.97 (0.69, 1.38)
T3	712/690	>25.56	1.10 (0.86, 1.40)	0.86 (0.61, 1.21)	0.79 (0.53, 1.17)
Total MUFAs					
T1	717/690	≤22.09	1.00	1.00	1.00
T2	669/691	22.09–27.23	0.79 (0.60, 1.04)	0.69 (0.51, 0.94)	0.69 (0.49, 0.96)
T3	703/690	>27.23	0.90 (0.71, 1.15)	0.63 (0.44, 0.91)	0.67 (0.44, 1.01)
Total PUFAs					
T1	722/690	≤14.34	1.00	1.00	1.00
T2	708/691	14.34–19.24	0.92 (0.68, 1.24)	0.74 (0.51, 1.09)	0.77 (0.48, 1.23)
T3	659/690	>19.24	0.76 (0.57, 1.02)	0.62 (0.47, 0.81)	0.68 (0.48, 0.96)
The ratio of UFAs to SFAs[a]					
≤1.2	227/226	–	1.00	1.00	1.00
1.2–2.5	1,562/1,508	–	0.97 (0.74, 1.26)	0.91 (0.68, 1.22)	1.05 (0.68, 1.60)
≥2.5	300/337	–	0.94 (0.65, 1.38)	0.89 (0.70, 1.12)	1.08 (0.69, 1.68)
Subtypes of fatty acids					
SFA 4:0					
T1	641/685	≤0.234	1.00	1.00	1.00
T2	774/687	0.234–0.462	1.17 (0.92, 1.49)	1.30 (0.98, 1.71)	1.28 (0.97, 1.69)
T3	664/685	>0.462	0.98 (0.73, 1.32)	1.08 (0.75, 1.54)	1.11 (0.73, 1.70)
SFA 6:0					
T1	665/682	≤0.161	1.00	1.00	1.00
T2	744/682	0.161–0.307	1.07 (0.87, 1.30)	1.13 (0.86, 1.48)	1.07 (0.81, 1.41)
T3	666/682	>0.307	0.96 (0.69, 1.34)	0.97 (0.67, 1.40)	1.01 (0.67, 1.52)
SFA 8:0					
T1	688/688	≤0.140	1.00	1.00	1.00
T2	706/689	0.140–0.247	0.92 (0.74, 1.13)	0.98 (0.75, 1.28)	1.03 (0.76, 1.40)
T3	690/688	>0.247	0.99 (0.73, 1.32)	1.05 (0.77, 1.43)	1.15 (0.81, 1.62)
SFA 10:0					
T1	668/690	≤0.290	1.00	1.00	1.00
T2	762/689	0.290–0.518	1.06 (0.89, 1.25)	1.13 (0.89, 1.44)	1.14 (0.85, 1.52)
T3	658/690	>0.518	0.91 (0.68, 1.20)	1.01 (0.73, 1.41)	1.07 (0.71, 1.60)
SFA 12:0					
T1	697/689	≤0.404	1.00	1.00	1.00
T2	689/690	0.404–0.783	0.95 (0.73, 1.23)	1.00 (0.73, 1.37)	0.93 (0.61, 1.41)
T3	703/689	>0.783	0.96 (0.70, 1.33)	1.02 (0.78, 1.34)	1.02 (0.78, 1.32)
SFA 14:0					
T1	655/690	≤1.344	1.00	1.00	1.00
T2	734/691	1.344–2.163	1.01 (0.78, 1.31)	1.03 (0.77, 1.36)	0.99 (0.76, 1.28)
T3	700/690	>2.163	1.00 (0.74, 1.35)	0.93 (0.68, 1.28)	0.90 (0.63, 1.28)
SFA 16:0					
T1	638/690	≤11.242	1.00	1.00	1.00
T2	724/691	11.242–14.124	1.11 (0.78, 1.56)	0.96 (0.66, 1.40)	0.98 (0.63, 1.51)
T3	727/690	>14.124	1.10 (0.85, 1.44)	0.78 (0.56, 1.09)	0.72 (0.50, 1.04)
SFA 18:0					
T1	621/690	≤4.593	1.00	1.00	1.00
T2	706/691	4.593–6.065	1.02 (0.76, 1.38)	0.91 (0.67, 1.23)	0.89 (0.61, 1.30)

(Continued)

TABLE 3 (Continued)

Dietary intakes	NASH/non-NASH	Levels, g/day[b]	Model 1	Model 2	Model 3
T3	762/690	>6.065	1.20 (0.92, 1.56)	0.84 (0.59, 1.20)	0.74 (0.47, 1.15)
MUFA 16:1					
T1	667/690	≤0.775	1.00	1.00	1.00
T2	696/691	0.775–1.132	1.09 (0.87, 1.35)	0.90 (0.68, 1.18)	0.97 (0.68, 1.38)
T3	726/690	>1.132	1.23 (0.97, 1.56)	0.84 (0.60, 1.16)	0.84 (0.56, 1.27)
MUFA 18:1					
T1	717/690	≤20.691	1.00	1.00	1.00
T2	677/691	20.691–25.586	0.79 (0.60, 1.06)	0.67 (0.49, 0.92)	0.65 (0.46, 0.93)
T3	695/690	>25.586	0.95 (0.75, 1.19)	0.66 (0.47, 0.92)	0.70 (0.48, 1.01)
MUFA 20:1					
T1	705/690	≤0.205	1.00	1.00	1.00
T2	676/691	0.205–0.296	0.99 (0.76, 1.30)	0.82 (0.62, 1.08)	0.84 (0.63, 1.13)
T3	707/690	>0.296	1.02 (0.78, 1.34)	0.73 (0.52, 1.01)	0.77 (0.52, 1.15)
MUFA 22:1					
T1	659/651	≤0.008	1.00	1.00	1.00
T2	629/652	0.008–0.023	1.04 (0.87, 1.24)	0.92 (0.77, 1.10)	1.08 (0.84, 1.39)
T3	692/651	>0.023	1.13 (0.97, 1.32)	0.94 (0.68, 1.29)	1.08 (0.75, 1.56)
PUFA 18:2					
T1	728/690	≤12.745	1.00	1.00	1.00
T2	695/691	12.745–17.031	0.90 (0.65, 1.25)	0.73 (0.47, 1.12)	0.74 (0.45, 1.22)
T3	666/690	>17.031	0.78 (0.57, 1.05)	0.64 (0.47, 0.86)	0.69 (0.47, 1.00)
PUFA 18:3					
T1	690/690	≤1.244	1.00	1.00	1.00
T2	710/691	1.244–1.776	1.10 (0.80, 1.49)	1.01 (0.71, 1.43)	1.08 (0.73, 1.61)
T3	689/690	>1.776	0.85 (0.62, 1.15)	0.66 (0.49, 0.89)	0.68 (0.48, 0.95)
PUFA 18:4					
T1	500/479	≤0.001	1.00	1.00	1.00
T2	518/479	0.001–0.004	0.97 (0.66, 1.42)	0.91 (0.62, 1.35)	0.92 (0.58, 1.46)
T3	467/479	>0.004	1.11 (0.75, 1.65)	1.05 (0.66, 1.66)	1.01 (0.61, 1.69)
PUFA 20:4					
T1	657/688	≤0.093	1.00	1.00	1.00
T2	694/688	0.093–0.164	1.00 (0.71, 1.42)	0.83 (0.54, 1.26)	0.77 (0.48, 1.26)
T3	736/688	>0.164	1.20 (0.94, 1.54)	0.85 (0.63, 1.15)	0.75 (0.53, 1.08)
PUFA 20:5					
T1	654/664	≤0.006	1.00	1.00	1.00
T2	724/664	0.006–0.013	1.19 (0.95, 1.49)	1.05 (0.87, 1.27)	1.02 (0.82, 1.27)
T3	638/664	>0.013	1.11 (0.83, 1.49)	0.94 (0.65, 1.35)	0.99 (0.68, 1.43)
PUFA 22:5					
T1	678/673	≤0.014	1.00	1.00	1.00
T2	680/674	0.014–0.023	1.04 (0.73, 1.48)	0.93 (0.64, 1.36)	0.85 (0.59, 1.23)
T3	696/673	>0.023	1.09 (0.82, 1.45)	0.83 (0.58, 1.19)	0.83 (0.55, 1.23)
PUFA 22:6					
T1	603/644	≤0.009	1.00	1.00	1.00
T2	657/645	0.009–0.045	1.14 (0.89, 1.46)	1.00 (0.71, 1.41)	1.01 (0.71, 1.43)
T3	697/644	>0.045	1.34 (1.08, 1.66)	1.15 (0.83, 1.59)	1.10 (0.74, 1.63)

NASH, non-alcoholic steatohepatitis; T1, first tercile; T2, second tercile; T3, third tercile; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; and UFAs, unsaturated fatty acids.

Model 1: unadjusted; model 2: adjusted for sex, age, and BMI; model 3: model 2 additionally adjusted for ethnicity, marital status, education levels, family income-to-poverty ratio, waist circumference, smoking status, regular activities, use of oral corticosteroid, energy intakes, HEI-2015, ALT, ALP, AST, GGT, hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, and cancer.

[a]The ratio of UFAs to SFAs was calculated as (PUFAs + MUFAs)/SFAs.

[b]Dietary intakes of fatty acids were adjusted for energy intakes using the residuals method.

TABLE 4 Odds ratios (ORs) and 95% confidence intervals (CIs) of non-alcoholic steatohepatitis by terciles of dietary total polyunsaturated fatty acids among controls stratified by covariates.

Terciles of dietary total PUFAs	NASH/non-NASH	OR (95% CI)[b]			*p*-interaction
		T1	T2	T3	
Sex					0.776
Men	1,100/915	1.00	0.67 (0.35, 1.29)	0.56 (0.34, 0.90)	
Women	989/1,157	1.00	0.87 (0.57, 1.33)	0.79 (0.50, 1.23)	
Age, years					0.083
<60	1,237/1,405	1.00	0.60 (0.35, 1.03)	0.68 (0.47, 0.98)	
≥60	852/667	1.00	1.30 (0.75, 2.24)	0.72 (0.43, 1.21)	
Smoking status					0.217
Never smoker	1,214/1,303	1.00	0.77 (0.49, 1.22)	0.66 (0.43, 1.02)	
Former smoker	572/386	1.00	0.67 (0.27, 1.64)	0.51 (0.25, 1.06)	
Current smoker	303/383	1.00	0.59 (0.29, 1.20)	0.81 (0.40, 1.64)	
Regular exercise					0.053
Yes	883/1,097	1.00	0.72 (0.39, 1.33)	0.53 (0.34, 0.83)	
No	1,206/975	1.00	0.79 (0.46, 1.35)	0.89 (0.53, 1.48)	
HEI-2015					0.997
≤median	1,064/1,016	1.00	0.49 (0.29, 0.82)	0.58 (0.34, 0.98)	
>median	1,025/1,056	1.00	1.34 (0.72, 2.51)	0.83 (0.48, 1.44)	
BMI, kg/m^2					0.004
<30	798/1,608	1.00	1.10 (0.70, 1.73)	1.00 (0.70, 1.43)	
≥30	1,280/453	1.00	0.48 (0.24, 0.96)	0.41 (0.22, 0.75)	
Waist circumference, cm[a]					0.847
<102 or 88	436/1,234	1.00	0.84 (0.50, 1.41)	0.67 (0.43, 1.05)	
≥102 or 88	1,600/789	1.00	0.76 (0.42, 1.38)	0.71 (0.44, 1.12)	
Hypertension					0.862
Yes	1,132/722	1.00	0.90 (0.48, 1.67)	0.46 (0.23, 0.94)	
No	957/1,350	1.00	0.71 (0.46, 1.09)	0.86 (0.61, 1.21)	
Diabetes mellitus					0.966
Yes	659/218	1.00	0.69 (0.25, 1.88)	0.44 (0.12, 1.55)	
No	1,430/1,854	1.00	0.78 (0.46, 1.31)	0.71 (0.47, 1.10)	
Dyslipidemia					0.197
Yes	1,632/1,121	1.00	0.76 (0.42, 1.36)	0.62 (0.43, 0.90)	
No	403/850	1.00	0.84 (0.41, 1.75)	0.95 (0.54, 1.66)	
Cardiovascular disease					0.086
Yes	276/166	1.00	1.80 (0.77, 4.17)	0.86 (0.35, 2.10)	
No	1,757/1,740	1.00	0.72 (0.44, 1.16)	0.67 (0.46, 0.97)	
Cancer					0.305
Yes	238/177	1.00	0.83 (0.28, 2.48)	0.39 (0.14, 1.07)	
No	1,794/1,730	1.00	0.77 (0.46, 1.29)	0.71 (0.49, 1.05)	

NASH, non-alcoholic steatohepatitis; PUFAs, polyunsaturated fatty acids; T1, first tercile; T2, second tercile; T3, third tercile; HEI-2015, healthy eating index-2015; and BMI, body mass index.

[a]Cut-off points of waist circumference were 102 cm for men and 88 cm for women.

[b]Adjusted for age, sex, BMI, ethnicity, marital status, education levels, family income-to-poverty ratio, waist circumference, smoking status, regular activities, use of oral corticosteroid, energy intakes, HEI-2015, ALT, ALP, AST, GGT, hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, and cancer. Stratified factors were not included in the corresponding models.

Discussion

In this nationally representative study with 4,161 adults in the United States, we explored the associations between risk of NASH and dietary intakes of fatty acids, including total SFAs, MUFAs, PUFAs, their common subtypes, and the ratio of UFAs to SFAs. The dietary intakes of total PUFAs, as well as its subtype of PUFA 18:3, were inversely associated with risk of NASH,

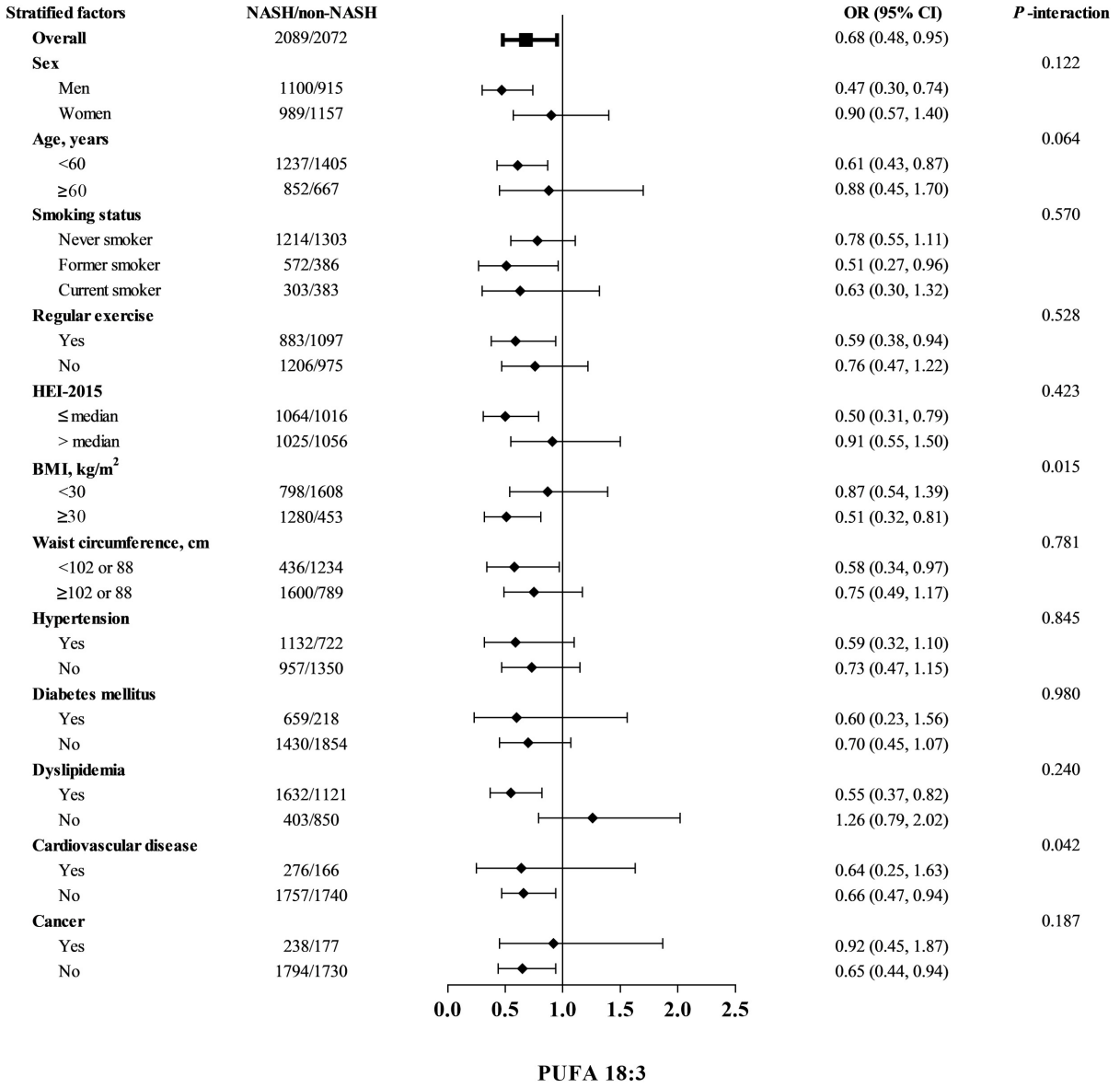


FIGURE 2
Odds ratios (ORs) and 95% confidence intervals (CIs) of non-alcoholic steatohepatitis by terciles of dietary PUFA 18:3 among controls stratified by covariates. Abbreviations: PUFA, polyunsaturated fatty acid NASH, non-alcoholic steatohepatitis; HEI-2015, healthy eating index-2015; and BMI, body mass index. Cut-off points of waist circumference were 102 cm for men and 88 cm for women. Odds ratios were adjusted for age, sex, BMI, ethnicity, marital status, education levels, family income-to-poverty ratio, waist circumference, smoking status, regular activities, use of oral corticosteroid, energy intakes, HEI-2015, ALT, ALP, AST, GGT, hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, and cancer. Stratified factors were not included in the corresponding models.

independent of several potential covariates, especially in those with obesity (BMI $\geq$ 30 kg/m^2). No significant associations were observed between NASH risk and dietary total SFAs, MUFAs, their subtypes as well as the ratio of UFAs to SFAs.

A few studies have explored the relationships between fatty acids and risk of liver diseases or liver-related indices; however, the results were still inconsistent and inconclusive. For example, high abundance of hepatic total SFAs and MUFAs was observed in humans with NAFLD and mice with NASH, which implied that higher total SFAs and MUFAs may be associated with hepatic lipotoxicity and inflammation (25). However, intervention study using diets full with MUFAs showed decreased cholesterol, triglycerides, and increased HDL-cholesterol levels in participants with NAFLD (26). In this study, no significant associations were found between dietary total SFAs, MUFAs, and NASH risk. In addition, an animal study found that moderate intakes of fatty acids with the high ratio of UFAs to SFAs could inhibit liver lipogenesis and steatosis (27),

although no significant association was found between the ratio of UFAs to SFAs and NASH risk in our study. Further studies need to be conducted to confirm these findings.

In regard to the relationship between total PUFAs and risk of NAFLD, a systematic review and meta-analysis of 13 studies, consisting of 668 patients with NAFLD, found that total PUFAs or fish oil supplementation may affect serum ALT levels and improve liver function (28). Similar associations could also be observed in other studies of dietary or supplementation with n-3 fatty acids (29–32). However, contradictory results could also be found. A case–control study conducted in 971 Chinese Han adults found that total PUFAs intakes were positively associated with the risk of NAFLD (10). Another cross-sectional study of 233 American children found that dietary long-chain n-3 fatty acids were inversely associated with portal and lobular inflammation, although no significant effects could be found on NASH, which was assessed using serum ALT and histological parameters (33).

The reasons including sample sizes, population, methods of dietary assessment, detection methods of NAFLD may account for diversities among the findings of the aforementioned studies. Particularly, none of those studies have explored the associations of their subtypes with risk of NASH, which was necessary since different fatty acids subtypes exerted different or even opposite effects on liver health (15), due to discrepancies of the length of carbon chain, straight or branched chain, position and numbers of double bonds (34). To the best of our knowledge, this is the first study to investigate associations between dietary total SFAs, MUFAs, PUFAs, their subtypes, the ratio of UFAs to SFAs, and risk of NASH. No significant associations were observed between NASH risk and dietary total SFAs, MUFAs, their subtypes as well as the ratio of UFAs to SFAs. However, dietary intakes of total PUFAs, as well as its subtype of PUFA 18:3, were inversely associated with risk of NASH, independent of several potential covariates, especially in those with obesity (BMI $\geq$ 30 kg/m^2). Several biologic mechanisms could explain the favorable associations between dietary intakes of total PUFAs, its subtype of PUFA 18:3, and the risk of NASH. NASH was characterized as lipid deposition and hepatic inflammation. Oxidative stress, insulin resistance, lipid peroxidation, abnormal secretion of proinflammatory cytokines and adipokines, and intestinal dysbiosis played important roles in the occurrence and development of NAFLD (8, 35–38). In line with our findings on the protective impact of total PUFAs on NASH, total PUFAs have been shown to exert anti-inflammatory effects in both *in vitro* and *in vivo* studies. Supplementation with n-3 PUFAs inhibited lipogenesis, attenuated hepatic oxidative stress, decreased inflammation and increased insulin sensitivity, further to preserve hepatic architecture and prevent hepatic steatosis (39). The n-3 PUFAs could downregulate sterol regulatory element-binding protein-1c (SREBP-1c) and upregulate peroxisome proliferator-activated receptor-alpha (PPAR-α) function, and therefore decreased *de novo* lipogenesis and increased free fatty acids oxidation, improved the biochemical and ultrasonographic manifestations of patients with NAFLD (40). Rats fed with a high-fat, high-calorie solid diet were observed with an increased expression of hepatic adiponectin and PPAR-α, a reduction of tumor necrosis factor-alpha (TNF-α), and therefore an improvement of fatty liver and the degree of liver injury after supplementation of n-3 PUFAs (41).

Polyunsaturated fatty acid 18:3, so called α-linolenic acid, was a kind of important n-3 PUFAs with anti-inflammatory and antioxidant effects for the human body. In a 6-month, randomized, placebo-controlled, double-blind trial, supplementation of n-3 PUFAs impacted on plasma lipid profile in patients with NASH, which was specific in reduction of triglycerides, and therefore improved plasma lipidomic and hepatic proteomic markers of lipogenesis, mitochondrial functions and endoplasmic reticulum stress (42, 43). Further mechanisms about effects of PUFA 18:3 on inflammation need to be certified in future studies.

There may be several kinds of common risk factors and links between overweight, obesity, other related metabolic diseases and NAFLD (44). In our study, an inverse association of dietary intakes of PUFA 18:3 with NASH was observed only among participants without cardiovascular disease. Increasing dietary intakes of PUFA 18:3 may reduce the risk of NASH in participants without presence of cardiovascular disease. The BMI was another risk factor for NASH, and decreases in hepatic fat content were partially attributed to favorable changes in BMI (45). Interactions could be found between dietary PUFAs and obesity on NASH risk in our study. Stronger associations of dietary PUFAs intakes with NASH risk were found in participants with obesity rather than those without obesity, suggesting that obese people might benefit more from increasing dietary intakes of PUFAs.

Our study has notable strengths. First, our observational study was based on a national, representative population with large sample size in the United States. Complex sampling design as well as appropriate sample weight method we conducted increased the reliability and generalizability of our findings. In addition, NASH was diagnosed by transient elastography, which was a non-invasive and fast method with high sensitivity and specificity by comparison with liver biopsy (17–19).

Several limitations need to be considered. First, although we adjusted for multiple potential confounders, including demographic information, lifestyle, medication use and history of chronic diseases, residual confounding cannot be eliminated fully. Furthermore, stratified analysis performed in our study may result in potential statistical power loss, it was desirable to conduct studies with larger sample sizes to validate our finding in the future. In addition, since the progression of NASH was long, dietary assessment in a long term was more appropriate to explore the relationships between dietary intakes of fatty acids and risks of NASH. However, in the analysis of this

study, dietary intakes of fatty acids were assessed with a 24-h food recall method in two inconsecutive days, future studies with repeated dietary assessment in a long term are warranted. Moreover, restricted by the observational study design, we cannot definitively exclude the possibility that our findings may be affected by residual confounding and reverse causality. Further prospective or interventional studies need to be carried out to verify our finding about associations between dietary fatty acids and NASH.

Conclusion

In conclusion, inverse associations were observed between dietary intakes of total PUFAs, as well as its subtype of PUFA 18:3, and risk of NASH after adjusting potential confounders. Further large prospective studies need to be conducted to confirm our findings.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by National Center for Health Statistics. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Z-YL and H-LZ created the analytic design. Z-YL performed data extraction. X-TL, Z-YL, Y-DW, and T-TZ analyzed the data. X-TL drafted the manuscript. X-TL, Z-YL, and H-LZ critically revised the manuscript. All authors read and approved the final manuscript.

Funding

This work was funded by the Basic and Applied Basic Research Foundation of Guangdong Province, China (Grant No. 2020A1515110682).

Acknowledgments

We thank all the participants, the investigators, and the staff of the National Health and Nutrition Examination Survey for their valuable contribution.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.952451/full#supplementary-material

References

1. Paik JM, Golabi P, Younossi Y, Mishra A, Younossi ZM. Changes in the global burden of chronic liver diseases from 2012 to 2017: the growing impact of Nafld. *Hepatology*. (2020) 72:1605–16. doi: 10.1002/hep.31173

2. Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol*. (2011) 9:524–30e1;quize60. doi: 10.1016/j.cgh.2011.03.020

3. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, et al. Modeling Nafld disease Burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the Period 2016–2030. *J Hepatol*. (2018) 69:896–904. doi: 10.1016/j.jhep.2018.05.036

4. Komolafe O, Buzzetti E, Linden A, Best LM, Madden AM, Roberts D, et al. Nutritional supplementation for nonalcohol-related fatty liver disease: a network meta-analysis. *Cochrane Database Syst Rev*. (2021) 7:CD013157. doi: 10.1002/14651858.CD013157.pub2

5. Kardashian A, Dodge JL, Terrault NA. Food insecurity is associated with mortality among U.S. Adults with nonalcoholic fatty liver disease and advanced

fibrosis. *Clin Gastroenterol Hepatol.* (2021) S1542–3565:1267–1262. doi: 10.1016/j.cgh.2021.11.029

6. Piras C, Noto A, Ibba L, Deidda M, Fanos V, Muntoni S, et al. Contribution of metabolomics to the understanding of nafld and nash syndromes: a systematic review. *Metabolites.* (2021) 11:694. doi: 10.3390/metabo11100694

7. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of nafld and nash: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109

8. Musso G, Cassader M, Paschetta E, Gambino R. Bioactive lipid species and metabolic pathways in progression and resolution of nonalcoholic steatohepatitis. *Gastroenterology.* (2018) 155:282–302e8. doi: 10.1053/j.gastro.2018.06.031

9. Willis SA, Bawden SJ, Malaikah S, Sargeant JA, Stensel DJ, Aithal GP, et al. The role of hepatic lipid composition in obesity-related metabolic disease. *Liver Int.* (2021) 41:2819–35. doi: 10.1111/liv.15059

10. Xie Y, Tian H, Xiang B, Li D, Liu J, Cai Z, et al. Total polyunsaturated fatty acid intake and the risk of non-alcoholic fatty liver disease in chinese han adults: a secondary analysis based on a case-control study. *BMC Gastroenterol.* (2021) 21:451. doi: 10.1186/s12876-021-02039-2

11. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis.* (2010) 42:503–8. doi: 10.1016/j.dld.2009.08.002

12. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* (2006) 6:33. doi: 10.1186/1471-230X-6-33

13. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology.* (2009) 137:865–72. doi: 10.1053/j.gastro.2009.06.005

14. Poynard T, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, et al. The diagnostic value of biomarkers (Steatotest) for the prediction of liver steatosis. *Comp Hepatol.* (2005) 4:10. doi: 10.1186/1476-5926-4-10

15. Yamada K, Mizukoshi E, Sunagozaka H, Arai K, Yamashita T, Takeshita Y, et al. Characteristics of hepatic fatty acid compositions in patients with nonalcoholic steatohepatitis. *Liver Int.* (2015) 35:582–90. doi: 10.1111/liv.12685

16. Centers for Disease Control and Prevention. *National Health and NutritionExamination Survey (NHANES) 2017–2018 Data Documentation, Codebook, and Frequencies*. (2020). Available online at: https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/LUX_J.htm (accessed June 13, 2022).

17. Myers RP, Pollett A, Kirsch R, Pomier-Layrargues G, Beaton M, Levstik M, et al. Controlled attenuation parameter (Cap): a noninvasive method for the detection of hepatic steatosis based on transient elastography. *Liver Int.* (2012) 32:902–10. doi: 10.1111/j.1478-3231.2012.02781.x

18. de Ledinghen V, Wong GL, Vergniol J, Chan HL, Hiriart JB, Chan AW, et al. Controlled attenuation parameter for the diagnosis of steatosis in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol.* (2016) 31:848–55. doi: 10.1111/jgh.13219

19. Sasso M, Audiere S, Kemgang A, Gaouar F, Corpechot C, Chazouilleres O, et al. Liver steatosis assessed by controlled attenuation parameter (Cap) measured with the Xl probe of the fibroscan: a pilot study assessing diagnostic accuracy. *Ultrasound Med Biol.* (2016) 42:92–103. doi: 10.1016/j.ultrasmedbio.2015.08.008

20. Siddiqui MS, Vuppalanchi R, Van Natta ML, Hallinan E, Kowdley KV, Abdelmalek M, et al. Vibration-controlled transient elastography to assess fibrosis and steatosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* (2019) 17:156.e–63.e. doi: 10.1016/j.cgh.2018.04.043

21. Centers for Disease Control and Prevention. *National Health and Nutrition Examination Survey (NHANES) 2017-2018 Procedure Manuals*. (2020). Available online at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?BeginYear=2017 (accessed June 13, 2022).

22. Centers for Disease Control and Prevention. *National Health and NutritionExamination Survey (NHANES) 2017-2018 Laboratory Methods*. (2020). Available online at: https://wwwn.cdc.gov/Nchs/Nhanes/continuousnhanes/labmethods.aspx?BeginYear=2017 (accessed June 13, 2022).

23. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* (1986) 124:17–27. doi: 10.1093/oxfordjournals.aje.a114366

24. Kim D, Konyn P, Cholankeril G, Ahmed A. Physical activity is associated with nonalcoholic fatty liver disease and significant fibrosis measured by fibroscan. *Clin Gastroenterol Hepatol.* (2022) 20: e1438–e1455. doi: 10.1016/j.cgh.2021.06.029

25. Spooner MH, Jump DB. Omega-3 Fatty acids and nonalcoholic fatty liver disease in adults and children: where do we stand? *Curr Opin Clin Nutr Metab Care.* (2019) 22:103–10. doi: 10.1097/MCO.0000000000000539

26. Barrera F, George J. The role of diet and nutritional intervention for the management of patients with nafld. *Clin Liver Dis.* (2014) 18:91–112. doi: 10.1016/j.cld.2013.09.009

27. Siddiqui RA, Xu Z, Harvey KA, Pavlina TM, Becker MJ, Zaloga GP. Comparative study of the modulation of fructose/sucrose-induced hepatic steatosis by mixed lipid formulations varying in unsaturated fatty acid content. *Nutr Metab (Lond).* (2015) 12:41. doi: 10.1186/s12986-015-0038-x

28. Yu L, Yuan M, Wang L. The effect of Omega-3 unsaturated fatty acids on non-alcoholic fatty liver disease: a systematic review and meta-analysis of rcts. *Pak J Med Sci.* (2017) 33:1022–8. doi: 10.12669/pjms.334.12315

29. Musa-Veloso K, Venditti C, Lee HY, Darch M, Floyd S, West S, et al. Systematic review and meta-analysis of controlled intervention studies on the effectiveness of long-chain omega-3 fatty acids in patients with nonalcoholic fatty liver disease. *Nutr Rev.* (2018) 76:581–602. doi: 10.1093/nutrit/nuy022

30. Mitrovic M, Sistilli G, Horakova O, Rossmeisl M. Omega-3 phospholipids and obesity-associated nafld: potential mechanisms and therapeutic perspectives. *Eur J Clin Invest.* (2022) 52:e13650. doi: 10.1111/eci.13650

31. Lu W, Li S, Li J, Wang J, Zhang R, Zhou Y, et al. Effects of omega-3 fatty acid in nonalcoholic fatty liver disease: a meta-analysis. *Gastroenterol Res Pract.* (2016) 2016:1459790. doi: 10.1155/2016/1459790

32. Corte CD, Iasevoli S, Strologo AD, Sanseviero M, Nobili V. Omega-3 fatty acids and fatty liver disease in children. *Adv Food Nutr Res.* (2018) 85:59–77. doi: 10.1016/bs.afnr.2018.03.001

33. St-Jules DE, Watters CA, Brunt EM, Wilkens LR, Novotny R, Belt P, et al. Estimation of fish and omega-3 fatty acid intake in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr.* (2013) 57:627–33. doi: 10.1097/MPG.0b013e3182a1df77

34. Kazaz S, Miray R, Lepiniec L, Baud S. Plant monounsaturated fatty acids: diversity, biosynthesis, functions and uses. *Prog Lipid Res.* (2022) 85:101138. doi: 10.1016/j.plipres.2021.101138

35. Videla LA, Valenzuela R. Perspectives in liver redox imbalance: toxicological and pharmacological aspects underlying iron overloading, nonalcoholic fatty liver disease, and thyroid hormone action. *Biofactors.* (2022) 48:400–15. doi: 10.1002/biof.1797

36. Pafili K, Roden M. Nonalcoholic fatty liver disease (Nafld) from pathogenesis to treatment concepts in humans. *Mol Metab.* (2021) 50:101122. doi: 10.1016/j.molmet.2020.101122

37. Liu W, Baker RD, Bhatia T, Zhu L, Baker SS. Pathogenesis of nonalcoholic steatohepatitis. *Cell Mol Life Sci.* (2016) 73:1969–87. doi: 10.1007/s00018-016-2161-x

38. Byrne CD. Fatty liver: role of inflammation and fatty acid nutrition. *Prostaglandins Leukot Essent Fatty Acids.* (2010) 82:265–71. doi: 10.1016/j.plefa.2010.02.012

39. Jeyapal S, Kona SR, Mullapudi SV, Putcha UK, Gurumurthy P, Ibrahim A. Substitution of linoleic acid with alpha-linolenic acid or long chain N-3 polyunsaturated fatty acid prevents western diet induced nonalcoholic steatohepatitis. *Sci Rep.* (2018) 8:10953. doi: 10.1038/s41598-018-29222-y

40. Bouzianas DG, Bouziana SD, Hatzitolios AI. Potential treatment of human nonalcoholic fatty liver disease with long-chain omega-3 polyunsaturated fatty acids. *Nutr Rev.* (2013) 71:753–71. doi: 10.1111/nure.12073

41. Svegliati-Baroni G, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, et al. A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-alpha and n-3 polyunsaturated fatty acid treatment on liver injury. *Am J Pathol.* (2006) 169:846–60. doi: 10.2353/ajpath.2006.050953

42. Nogueira MA, Oliveira CP, Ferreira Alves VA, Stefano JT, Rodrigues LS, Torrinhas RS, et al. Omega-3 polyunsaturated fatty acids in treating non-alcoholic steatohepatitis: a randomized, double-blind, placebo-controlled trial. *Clin Nutr.* (2016) 35:578–86. doi: 10.1016/j.clnu.2015.05.001

43. Okada L, Oliveira CP, Stefano JT, Nogueira MA, Silva I, Cordeiro FB, et al. Omega-3 pufa modulate lipogenesis, er stress, and mitochondrial dysfunction markers in nash - proteomic and lipidomic insight. *Clin Nutr.* (2018) 37:1474–84. doi: 10.1016/j.clnu.2017.08.031

44. Allen AM, Therneau TM, Larson JJ, Coward A, Somers VK, Kamath PS. Nonalcoholic fatty liver disease incidence and impact on metabolic burden and death: A 20 year-community study. *Hepatology.* (2018) 67:1726–36. doi: 10.1002/hep.29546

45. Lee CH, Fu Y, Yang SJ, Chi CC. Effects of omega-3 polyunsaturated fatty acid supplementation on non-alcoholic fatty liver: a systematic review and meta-analysis. *Nutrients.* (2020) 12:2769. doi: 10.3390/nu12092769

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 26 July 2022
DOI 10.3389/fnut.2022.938645

OPEN ACCESS

EDITED BY
Zheqing Zhang,
Southern Medical University, China

REVIEWED BY
Huan Fan,
Capital Medical University, China
Heng Piao,
Affiliated Cancer Hospital of Zhengzhou University, China

*CORRESPONDENCE
Zhao-Yan Liu
liuzhy235@mail.sysu.edu.cn
Hui-Lian Zhu
zhuhl@mail.sysu.edu.cn

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 07 May 2022
ACCEPTED 20 June 2022
PUBLISHED 26 July 2022

CITATION
Zhu T, Lu X-T, Liu Z-Y and Zhu H-L (2022) Dietary linoleic acid and the ratio of unsaturated to saturated fatty acids are inversely associated with significant liver fibrosis risk: A nationwide survey.
Front. Nutr. 9:938645.
doi: 10.3389/fnut.2022.938645

Dietary linoleic acid and the ratio of unsaturated to saturated fatty acids are inversely associated with significant liver fibrosis risk: A nationwide survey

Tingting Zhu[1,2,3,4], Xiao-Ting Lu[1,2], Zhao-Yan Liu[1,2]* and Hui-Lian Zhu[1,2]*

[1]Department of Nutrition, School of Public Health, Sun Yat-sen University, Guangzhou, China, [2]Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou, China, [3]Department of Food Science and Engineering, School of Food Science and Engineering, Hainan Tropical Ocean University, Sanya, China, [4]Collaborative Innovation Center of Provincial and Ministerial Co-construction for Marine Food Deep Processing, Hainan Tropical Ocean University, Sanya, China

Since no pharmaceuticals have been proven to effectively reduce liver fibrosis, dietary fatty acids may be beneficial as one of the non-pharmaceutical interventions due to their important roles in liver metabolism. In this cross-sectional study, we analyzed the data from the 2017–2018 cycle of National Health and Nutrition Examination Survey to examine the associations between the proportion and composition of dietary fatty acid intakes with significant liver fibrosis among US population. The dietary fatty acid consumptions were calculated based on two 24-h dietary recalls. Significant liver fibrosis was diagnosed based on liver stiffness measurement value derived from the vibration controlled transient elastography. Multivariate logistic regression analysis and sensitivity analysis were performed to assess the association between dietary fatty acid consumption and significant liver fibrosis risk. Finally, restricted cubic spline analysis was carried out to explore the dose–response between polyunsaturated fatty acids (PUFA) or linoleic acid intakes and the risk of significant liver fibrosis. The results showed that the multivariate adjusted odds ratios (95% confidence intervals) of significant liver fibrosis were 0.34 (0.14–0.84), 0.68 (0.50–0.91), and 0.64 (0.47–0.87) for the highest level of unsaturated to saturated fatty acid ratio, dietary PUFA, and linoleic acid intakes compared to the lowest reference, respectively. The sensitivity analysis and restricted cubic spline analysis produced similar results, reinforcing the inverse association of unsaturated to saturated fatty acid ratio, PUFA, and linoleic acid consumptions with significant liver fibrosis risk. However, other dietary fatty acids did not show the statistically significant association with significant liver fibrosis. In conclusion, dietary linoleic acid may play a key role in the inverse association between the unsaturated to saturated fatty acid ratio and the risk of significant liver fibrosis. Further studies are needed to confirm these findings.

KEYWORDS

dietary fatty acids, ratio of unsaturated to saturated fatty acids, dietary fatty acid components, significant liver fibrosis, nationwide study

Introduction

Liver fibrosis, the result of wound healing response to chronic liver injury (1), is prevalent worldwide and can be related to the kinds of chronic liver diseases (CLD) (2). Furthermore, liver fibrosis is known as the main reason for liver disease-related morbidity and mortality (3). Among the CLD, non-alcoholic fatty liver disease (NAFLD) is a representative one, which contains a series of proceeding liver damages, ranging from simple hepatic steatosis to non-alcoholic steatohepatitis and fibrosis, cirrhosis, and even cancer (4). NAFLD-related advanced fibrosis has been reported to have an accelerated increasing trend in the US population (5). Large-scale observational studies have demonstrated that a progressive stage of fibrosis, ranging from significant fibrosis to cirrhosis, is the most powerful histological predictor of hepatic all-cause mortality in NAFLD (6, 7). In addition, the development of liver fibrosis into a more progressive stage mainly occurs when existing chronically liver damage due to infectious, metabolic, toxic/drug-induced, cholestatic, or autoimmune insult (8). Since there are still no approved antifibrotic pharmaceuticals for liver fibrosis (8), the non-medical elements are critical to delaying or even reversing the progression of liver fibrosis. Cost-effective modifiable dietary nutrients are considered to be one of them.

The liver is an important organ for the metabolic regulation of dietary fat, 15% of liver triacylglycerol comes from the diet (9). Among the dietary fat, the fatty acid compositions are relevant to hepatic lipogenesis because of their different metabolic and functional activities (10). The dietary fatty acid compositions can be distinguished by both degrees of the number of carbon atoms and configuration of the saturation (11). Based on the number of double bonds, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), which are further sub-categorized into their specific fatty acid components.

Dietary fatty acids can regulate the distribution of fat in the human body, independent of body weight change (12), and take part in the metabolic pathways (13, 14). According to the results of a randomized controlled trial, a hypercaloric SFA-rich diet led to a remarkable increase in hepatic fat; by contrast, a PUFA-enriched diet did not increase hepatic fat, albeit similar weight gain in both groups (12). Several studies have also observed that SFA can induce endoplasmic reticulum stress and result in liver damage (15, 16), whereas n-3 PUFA showed protective activities to the pathological conditions in NAFLD, macrosteatotic livers, and acute hepatitis (17–19). Nevertheless, contradictory results were also found. The subjects with human immunodeficiency virus (HIV) were reported to be lower odds of having liver fibrosis when consuming lauric and myristic SFA intermediately (20). Additionally, the mice fed with additional eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids represented high expression of tissue inhibitor of metalloproteinase (TIMP)-1 and transforming growth factor (TGF)-β profibrogenic genes, and more severe fibrosis score (21).

Based on the contradictory results of PUFA, SFA, and their specific components, it is thus necessary to advance our understanding of the association of dietary fatty acids with liver fibrosis, especially for the specific fatty acid components. However, to our knowledge, there are sparse epidemiologic studies assessing the associations between specific fatty acid components and liver fibrosis in a large-scale population that is representative nationally, possibly due to the lack of suitable screening techniques for liver fibrosis among such large-scale population (22). Liver biopsy, as the gold standard for liver fibrosis evaluation, with the shortcoming of invasiveness, poor acceptability, not-easy handling, and so on, is not well-suitable for the large-scale population survey. Until the appearance of vibration controlled transient elastography (VCTE), with the advantages of non-invasiveness, better acceptability, and accurate technique, VCTE has been widely used as the non-invasive standard tool for evaluating hepatic fibrosis by liver stiffness measurement (LSM) (23). VCTE was first used as a part of the survey process in the 2017–2018 cycle of National Health and Nutrition Examination Survey (NHANES) (24). Using the more accessible and accurate diagnostic technique will provide a valid assessment of the population-based burden of liver fibrosis in the United States. Moreover, 2015–2020 dietary guidelines for Americans recommend that adults keep within saturated fat limits and replace SFA with unsaturated fatty acids. The ratio of unsaturated fatty acids (UFA) to SFA was first added to healthy eating index-2010 (HEI-2010) to evaluate diet and retained in the HEI-2015. However, scarce epidemiologic studies have referred to the ratio of UFA to SFA. Therefore, herein, we tried to estimate whether the components of dietary fatty acids or the ratio of UFA to SFA were associated with significant liver fibrosis assessed by VCTE among US adults.

Abbreviations: CLD, chronic liver diseases; NAFLD, non-alcoholic fatty liver disease; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic; DHA, docosahexaenoic; NHANES, National Health and Nutrition Examination Survey; VCTE, vibration controlled transient elastography; LSM, liver stiffness measurement; CAP, controlled attenuation parameter; MEC, Mobile Examination Center; DGAs, Dietary Guidelines for Americans; HEI, healthy eating index; BMI, body mass index; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin A1c; CVD, cardiovascular disease; PHQ-9, Patient Health Questionnaire; SE, standard error; OR, odds ratio; CI, confidence interval; RCS, restricted cubic spline; HIV, human immunodeficiency virus; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase.

Materials and methods

Data source

The cross-sectional study was conducted using the data from the 2017–2018 cycle of NHANES, which can be attained on the NHANES website (http://www.cdc.gov/nchs/nhanes.htm). The NHANES data are a multi-stage, stratified, cluster sample representative of the US non-institutionalized civilians (24). The data collection and methodology of NHANES have been reported in detail previously (25). Briefly, NHANES is comprised of questionnaires to obtain the demographic, socioeconomic, dietary, health-associated information, and a standardized physical examination to obtain the equipment-needed indexes. The National Center for Health Statistics Research Ethics Review Board approved the protocol of NHANES and all participants have provided written informed consent before data collection.

Study population and design

The participants with age older than 18 years in the 2017–2018 NHANES cycle (n=5,856) and finished both the survey and medical examination were included. We excluded the participants if they did not have complete VCTE data ($n = 737$) and dietary data ($n = 445$). We also excluded the participants if they were examined with the presence of hepatitis C antibodies ($n = 44$) and hepatitis B surface antigen ($n = 18$), and if they had significant consumptions of alcohol (>30 g/d in men and >20 g/d in women) ($n = 451$). The final enrolled participants were 4,161 (Figure 1).

Definition of significant liver fibrosis

Liver fibrosis was assessed using LSM data derived from VCTE with controlled attenuation, which was performed in the NHANES Mobile Examination Center (MEC). The VCTE measurements were taken using FibroScan® model 502 V2 Touch (Echosens, Paris, France). The equipment can simultaneously measure the ultrasound attenuation and record the controlled attenuation parameter (CAP). CAP can be calculated only if the LSM is valid. The detailed VCTE examination procedure has been reported previously (26).

The liver stiffness is derived by wave velocity when it passes through the liver tissue with 50 Hz by mechanical vibration. Complete examination should meet the conditions: fasting time of at least 3 h, 10, or more complete LSM (E), and liver stiffness interquartile range/ median E <30% (27). LSM ranges from 1.5 to 75 kPa, with higher values indicating more severe fibrosis (24). According to the previous studies (28, 29), LSM value higher than 8 kPa derived from VCTE was considered as significant liver fibrosis.

Diet intake assessment

In the NHANES study, the daily average intakes of total energy, total fat, and dietary fatty acids were calculated based on two 24-h dietary recalls. The first dietary recall was administered in person at the NHANES MEC, and the second dietary recall was administered over the telephone 3 to 10 days later, which were conducted by trained interviewers. If the subjects did not complete the second dietary recall interview, only the first dietary recall was used as the average value. Quality control was used for completeness of recalls, missing information, inconsistent reports, and unclear notes. The ratio of UFA to SFA, which was first listed in HEI-2010 and retained in HEI-2015, was calculated as (PUFA + MUFA)/SFA. In terms of n-3 and n-6 PUFA, due to no specific classification of linolenic acid in NHANES (30), it mainly includes alpha-linolenic acid (n-3 PUFA) and a small part of gamma-linolenic acid (n-6 PUFA). Therefore, in this study, linolenic acid, together with stearidonic acid, eicosapentaenoic acid, clupanodonic acid, and docosahexaenoic acid were defined as n-3 PUFA, and linoleic acid and arachidonic acid were defined as n-6 PUFA. Additionally, aligned with the 2015–2020 Dietary Guidelines for Americans (DGAs), the HEI-2015 includes 13 components that sum to score of 100 in maximum to evaluate dietary quality (31).

Collection of covariates

The following variates were evaluated for each participant: [1] Demographic information including age, gender (male, female), family income-to-poverty ratio (<1.0, 1.0–3.0, >3.0), education levels (less than high school, high school or equivalent, college or above), marital status (married/living with partner, widowed/divorced/separated, never married), ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and others) were collected. [2] Laboratory parameters including platelet count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), alkaline phosphatase (ALP), albumin, and total bilirubin were tested. The methods to assessing laboratory parameters have been described elsewhere in detail (32). [3] Medical conditions. Prevalent pre-hypertension was defined systolic blood pressure between 120 and 139 mmHg or diastolic blood pressure between 80 and 89 mmHg. Prevalent hypertension was diagnosed as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or taking antihypertensive medications by self-report. Prevalent prediabetes was defined as without diabetes mellitus, but with fasting plasma glucose level of 100 to 125 mg/dl, or 2-h plasma glucose level of 140

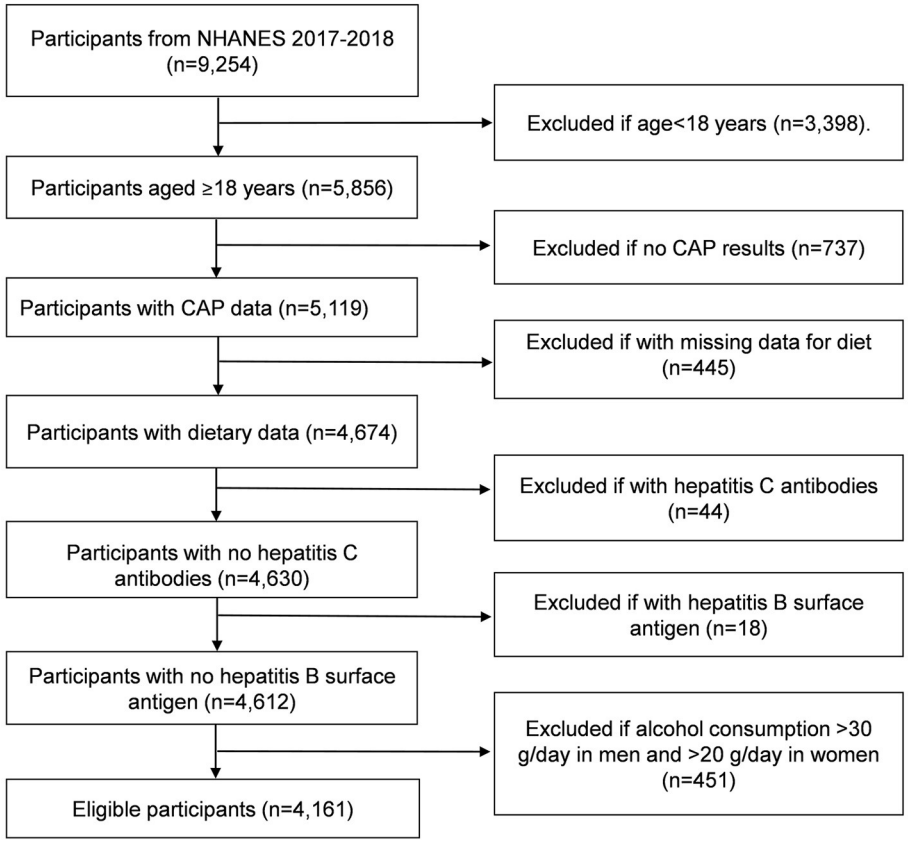


FIGURE 1
Flowchart of participants from 2017 to 2018 cycle of National Health and Nutrition Examination Survey (NHANES). CAP, controlled attenuation parameter.

to 199 mg/dl, or glycated hemoglobin A1c (HbA1c) level of 5.7 to 6.4% or prediabetes diagnosis by self-report. Diabetes mellitus was defined as a fasting plasma glucose level ≥126 mg/dl, HbA1c level ≥6.5%, and/or use of a hypoglycemic agent or insulin or self-reported diabetes diagnosis. Prevalent cardiovascular disease (CVD) was defined if with the condition of coronary heart disease, stroke, angina, heart attack, or congestive heart failure by self-report. History of cancer was defined as self-reported physician diagnosis of any kind of cancer during the lifetime. Prevalent dyslipidemia was defined if total cholesterol ≥200 mg/dl, or triglyceride ≥150 mg/dl, or low-density lipoprotein-cholesterol ≥130 mg/dl, or high-density lipoprotein-cholesterol <40 mg/dl for men, high-density lipoprotein-cholesterol <50 mg/dl for women, or self-reported use of prescribed lipid-modifying medication. Those who took oral corticosteroid over 180 days were defined as having used oral corticosteroid. Depression was evaluated by the Patient Health Questionnaire (PHQ-9). We categorized depression status as less depression (0–4), mild depression (5–9), and major depression (≥10) according to the PHQ-9 score (33). [4] Body measurement and lifestyle factors. We defined current smokers as the participants who reported having smoked at least 100 cigarettes in their lifetime and still kept the habit of smoking at the time of the interview. Former smokers were those who had quit smoking before the interview. Non-smokers were those who smoked <100 cigarettes during their lifetime. Height, weight, and waist circumference were measured, and body mass index (BMI) was defined as measured weight in kilograms divided by measured height in meters squared. The sleep status was evaluated by sleep duration at night and self-reported sleep disorder. Regular exercise was defined as continuous exercise in moderate or vigorous intensity for at least 10 min in a typical week, causing an increase in breathing or heart rate at varying degrees.

Statistical analysis

Because of a complex, multi-stage, cluster-sampling design applied by NHANES, we conducted appropriate sample weights to constitute representative population-level data for the US civilian (34). Demographic information, laboratory parameters,

TABLE 1 Baseline characteristics of the participants.

Variables	All ($n = 4,161$)
Demographic information	
Age, years	47.5 ± 0.8
Sex (Male), *n* (%)	2,015 (48.6)
Family income-to-poverty ratio, *n* (%)	
<1.0	642 (12.1)
1.0–3.0	1,727 (38.6)
>3.0	1,290 (49.3)
Education levels, *n* (%)	
Less than high school	753 (10.4)
High school or equivalent	956 (27.0)
College or above	2,225 (58.7)
Marital status, *n* (%)	
Married/living with partner	2,340 (60.0)
Widowed/divorced/separated	885 (18.0)
Never married	711 (18.1)
Ethnicity, *n* (%)	
Non-Hispanic white	1,442 (62.6)
Non-Hispanic black	971 (11.4)
Mexican American	582 (9.1)
Others	1,166 (17.0)
Laboratory parameters	
Platelet count, 10^9/L	245.4 ± 2.4
ALT, IU/L	22.5 ± 0.3
AST, IU/L	21.5 ± 0.2
GGT, IU/L	27.8 ± 0.6
ALP, IU/L	77.4 ± 0.7
Albumin, g/L	41.0 ± 0.2
Total Bilirubin, μmol/L	8.1 ± 0.1
Medical conditions	
Prevalent pre-hypertension, *n* (%)	905 (23.1)
Prevalent hypertension, *n* (%)	1,854 (38.3)
Prevalent prediabetes, *n* (%)	1,588 (39.0)
Prevalent diabetes, *n* (%)	877 (15.4)
Prevalent CVD, *n* (%)	442 (8.3)
History of cancer, *n* (%)	415 (10.6)
Dyslipidemia, *n* (%)	2,753 (65.0)
Use of oral corticosteroid ≥180 days, *n* (%)	43 (0.9)
PHQ-9 score, *n* (%)	
0–4	3,163 (76.9)
5–9	649 (15.0)
≥10	349 (8.1)
Body measurement and life style factors	
BMI, kg/m^2	29.8 ± 0.3
Waist circumference, cm	100.7 ± 0.8
Smoking status, *n* (%)	
Non-smoker	2,517 (60.2)
Former smoker	958 (24.1)
Current smoker	686 (15.7)
Regular exercise, *n* (%)	1,980 (53.8)
Sleep duration <8 h/day, *n* (%)	2,143 (54.3)
History of sleep disorder, *n* (%)	1,140 (29.2)
Dietary information	
HEI-2015	51.2 ± 0.8
Energy intake, kcal/day	2040.4 ± 16.5
Ratio of UFA to SFA, *n* (%)	
≤1.2	453 (12.0)
1.2–2.5	3,070 (76.1)
≥2.5	637 (12.0)
Total fat, g/day	73.95 ± 0.45
SFA, g/day	24.12 ± 0.27
UFA, g/day	42.55 ± 0.32
MUFA, g/day	25.34 ± 0.20
PUFA, g/day	17.20 ± 0.19
n-3 PUFA, g/day	1.73 ± 0.03
n-6 PUFA, g/day	15.38 ± 0.16

Data were expressed as mean ± SE for continuous variables or as n (weighted %) for categorical variables.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; CVD, cardiovascular disease; PHQ-9, Patient Health Questionnaire; BMI, body mass index; HEI-2015, healthy eating index-2015; UFA, unsaturated fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

medical conditions and body measurement, lifestyle factors, and dietary information were presented as mean ± standard error (SE) for continuous variables and counts (weighted frequencies) for categorical variables in the baseline.

The dietary fatty acid intakes including UFA, SFA, PUFA, MUFA, n-3, n-6 PUFA, and their specific components were analyzed by energy-adjusted method (35). We used multivariate logistic regression model to consider the association between dietary fatty acid intakes and significant liver fibrosis risk. Model 1 was only adjusted for age and gender. Model 2 was adjusted for age, sex, family income-to-poverty ratio, education level, marital status, ethnicity, and laboratory parameters including platelet count, ALT, AST, GGT, ALP, albumin, total bilirubin, medical conditions including pre-hypertension, hypertension, diabetes, prediabetes, CVD, history of cancer, dyslipidemia, use of oral corticosteroid over 180 days, depression status, body measurement and life style factors including smoking status, BMI, waist circumference, regular exercise, HEI-2015, energy intake, sleep duration, and history of sleep disorders. The results were presented with odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

With the consideration of the higher risk of liver fibrosis in those with history of cancer and with use of oral corticosteroid

over 180 days, sensitivity analyses were performed by excluding the participants with history of cancer without or with oral corticosteroid administration over 180 days, respectively. Furthermore, after confirming the non-linear relationship of significant liver fibrosis with PUFA and linoleic acid, we used restricted cubic spline (RCS) with 4 knots located at the 5, 35, 65, and 95th centiles to flexibly model the association of PUFA or linoleic acid with significant liver fibrosis risk, with the same adjusted variables as those in the multiple logistic regression model 2. Data were analyzed using the R software 4.1.2 (R Foundation Vienna, Austria), SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA) and the GraphPad Prism 7.0 (La Jolla, California), considering p-value <0.05 to be statistically significant.

Results

Study characteristics

Among 9,254 participants from NHANES 2017–2018 cycle, 4,161 participants were enrolled. The flowchart of study population is shown in Figure 1. Table 1 describes the baseline characteristics of the enrolled participants, including demographic information, laboratory parameters, medical conditions and body measurement, life style factors, and dietary information. In brief, the participants included 48.6% male, with average age of 47.5 ± 0.8 years. Other demographic variates showed that 10.4% participants were without high school education and 18.1% participants were without marriage. Of note, participants had the higher BMI of 29.8 ± 0.3 kg/m^2 and the higher waist circumference of 100.7 ± 0.8 cm. Additionally, 65.0% participants endured dyslipidemia. As for dietary intakes, energy intake was 2,040.4 ± 16.5 kcal/day, and the mean dietary SFA, UFA, MUFA, PUFA, n-3 PUFA, and n-6 PUFA are also listed in Table 1, respectively. With regard to the ratio of UFA to SFA, most participants ranged from 1.2 to 2.5 (76.1%).

Multivariate analysis and sensitivity analysis

We used the multivariate logistic regression model to explore the associations between dietary fatty acid intakes and the risk of significant liver fibrosis. Higher UFA to SFA ratio was inversely associated with significant liver fibrosis risk. Specifically, the ORs (95% CIs) of significant liver fibrosis were 0.47 (0.25–0.88) in model 1 and 0.34 (0.14–0.84) in model 2 for the ratio of UFA to SFA (≥2.5) vs. the reference; 0.54 (0.34–0.87) in model 1 and 0.47 (0.29–0.74) in model 2 for the ratio of UFA to SFA (1.2–2.5) when compared to the reference, respectively (Table 2). The ORs (95% CIs) of significant liver fibrosis based on tertiles of SFA, UFA, MUFA, PUFA, n-3, and n-6 PUFA are also presented in Table 2. The ORs (95% CIs) of significant liver fibrosis for the highest tertile vs. the reference tertile were 0.74 (0.57–0.95) in model 1 and 0.68

TABLE 2 Multivariate logistic regression model considering dietary fatty acid intakes and the risk of significant liver fibrosis in participants, NHANES 2017–2018 (n = 4161).

	Significant liver fibrosis			
	OR1 (95% CI) [a]	p-value	OR2 (95% CI) [b]	p-value
Ratio of UFA to SFA				
≤1.2	1.00		1.00	
1.2–2.5	0.54 (0.34–0.87)	0.014	0.47 (0.29–0.74)	0.003
≥2.5	0.47 (0.25–0.88)	0.021	0.34 (0.14–0.84)	0.023
Total fat, g/day				
T1 (≤66.23)	1.00		1.00	
T2 (66.24–78.94)	0.93 (0.62–1.39)	0.706	1.08 (0.67–1.75)	0.723
T3 (≥78.95)	1.21 (0.86–1.71)	0.248	1.08 (0.71–1.66)	0.698
SFA, g/day				
T1 (≤19.99)	1.00		1.00	
T2 (20.00–25.58)	0.92 (0.57–1.48)	0.707	0.90 (0.40–2.00)	0.780
T3 (≥25.59)	1.49 (1.03–2.18)	0.038	1.47 (0.83–2.63)	0.175
UFA, g/day				
T1 (≤37.13)	1.00		1.00	
T2 (37.14–46.06)	0.91 (0.64–1.30)	0.569	0.91 (0.59–1.40)	0.636
T3 (≥46.07)	1.06 (0.77–1.44)	0.718	0.98 (0.66–1.45)	0.900
MUFA, g/day				
T1 (≤ 22.06)	1.00		1.00	
T2 (22.07–27.28)	1.09 (0.68–1.75)	0.701	1.14 (0.64–2.01)	0.643
T3 (≥27.29)	1.32 (0.90–1.93)	0.138	1.18 (0.77–1.81)	0.433
PUFA, g/day				
T1 (≤ 14.27)	1.00		1.00	
T2 (14.28–19.14)	1.10 (0.78–1.55)	0.561	1.13 (0.74–1.74)	0.550
T3 (≥19.15)	0.74 (0.57–0.95)	0.021	0.68 (0.50–0.91)	0.012
n−3 PUFA, g/day				
T1 (≤1.34)	1.00		1.00	
T2 (1.35–1.91)	1.02 (0.75–1.39)	0.871	1.04 (0.68–1.60)	0.847
T3 (≥1.92)	0.85 (0.62–1.16)	0.275	0.91 (0.61–1.35)	0.607
n−6 PUFA, g/day				
T1 (≤12.74)	1.00		1.00	
T2 (12.75–17.08)	1.07 (0.76–1.52)	0.677	1.08 (0.70–1.67)	0.721
T3 (≥17.09)	0.70 (0.53–0.93)	0.016	0.64 (0.47–0.88)	0.009

[a]The adjusted variables were age and gender in model 1.

[b]The adjusted variables were age, sex, family income-to-poverty ratio, education level, marital status, ethnicity, platelet count, ALT, AST, GGT, ALP, albumin, total bilirubin, pre-hypertension, hypertension, diabetes, prediabetes, CVD, history of cancer, dyslipidemia, use of oral corticosteroid over 180 days, depression status, smoking status, BMI, waist circumference, regular exercise, HEI-2015, energy intake, sleep duration, and history of sleep disorders in model 2.

T1, first tertile; T2, second tertile; T3, third tertile; UFA, unsaturated fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

TABLE 3 Odds ratios (ORs) and 95% confidence intervals (CIs) for risk of significant liver fibrosis based on tertiles of dietary intakes of fatty acid components.

	Significant liver fibrosis			
	OR1 (95% CI) [a]	*p*- value	OR2 (95% CI) [b]	*p*-value
Linoleic acid (18:2), g/day				
T1 (≤12.62)	1.00		1.00	
T2 (12.63–16.92)	1.04 (0.74–1.47)	0.791	1.07 (0.70–1.63)	0.749
T3 (≥16.93)	0.69 (0.52–0.91)	0.011	0.64 (0.47–0.87)	0.008
Linolenic acid (18:3), g/day				
T1 (≤1.24)	1.00		1.00	
T2 (1.25–1.77)	1.03 (0.74–1.43)	0.847	1.06 (0.74–1.52)	0.745
T3 (≥1.78)	0.77 (0.56–1.06)	0.103	0.73 (0.48–1.11)	0.127
Arachidonic acid (20:4), g/day				
T1 (≤0.09)	1.00		1.00	
T2 (0.10–0.17)	0.91 (0.60–1.37)	0.626	0.85 (0.47–1.52)	0.555
T3 (≥0.18)	1.44 (1.13–1.84)	0.007	1.18 (0.80–1.73)	0.373
Butyric acid (4:0), g/day				
T1 (≤0.24)	1.00		1.00	
T2 (0.25–0.46)	0.87 (0.62–1.22)	0.397	0.94 (0.64–1.38)	0.748
T3 (≥0.47)	0.97 (0.60–1.57)	0.900	1.19 (0.64–2.20)	0.561
Caproic acid (6:0), g/day				
T1 (≤0.16)	1.00		1.00	
T2 (0.17–0.30)	0.97 (0.69–1.36)	0.833	1.01 (0.70–1.45)	0.957
T3 (≥0.31)	1.16 (0.69–1.94)	0.549	1.39 (0.69–2.77)	0.329
Caprylic acid (8:0), g/day				
T1 (≤0.14)	1.00		1.00	
T2 (0.15–0.25)	1.04 (0.75–1.43)	0.808	1.16 (0.68–1.97)	0.572
T3 (≥0.26)	1.26 (0.84–1.89)	0.238	1.48 (0.80–2.75)	0.197
Capric acid (10:0), g/day				
T1 (≤0.29)	1.00		1.00	
T2 (0.30–0.51)	1.08 (0.78–1.48)	0.631	1.24 (0.87–1.77)	0.207
T3 (≥0.52)	1.29 (0.82–2.03)	0.258	1.71 (0.87–3.33)	0.110
Lauric acid (12:00), g/day				
T1 (≤0.40)	1.00		1.00	
T2 (0.41–0.79)	1.05 (0.74–1.51)	0.759	1.20 (0.71–2.02)	0.481
T3 (≥0.80)	1.12 (0.75–1.68)	0.551	1.28 (0.78–2.10)	0.299
Myristic acid (14:00), g/day				
T1 (≤1.37)	1.00		1.00	
T2 (1.38-2.17)	1.01 (0.70–1.45)	0.970	1.04 (0.62–1.76)	0.873
T3 (≥2.18)	1.21 (0.75–1.96)	0.404	1.40 (0.78–2.54)	0.242
Palmitic acid (16:0), g/day				
T1 (≤11.33)	1.00		1.00	
T2 (11.34–14.15)	0.87 (0.55–1.36)	0.511	0.82 (0.39–1.74)	0.577
T3 (≥14.16)	1.48 (0.98–2.25)	0.062	1.39 (0.72–2.69)	0.303
Stearic acid (18:0), g/day				
T1 (≤4.68)	1.00		1.00	
T2 (4.69–6.16)	0.85 (0.54–1.33)	0.443	0.69 (0.41–1.17)	0.155
T3 (≥6.17)	1.68 (1.19–2.36)	0.006	1.34 (0.74-—2.43)	0.305
Palmitoleic acid (16:1), g/day				
T1 (≤0.78)	1.00		1.00	
T2 (0.79–1.14)	0.96 (0.66–1.41)	0.840	0.87 (0.52–1.45)	0.558
T3 (≥1.15)	1.43 (0.98–2.08)	0.061	1.02 (0.60–1.73)	0.947
Oleic acid (18:1), g/day				
T1 (≤20.68)	1.00		1.00	
T2 (20.69–25.68)	1.14 (0.68–1.89)	0.595	1.19 (0.65–2.16)	0.554
T3 (≥25.69)	1.25 (0.85–1.85)	0.236	1.06 (0.68–1.67)	0.778

[a] The adjusted variables were age and gender in model 1.

[b] The adjusted variables were age, sex, family income-to-poverty ratio, education level, marital status, ethnicity, platelet count, ALT, AST, GGT, ALP, albumin, total bilirubin, pre-hypertension, hypertension, diabetes, prediabetes, CVD, history of cancer, dyslipidemia, use of oral corticosteroid over 180 days, depression status, smoking status, BMI, waist circumference, regular exercise, HEI-2015, energy intake, sleep duration, and history of sleep disorders in model 2.

T1, first tertile; T2, second tertile; T3, third tertile.

(0.50–0.91) in model 2 for PUFA intake and 0.70 (0.53–0.93) in model 1 and 0.64 (0.47–0.88) in model 2 for n-6 PUFA intake. Additionally, the ORs (95% CIs) of significant liver fibrosis were 1.49 (1.03–2.18) for the highest tertile of SFA intake vs. lowest tertile in only age and gender adjusted model 1, which did not show statistically significant association consistently in model 2. Thus, we further explored the association of the main components of dietary fatty acid intakes with significant liver fibrosis risk (Table 3). The ORs (95% CIs) of significant liver fibrosis were 0.69 (0.52–0.91) in model 1 and 0.64 (0.47–0.87) in model 2 for linoleic acid intake (the highest tertile) when compared to the reference tertile. In addition, in only age- and gender-adjusted model 1, for the highest tertile vs. lowest tertile, the ORs (95% CIs) of significant liver fibrosis were 1.44 (1.13–1.84) for arachidonic acid intake and 1.68 (1.19–2.36) for stearic acid intake, which did not

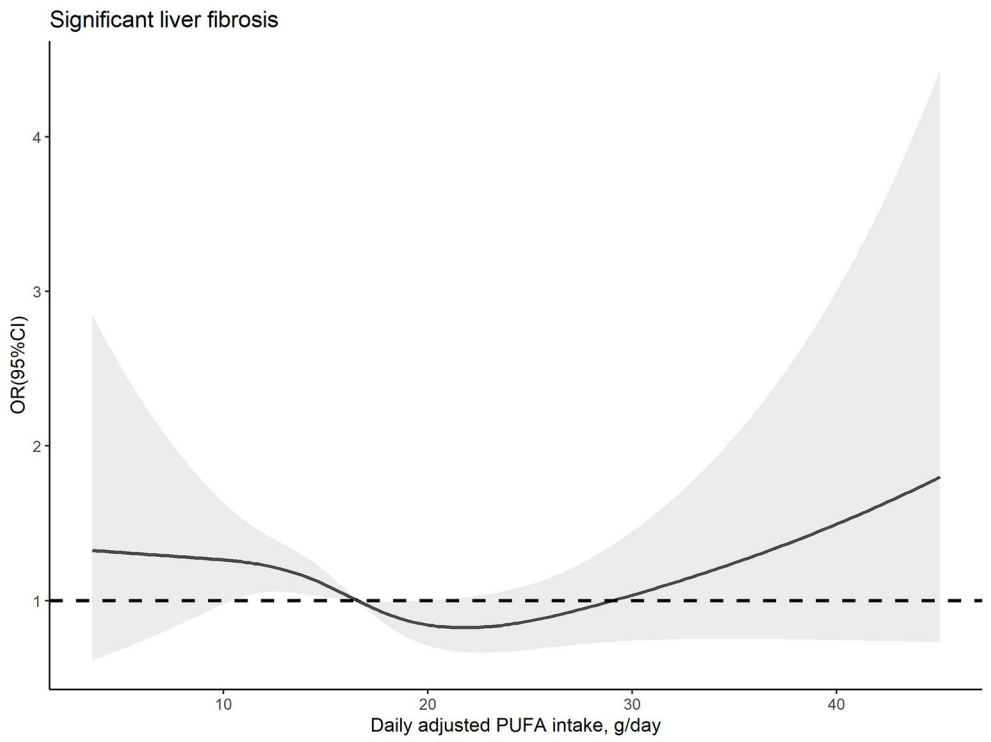


FIGURE 2
Dose–response relationship between dietary PUFA intake and significant liver fibrosis. The solid line and shadow area represent the estimated odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). The adjusted variables were the same as those in model 2, including age, sex, family income-to-poverty ratio, education level, marital status, ethnicity, platelet count, ALT, AST, GGT, ALP, albumin, total bilirubin, pre-hypertension, hypertension, diabetes, prediabetes, CVD, history of cancer, dyslipidemia, use of oral corticosteroid over 180 days, depression status, smoking status, BMI, waist circumference, regular exercise, HEI-2015, energy intake, sleep duration, and history of sleep disorders.

show statistically significant association consistently in model 2. Except for the fatty acid components mentioned above, other components did not show the statistically significant association with significant liver fibrosis. Moreover, based on the results of multiple logistic regression, we further observed a stable relationship between dietary fatty acid intakes and significant liver fibrosis by sensitivity analysis. The results of sensitivity analyses (Supplementary Figures S1, S2, Supplementary Tables S1, S2) had the same pattern with that in Tables 2, 3, reinforcing the inverse association of unsaturated to saturated fatty acid ratio, PUFA, and linoleic acid consumptions with significant liver fibrosis risk.

Dose–response analysis

The dose–response relationships between PUFA or linoleic acid intake and the risk of significant liver fibrosis are shown in Figures 2, 3, respectively. The similar U-shaped associations were observed between PUFA or linoleic acid intake and the risk of significant liver fibrosis, showing that the PUFA intake ranging from 16.70 to 19.83 g/day or linoleic acid intake ranging from 14.71 to 20.29 g/day was inversely associated with the risk of significant liver fibrosis, respectively (p for non-linearity <0.05).

Discussion

In this cross-sectional study, after adjusting multiple potential confounders, we pointed out the inverse association between the ratio of UFA to SFA and significant liver fibrosis risk, and further demonstrated the protective factors of total PUFA and its specific component linoleic acid for significant liver fibrosis in general US adults. Sensitivity analysis and RCS analysis showed similar results, reinforcing the significant inverse associations between the UFA to SFA ratio, PUFA, linoleic acid, and significant liver fibrosis risk. However, SFA, UFA, MUFA, and their specific components did not show the statistically significant association with significant liver fibrosis risk.

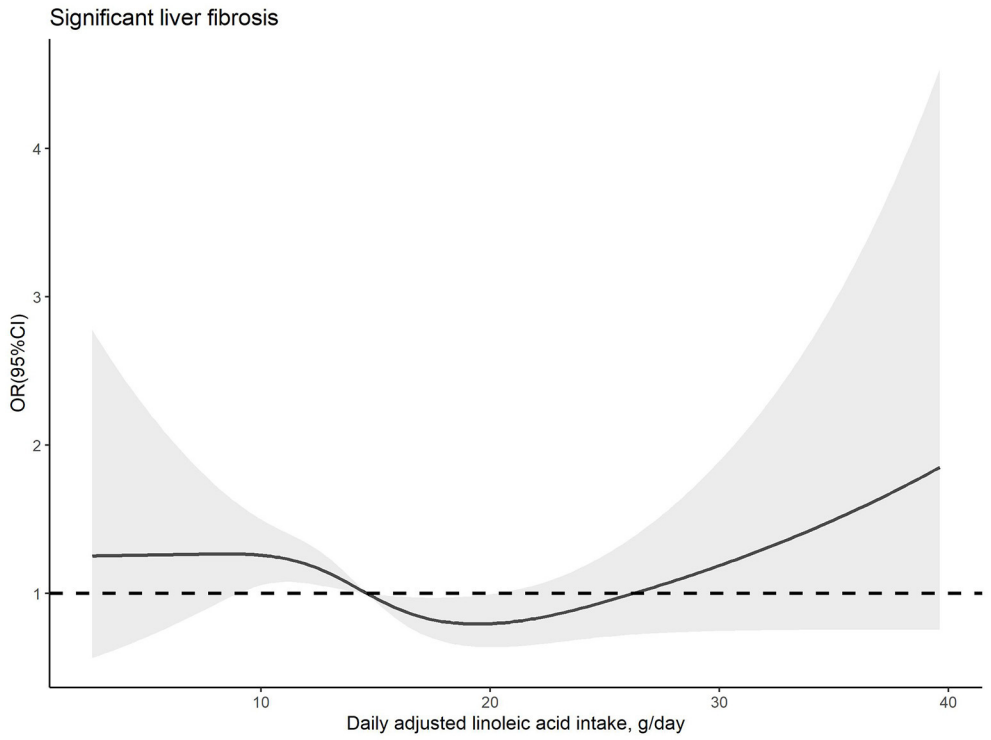


FIGURE 3
Dose–response relationship between dietary linoleic acid intake and significant liver fibrosis. The solid line and shadow area represent the estimated odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). The adjusted variables were the same as those in model 2, including age, sex, family income-to-poverty ratio, education level, marital status, ethnicity, platelet count, ALT, AST, GGT, ALP, albumin, total bilirubin, pre-hypertension, hypertension, diabetes, prediabetes, CVD, history of cancer, dyslipidemia, use of oral corticosteroid over 180 days, depression status, smoking status, BMI, waist circumference, regular exercise, HEI-2015, energy intake, sleep duration, and history of sleep disorders.

With the effective treatment available for hepatitis B and C, the worldwide prevalence of NAFLD or non-alcoholic steatohepatitis is currently the leading causes of liver fibrosis (36). Accumulation of excess liver fat is the basis for the contribution of NAFLD; of note, the quality of dietary fatty acids may take a role in the accumulation of liver fat (37, 38). The aforementioned study reported that a hypercaloric SFA-rich diet led to a remarkable increase in hepatic fat; by contrast, a PUFA-enriched diet did not show an increase in hepatic fat (12). Moreover, the 2015–2020 dietary guidelines for Americans recommend replacing total SFA with total UFA and keeping within saturated fat limits, and similar recommendations can also be found in the 2019 Canada's Food Guide (39). Additionally, a recent meta-analysis demonstrated that the replacement of SFA with PUFA reduced the cardiovascular disease risk, and replacement with MUFA was not clear due to the limited data (40). To the best of our knowledge, this study was one of the first studies to explore the association between the UFA to SFA ratio and liver fibrosis risk. As expected, statistically significant inverse association was observed between the UFA to SFA ratio and significant liver fibrosis risk, with a reduction of about 66% in the odds for liver fibrosis risk after adjustment of multiple potential confounders (Table 2). Inconsistent with our findings, an Italian longitudinal study (41), the epidemiologic study referred to the UFA to SFA ratio, indicated that higher UFA to SFA ratio increased total mortality but marginally. Different sample sizes, ethnicity, age, and dietary patterns may be the reasons. Noteworthy, the MUFA intake was 42.1 ± 12.5 g/day, PUFA intake was 7.4 ± 2.6 g/day, and SFA intake was 20.8 ± 7.8 g/day at baseline in the study above, whereas it was 25.34 ± 0.20 g/day, 17.20 ± 0.19 g/day, and 24.12 ± 0.27 g/day, respectively, in this study (Table 1), which implied that the different amount of PUFA or MUFA intakes may influence the healthy effect of UFA to SFA ratio. Furthermore, in this study, PUFA, but not MUFA or SFA, had the statistically significant inverse association with significant liver fibrosis risk, suggesting that PUFA plays a crucial role in the aforementioned relationship.

The n-3 PUFA and n-6 PUFA are the principal series of PUFA, playing the important roles in the development of NAFLD. A cross-sectional study indicated both dietary n-3 and n-6 PUFA had inverse associations with NAFLD risk, using data

from NHANES 2007–2014 (42). Additionally, depletion of long-chain PUFA has been reported in non-alcoholic fatty liver (43). Several studies also presented favorable associations between n-3 fatty acid intakes and NAFLD risk (17, 44, 45). Nevertheless, some inconsistent results in terms of n-3 PUFA components were found in more progressive NAFLD, such as fibrosis. In Lytle et al.'s (46) study, it was DHA, but not EPA, that attenuated western diet-linked liver fibrosis by targeting TGF-β pathway, while in another animal study, higher expression of TIMP-1 and TGF-β pro-fibrogenic genes and more severe fibrosis score were found in EPA and DHA together with olive oil-fed mice than that in only olive oil-fed mice (21). Those studies above implied that the different fatty acid components may influence the prevalence of liver fibrosis specifically, due to their different chemical structures and biological effects. However, up to now, limited study has explored the association between fatty acid components and risk of liver fibrosis.

In our study, the components of PUFA, only linoleic acid, presented statistically significant less odds of having significant liver fibrosis. Furthermore, the components of SFA and MUFA were not significantly related to the risk of significant liver fibrosis in model 2, consistent with the result of SFA and MUFA in total. This is inconsistent with a cross-sectional study, which showed the components of SFA lauric acid and myristic acid, palmitoleic and oleic MUFA had inverse associations with liver fibrosis, but a similar association was not observed in higher quartile of the fatty acids above (20). The discrepancies may be partially due to the specific participants with HIV, who has the different nutritional situation from normal people. Moreover, dietary lipid consumptions of total SFA, oleic acid, and linoleic acid had no significant association with the risk of cirrhosis or liver cancer, according to the study using data from NHANES I (47). We suppose that the effects of dietary fatty acids on liver function may vary depending on the intakes of fatty acid components, the stages of liver diseases, and the characteristics of the study population. Thus, we further explored the dose–response association between PUFA or linoleic acid intakes and risk of significant liver fibrosis. The results of RCS predicted that the range estimation of PUFA and linoleic acid intakes for the inversed association with significant liver fibrosis risk.

With the typical Western diet style of higher n-6 PUFA consumption than n-3 PUFA consumption, linoleic acid, as the major n-6 PUFA, can also represent the most consumed fatty acid in PUFA (48). Although linoleic acid is the most consumed PUFA, scarce study has investigated the associations of linoleic acid intake with NAFLD, not to mention liver fibrosis. Only one aforementioned study observed moderate linoleic acid intake increased liver fibrosis risk in subjects with HIV infection (20), and the discrepancy with our study may be due to the special characteristics of participants. Some biological processes may explain the inverse associations between dietary intakes of linoleic acid and significant liver fibrosis partially. Liver fibrosis induced by chronic damage to the liver is related to the accumulation of extracellular matrix (ECM) proteins, which distorts the hepatic wound healing process by forming a fibrous scar, and can proceed to cirrhosis with nodules of regenerating hepatocytes (1). Hepatic stellate cells are the main cell types to produce ECM in liver, which can be triggered by fat-accumulated hepatocytes. Additionally then, activated hepatic stellate cells are migratory and process excessive ECM (49). Furthermore, one randomized controlled trial of 67 participants with abdominally obesity demonstrated that n-6 PUFA reduced liver fat and did not induce inflammation or oxidative stress (50). It is worth to note that the participants in our study were also with higher BMI (29.8 ± 0.3 kg/m^2) and waist circumference (100.7 ± 0.8 cm), similar to the subjects in the study above. Altogether, we speculate that linoleic acid may reduce the liver fat and thereby reducing the amount of ECM produced by activated hepatic stellate cells, further alleviating the process of liver fibrosis. Because of the observational study limitation, further studies are needed to verify these findings.

This study has several strengths. First, a large-scale and national representative sample was used in our study, which can increase the statistical power and reliability of the findings. Second, we adjusted a large number of potential confounders, including demographic information, laboratory and body measurement parameters, medical conditions, and lifestyle factors. Third, significant liver fibrosis is determined by highly accurate transient elastography, which is considered as the non-invasive standard tool for evaluating significant fibrosis (23). Fourth, we investigated the dose–response relationship between PUFA or linoleic acid intakes and the risk of significant liver fibrosis.

However, the study also includes some limitations. First, our study was a cross-sectional study, which cannot determine the causality between dietary fatty acid intakes and the incidence of significant liver fibrosis. In addition, the dietary data were obtained from two 24-h dietary recalls, and the influence of recall bias was hardly avoided. Finally, we did not perform a stratified analysis due to the limited sample size of participants with significant liver fibrosis, which may hinder its statistical power to clarify associations of dietary fatty acid intakes with significant liver fibrosis risk.

In conclusion, this study observed that the UFA to SFA ratio, dietary PUFA intake, and linoleic acid intake were inversely associated with significant liver fibrosis risk. Furthermore, consumptions of PUFA and specific linoleic acid were in a dose–response relationship with significant liver fibrosis risk, warranting further large-scale prospective studies in this area.

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 below: http://www.cdc.gov/nchs/nhanes.htm.

Ethics statement

The studies involving human participants were reviewed and approved by the National Center for Health Statistics Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

H-LZ and Z-YL designed the study and performed data analyses and reviewed and editing the manuscript. TZ drafted, reviewed, and edited the manuscript. X-TL contributed in elaborating the tables and figures. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (no. 81973016), Basic and Applied Basic Research Foundation of Guangdong Province (2020A1515110682), and China Postdoctoral Science Foundation (2020M683135).

Acknowledgments

We are grateful to all the staff, the investigators, and the participants of the National Health and Nutrition Examination Survey for their meritorious contribution.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.938645/full#supplementary-material

References

1. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest.* (2005) 115:209–18. doi: 10.1172/jci24282

2. Lai M, Afdhal NH. Liver fibrosis determination. *Gastroenterol Clin North Am.* (2019) 48:281–9. doi: 10.1016/j.gtc.2019.02.002

3. Lurie Y, Webb M, Cytter-Kuint R, Shteingart S, Lederkremer GZ. Non-invasive diagnosis of liver fibrosis and cirrhosis. *World J Gastroenterol.* (2015) 21:11567–83. doi: 10.3748/wjg.v21.i41.11567

4. Kardashian A, Dodge JL, Terrault NA. Food insecurity is associated with mortality among u.s. adults with nonalcoholic fatty liver disease and advanced fibrosis. *Clin Gastroenterol Hepatol.* (2021) 5:29. doi: 10.1016/j.cgh.2021.11.029

5. Kim D, Kim W, Adejumo AC, Cholankeril G, Tighe SP, Wong RJ, et al. Race/ethnicity-based temporal changes in prevalence of NAFLD-related advanced fibrosis in the United States, 2005–2016. *Hepatol Int.* (2019) 13:205–13. doi: 10.1007/s12072-018-09926-z

6. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *J Hepatol.* (2017) 67:1265–73. doi: 10.1016/j.jhep.2017.07.027

7. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2015) 149:389–97.e10.doi: 10.1053/j.gastro.2015.04.043

8. Friedman SL, Pinzani M. Hepatic fibrosis 2022: Unmet needs and a blueprint for the future. *Hepatology.* (2022) 75:473–88. doi: 10.1002/hep.32285

9. Ferramosca A, Zara V. Modulation of hepatic steatosis by dietary fatty acids. *World J Gastroenterol.* (2014) 20:1746–55. doi: 10.3748/wjg.v20.i7.1746

10. Vergani L. Fatty acids and effects on in vitro and in vivo models of liver steatosis. *Curr Med Chem.* (2019) 26:3439–56. doi: 10.2174/0929867324666170518101334

11. Morris MC, Tangney CC. Dietary fat composition and dementia risk. *Neurobiol Aging.* (2014) 35 Suppl 2:S59–64. doi: 10.1016/j.neurobiolaging.2014.03.038

12. Rosqvist F, Kullberg J, Ståhlman M, Cedernaes J, Heurling K, Johansson HE, et al. Overeating saturated fat promotes fatty liver and ceramides compared with polyunsaturated fat: a randomized trial. *J Clin Endocrinol Metab.* (2019) 104:6207–19. doi: 10.1210/jc.2019-00160

13. Wu K, Zhao T, Hogstrand C, Xu YC, Ling SC, Chen GH, et al. FXR-mediated inhibition of autophagy contributes to FA-induced TG accumulation and accordingly reduces FA-induced lipotoxicity. *Cell Commun Signal.* (2020) 18:47. doi: 10.1186/s12964-020-0525-1

14. Song YF, Hogstrand C, Ling SC, Chen GH, Luo Z. Creb-Pgc1α pathway modulates the interaction between lipid droplets and mitochondria and influences high fat diet-induced changes of lipid metabolism in the liver and isolated hepatocytes of yellow catfish. *J Nutr Biochem.* (2020) 80:108364. doi: 10.1016/j.jnutbio.2020.108364

15. Leamy AK, Egnatchik RA, Young JD. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. *Prog Lipid Res.* (2013) 52:165–74. doi: 10.1016/j.plipres.2012.10.004

16. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. *Endocrinology.* (2006) 147:943–51. doi: 10.1210/en.2005-0570

17. Masterton GS, Plevris JN, Hayes PC. Review article: omega-3 fatty acids - a promising novel therapy for non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* (2010) 31:679–92. doi: 10.1111/j.1365-2036.2010.04230.x

18. El-Badry AM, Moritz W, Contaldo C, Tian Y, Graf R, Clavien PA. Prevention of reperfusion injury and microcirculatory failure in macrosteatotic mouse liver by omega-3 fatty acids. *Hepatology.* (2007) 45:855–63. doi: 10.1002/hep.21625

19. Schmöcker C, Weylandt KH, Kahlke L, Wang J, Lobeck H, Tiegs G, et al. Omega-3 fatty acids alleviate chemically induced acute hepatitis by suppression of cytokines. *Hepatology.* (2007) 45:864–9. doi: 10.1002/hep.21626

20. de Almeida CF, da Silva PS, Cardoso CSA, Moreira NG, Antunes JC, de Andrade MM, et al. Relationship between Dietary fatty acid intake with non-alcoholic fatty liver disease and liver fibrosis in people with HIV. *Nutrients.* (2021) 13:3462. doi: 10.3390/nu13103462

21. Provenzano A, Milani S, Vizzutti F, Delogu W, Navari N, Novo E, et al. n-3 polyunsaturated fatty acids worsen inflammation and fibrosis in experimental nonalcoholic steatohepatitis. *Liver Int.* (2014) 34:918–30. doi: 10.1111/liv.12500

22. Paik JM, Deshpande R, Golabi P, Younossi I, Henry L, Younossi ZM. The impact of modifiable risk factors on the long-term outcomes of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* (2020) 51:291–304. doi: 10.1111/apt.15580

23. Castera L, Friedrich-Rust M, Loomba R. Non-invasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2019) 156:1264–81. doi: 10.1053/j.gastro.2018.12.036

24. Zhang X, Heredia NI, Balakrishnan M, Thrift AP. Prevalence and factors associated with NAFLD detected by vibration controlled transient elastography among US adults: results from NHANES 2017-2018. *PLoS ONE.* (2021) 16:e0252164. doi: 10.1371/journal.pone.0252164

25. Johnson CL, Paulose-Ram R, Ogden CL, Carroll MD, Kruszon-Moran D, Dohrmann SM, et al. National health and nutrition examination survey: analytic guidelines, 1999–2010. *Vital Health Stat 2.* (2013) 161:1–24.

26. de Lédinghen V, Vergniol J. Transient elastography (FibroScan). *Gastroenterol Clin Biol.* (2008) 32(6 Suppl 1):58–67.doi: 10.1016/s0399-8320(08)73994-0

27. National Health and Nutrition Examination Survey. (2020). Available online at: https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/LUX_J.htm (accessed March 20, 2022).

28. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, et al. Accuracy of fibroscan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2019) 156:1717–30. doi: 10.1053/j.gastro.2019.01.042

29. Kim D, Konyn P, Cholankeril G, Ahmed A. Physical activity is associated with nonalcoholic fatty liver disease and significant fibrosis measured by fibroscan. *Clin Gastroenterol Hepatol.* (2021). doi: 10.1016/j.cgh.2021.06.029

30. Naqvi AZ, Davis RB, Mukamal KJ. Dietary fatty acids and peripheral artery disease in adults. *Atherosclerosis.* (2012) 222:545–50. doi: 10.1016/j.atherosclerosis.2012.03.029

31. National Cancer Institute. Developing the Healthy Eating Index. (2019). Available online at: https://epi.grants.cancer.gov/hei/developing.html#f1b (accessed March 20, 2022).

32. National Center for Health Statistics. (2020). Available online at: https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Laboratory&CycleBegin (accessed March 20, 2022).

33. Kim D, Yoo ER Li AA, Tighe SP, Cholankeril G, Harrison SA, et al. Depression is associated with non-alcoholic fatty liver disease among adults in the United States. *Aliment Pharmacol Ther.* (2019) 50:590–8. doi: 10.1111/apt.15395

34. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–94. Series 1: programs and collection procedures. *Vital Health Stat.* (1994) 1:1–407.

35. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65(4 Suppl):1220S–8S. doi: 10.1093/ajcn/65.4.1220S

36. Tacke F, Weiskirchen R. Non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH)-related liver fibrosis: mechanisms, treatment and prevention. *Ann Transl Med.* (2021) 9:729. doi: 10.21037/atm-20-4354

37. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J, et al. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J Mol Endocrinol.* (2006) 36:485–501. doi: 10.1677/jme.1.01909

38. Tiikkainen M, Bergholm R, Vehkavaara S, Rissanen A, Häkkinen AM, Tamminen M, et al. Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes.* (2003) 52:701–7. doi: 10.2337/diabetes.52.3.701

39. Harrison S, Lemieux S, Lamarche B. Assessing the impact of replacing foods high in saturated fats with foods high in unsaturated fats on dietary fat intake among Canadians. *Am J Clin Nutr.* (2022) 115:877–85. doi: 10.1093/ajcn/nqab420

40. Hooper L, Martin N, Jimoh OF, Kirk C, Foster E, Abdelhamid AS. Reduction in saturated fat intake for cardiovascular disease. *Cochrane Database Syst Rev.* (2020) 5:Cd011737. doi: 10.1002/14651858.CD011737.pub2

41. Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Palasciano R, Capurso S. et al. Unsaturated fatty acids intake and all-causes mortality: a 85-year follow-up of the Italian longitudinal study on Aging. *Exp Gerontol.* (2005) 40:335–43. doi: 10.1016/j.exger.2005.01.003

42. Cui J, Li L, Ren L, Sun J, Zhao H, Sun Y. Dietary n-3 and n-6 fatty acid intakes and NAFLD: a cross-sectional study in the United States. *Asia Pac J Clin Nutr.* (2021) 30:87–98. doi: 10.6133/apjcn.202103_30(1).0011

43. Kotronen A, Seppänen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepää AL, et al. Hepatic stearoyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the nonalcoholic human fatty liver. *Diabetes.* (2009) 58:203–8. doi: 10.2337/db08-1074

44. Lu W, Li S, Li J, Wang J, Zhang R, Zhou Y, et al. Effects of Omega-3 fatty acid in nonalcoholic fatty liver disease: a meta-analysis. *Gastroenterol Res Pract.* (2016) 2016:1459790. doi: 10.1155/2016/1459790

45. Musa-Veloso K, Venditti C, Lee HY, Darch M, Floyd S, West S, et al. Systematic review and meta-analysis of controlled intervention studies on the effectiveness of long-chain omega-3 fatty acids in patients with nonalcoholic fatty liver disease. *Nutr Rev.* (2018) 76:581–602. doi: 10.1093/nutrit/nuy022

46. Lytle KA, Depner CM, Wong CP, Jump DB. Docosahexaenoic acid attenuates Western diet-induced hepatic fibrosis in Ldlr-/- mice by targeting the TGFβ-Smad3 pathway. *J Lipid Res.* (2015) 56:1936–46. doi: 10.1194/jlr.M061275

47. Ioannou GN, Morrow OB, Connole ML, Lee SP. Association between dietary nutrient composition and the incidence of cirrhosis or liver cancer in the United States population. *Hepatology.* (2009) 50:175–84. doi: 10.1002/hep.22941

48. Hodson L, Rosqvist F, Parry SA. The influence of dietary fatty acids on liver fat content and metabolism. *Proc Nutr Soc.* (2020) 79:30–41. doi: 10.1017/s0029665119000569

49. Moreira RK. Hepatic stellate cells and liver fibrosis. *Arch Pathol Lab Med.* (2007) 131:1728–34. doi: 10.5858/2007-131-1728-hscalf

50. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr.* (2012) 95:1003–12. doi: 10.3945/ajcn.111.030114

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 18 August 2022
DOI 10.3389/fnut.2022.956900

OPEN ACCESS

EDITED BY
Muzi Na,
The Pennsylvania State University (PSU), United States

REVIEWED BY
Guannan Bai,
Zhejiang University School of Medicine, China
Zhengyuan Wang,
Shanghai Municipal Center for Disease Control and Prevention (SCDC), China

*CORRESPONDENCE
Lin Xu
xulin27@mail.sysu.edu.cn

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 30 May 2022
ACCEPTED 25 July 2022
PUBLISHED 18 August 2022

CITATION
Wang J, Huang Y, Yang H, Lin Z, Campos AI, Rentería ME and Xu L (2022) Plasma polyunsaturated fatty acid concentrations and sleep apnea risk: A two-sample Mendelian randomization study.
Front. Nutr. 9:956900.
doi: 10.3389/fnut.2022.956900

Plasma polyunsaturated fatty acid concentrations and sleep apnea risk: A two-sample Mendelian randomization study

Jiao Wang[1], Yingyue Huang[1], Huiling Yang[2], Zihong Lin[3], Adrian I. Campos[4], Miguel E. Rentería[4] and Lin Xu[1,5]*

[1]School of Public Health, Sun Yat-sen University, Guangzhou, China, [2]Eastern-Fusion Master Studio of Hezhou, Hezhou, China, [3]Hezhou Research Institute of Longevity Health Science, Hezhou, China, [4]Department of Genetics & Computational Biology, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia, [5]Li Ka Shing Faculty of Medicine, School of Public Health, The University of Hong Kong, Hong Kong, Hong Kong SAR, China

Background: Previous observational studies have found that lower levels of circulating polyunsaturated fatty acids (PUFAs) were associated with a higher risk of sleep apnea (SA). However, the causality of the association remains unclear.

Materials and methods: We used the two-sample Mendelian randomization (MR) study to assess the causal association of omega-3 and omega-6 fatty acids with SA. Single-nucleotide polymorphisms (SNPs) predicting the plasma level of PUFAs at the suggestive genome-wide significance level ($p < 5 \times 10^{-6}$) were selected as instrumental variables (IVs) from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) (n = ~8,000) Consortium. For outcomes, the summary-level statistics of SA were obtained from the latest genome-wide association study (GWAS), which combined five cohorts with a total number of 25,008 SA cases and 172,050 snoring cases (total = 523,366).

Results: We found no association of α-linolenic acid (ALA) [odds ratio (OR) = 1.09 per% changed, 95% confidence interval (CI) 0.67–1.78], eicosapentaenoic acid (EPA) (OR = 0.94, 95% CI 0.88–1.01), docosapentaenoic acid (DPA) (OR = 0.95, 95% CI 0.88–1.02), and docosahexaenoic acid (DHA) (OR = 0.99, 95% CI 0.96–1.02) with the risk of SA using inverse-variance weighted (IVW) method. Moreover, for omega-6 PUFAs, no association between linoleic acid (LA) (OR = 0.98, 95% CI 0.96–1.01), arachidonic acid (AA) (1.00, 95% CI 0.99–1.01), and adrenic acid (AdrA) (0.93, 95% CI 0.71–1.21) with the risk of SA was found. Similarly, no associations of PUFAs with SA were found in single-locus MR analysis.

Conclusion: In the current study, we first found that there is no genetic evidence to support the causal role of omega-3 and omega-6 PUFAs in the

risk of SA. From a public health perspective, our findings refute the notion that consumption of foods rich in PUFAs or the use of PUFAs supplementation can reduce the risk of SA.

KEYWORDS

plasma polyunsaturated fatty acid, omega-3, omega-6, sleep apnea, Mendelian randomization

Introduction

Sleep apnea (SA), a common form of sleep-disordered breathing, is characterized by brief interruptions of breathing during sleep. SA affects almost one billion adults aged 30–69 years worldwide (1) and is linked to a higher risk of cardiovascular diseases (CVDs) (2), type 2 diabetes (3), and Alzheimer's disease (4). The development of SA involved a higher inflammatory response (5, 6). Therefore, apart from continuous positive airway pressure (CPAP), nutritional supplementation may be a possible alternative approach to decrease the risk of SA, i.e., supplementation with polyunsaturated fatty acids (PUFAs).

As the key components of cellular and intracellular membranes, PUFAs can be classified into omega-3 PUFAs and omega-6 PUFAs. Previous studies showed that PUFAs were associated with lower risks of CVDs (7), type 2 diabetes (8), and autoimmune disorders (9). PUFAs mainly include the plant-derived α-linolenic acid (ALA) and linoleic acid (LA), and seafood-derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which were associated with lower immune response (10, 11). Previous epidemiological studies showed that lower levels of circulating omega-3 PUFA were associated with a higher risk of SA (12, 13), suggesting that diet-sourced omega-3 PUFAs may be modifiable targets for SA prevention. Based on these findings and the tolerability and safety of PUFAs (14), it has been speculated that PUFAs supplementation may reduce inflammatory response and decrease the risk of SA, and intervention trials have been suggested (15).

In this situation, in which a rationale exists for studying the role of PUFAs in SA but the evidence is lacking, Mendelian randomization (MR) provides an alternative way of examining causal effects. MR is an instrumental variable (IV) approach that uses genetic variants allocated randomly at conception as IVs and thus is unlikely to be biased by common confounders, such as lifestyle, health status, and socioeconomic positions. Moreover, compared with randomized control trials (RCTs) which estimate effects during a short term, MR tends to reflect the effects of lifelong exposure to PUFAs (16). Previous MR studies showed that higher genetically predicted ALA and LA, and lower EPA and docosapentaenoic acid (DPA), were associated with a lower risk of type 2 diabetes (17). A higher genetically predicted LA was also associated with a lower risk of asthma (9). However, to date we found no MR studies regarding the association between PUFAs and SA. Using genetic variants [i.e., single nucleotide polymorphisms (SNPs)] from the genome-wide association study (GWAS) of PUFAs (18–20), we conducted two-sample MR studies to examine the effect of main omega-3 fatty acids (ALA, EPA, DPA, and DHA) and omega-6 fatty acids [LA, arachidonic acid (AA), and adrenic acid (AdrA)] on SA, using genetic summary statistics from a large multivariate genome-wide association study (GWAS) of SA.

Materials and methods

Study design and data sources

We did a two-sample MR study, using de-identified summary-level data that were publicly available. Information about the data sources and sample sizes used in this study are summarized in the appendix (**Supplementary Table 1**). An overview of the study design is displayed in **Figure 1**. Ethical approvals were obtained in all original studies.

Genetic associations with polyunsaturated fatty acids (exposure)

Genetic variants associated with plasma omega-3 and omega-6 PUFAs were obtained from the recent GWAS of plasma fatty acid in European ancestry. Single nucleotide polymorphisms (SNPs) that reach the suggestive significant genome-wide association level ($p \leq 5 \times 10^{-6}$) and had a minor allele frequency of 0.01 or more were included. In this MR study, four omega-3 (ALA, EPA, DPA, and DHA) and three omega-6 (LA, AA, and AdrA) PUFAs were included. The GWAS of omega-3 (20) and omega-6 (18) PUFAs were from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (n = 8,866 and 8,631). The unit of omega-3 and omega-6 was the percentage (%) of total fatty acids. Information about DHA and LA-related SNPs was obtained from an up-to-date meta-analysis of GWAS (19).

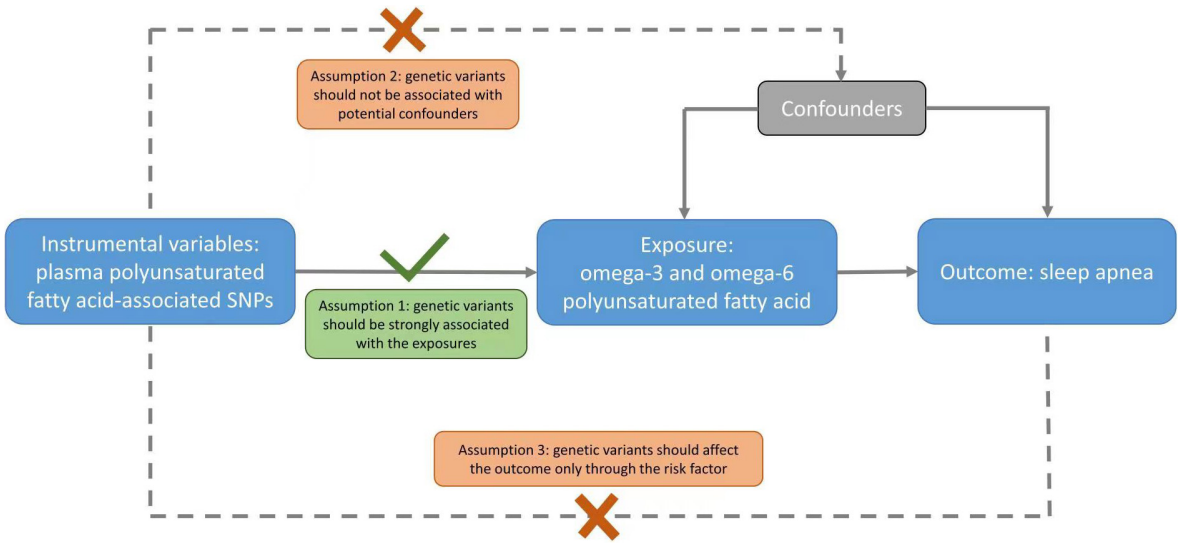


FIGURE 1
A schematic diagram of the Mendelian randomization (MR) assumptions underpinning an MR analysis of the association of plasma fatty acid levels with sleep apnea (SA). The dashed lines represent the possible violations of the MR assumptions.

To date, the best-characterized gene loci for PUFAs are the fatty acid desaturase (FADS) genes, such as FADS1, FADS2, and FADS3. These biologically relevant candidate genes encode the δ-5 and δ-6 desaturases, which are involved in the metabolic conversion of the LA to longer chain omega-6 PUFAs. The SNPs strongly associated with fatty acid were mainly allocated in chromosome 11q12.2 (i.e., in genes of C11orf9/10, FEN1, and FADS), and chromosome 6p24.2 (i.e., ELOVL2). From the meta-analysis of GWAS, the most highly associated SNPs on chromosome 11 explained 3.8% of the variance of ALA, 2.0% of the variance of EPA, and 8.6% of the variance of DPA (20). We further conducted the mechanistically informative analysis using the strongest SNP related to fatty acid biosynthesis in chromosome 11 or 6 for each PUFA, respectively, to minimize the pleiotropic effect (single-locus MR analysis), which method has been widely used in previous studies (17, 21).

Genetic associations with sleep apnea and snoring (outcome)

The genetic associations with SA were obtained from the most recent and largest GWAS of SA and snoring by Campos et al. (22), which used multi-trait analysis of GWAS (MTAG) (23) to boost statistical power, leveraging the high genetic correlations between SA and snoring. The SA multi-trait discovery GWAS combined five cohorts from the United Kingdom (UK Biobank; UKB), Canada (Canadian Longitudinal Study of Aging; CLSA), Australia (Australian Genetics of Depression Study; AGDS), the United States (Partner's Healthcare Biobank), and Finland genetic research (FinnGen) with a total number of 25,008 SA cases and 172,050 snoring cases (total = 523,366) with replication in an independent sample from 23andMe (total = 1,477,352 and cases = 175,522). Totally, 43 SNPs for SA explained 0.87% variance. For each cohort, SA was coded using either International Classification of Diseases Tenth Revision (ICD-10) codes *via* primary care records or self-reported diagnostic items *via* questionnaires (**Supplementary material**). All cohorts were restricted to European descent individuals with the adjustment for age, sex, batch (where relevant), and genetic ancestry principal components derived from genotype data [individual cohort details have been reported elsewhere (22)]. Since this GWAS paper is currently a pre-print, we used the latest published GWAS for sleep apnea from the FinnGen Study (24), and then repeated the analysis to validate our findings in the sensitivity analysis.

Statistical analysis

We estimated the F-statistic for each SNP as the square of the SNP-exposure association divided by the variance of the SNP-exposure association (25), and we generated the mean F-statistic for exposure (26). Independent variants ($r^2 < 0.01$) were selected using the "clump_data" function (EUR population) of the "MR-Base" R package. In the sensitivity analysis, we replicated the MR analysis using SNPs with a p-value $< 5 \times 10^{-8}$. We obtained MR estimates by meta-analyzing the SNP-specific Wald estimates (effect of SNP on outcome divided by SNP on exposure) using inverse-variance

weighted (IVW) with multiplicative random effects, which assumes balanced pleiotropy. The presence of heterogeneity due to pleiotropy was indicated by high Cochran's Q and I^2 statistics. To ensure the same effect allele was used for exposure and outcome for palindromic SNPs (coded A/T or C/G), we aligned them on effect allele frequency and the coding (forward or reverse). In sensitivity analyses, we obtained MR estimates using different methods with different assumptions, including the weighted median (WM) and MR-PRESSO. The WM (of SNP-specific Wald estimates) gives robust estimates if more than 50% of the information is derived from valid SNPs. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) is another way to identify horizontal pleiotropic outliers, and corrects for pleiotropy *via* outlier removal, if necessary (27). We obtained the empirical p-value for the MR-PRESSO global test *via* 10,000 simulations and used the outlier corrected estimate if outliers were found. The MR-Egger intercept test is used to statistically check the presence of horizontal pleiotropy. Since body mass index (BMI) could be a confounder in the associations between PUFAs and SA, multivariable MR (MVMR) was conducted to assess the associations of PUFAs with SA after adjustment for BMI (28). To adjust for multiple comparisons, the Bonferroni multiple testing correction was applied, and two-sided p-values of < 0.007 (0.05/7) were considered significant. Power was estimated using the approximation that the sample size for IV analysis to obtain a given power is the sample size for exposure on outcome divided by the r^2 for an instrument on exposure (29). The post-power calculation was based on the results of an online tool using several parameters, such as sample size of the outcome GWAS, variance explained by selected SNPs, and expected effect size.[1]

All statistical analyses were conducted in Stata version 13.1 (StataCorp LP, College Station, TX, United States) and R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) using the "TwoSampleMR," "MendelianRandomization," and "MRPRESSO" packages. The current MR study used publicly available summary data and does not require specific ethical approval.

Results

Totally, 6, 17, 12, and 12 SNPs associated with ALA, EPA, DPA, and DHA, respectively, were obtained at suggestive genome-wide significance (p-value $< 5 \times 10^{-6}$) after excluding linkage disequilibrium ($r^2 < 0.01$) with the average F-statistic range from 34 to 95. Similarly, 23, 10, and 5 SNPs associated with LA, AA, and AdrA, respectively, were identified, with the average F-statistic range from 41 to 145. Proxy SNPs were used when there were missing SNPs in the outcome dataset. No SNP was palindromic. **Supplementary Tables 2**, **3** summarize the information extracted for each SA-related SNP in the current study. Using the single-locus MR analysis, only the strongest SNP associated with fatty acid in chromosomes 11 or 6 were collected to predict the levels of PUFAs, with the average F-statistic range from 104 to 699. Rs174547, rs174546, and rs174550 in FADS1 were used as the genetic instruments of ALA/DPA, DHA, and AdrA, respectively. Rs99780 and rs472031 in FADS2 and FADS3 were used as the genetic instruments of LA and AA, respectively. Finally, rs174538 in C11orf10 was used as the genetic instrument of EPA.

In primary results (**Tables 1**, **2**), we found no association of ALA [odds ratio (OR) = 1.09 per% changed, 95% confidence interval (CI) 0.67–1.78], EPA (OR = 0.94, 95% CI 0.88–1.01), DPA (OR = 0.95, 95% CI 0.88–1.02), and DHA (OR = 0.99, 95% CI 0.96–1.02) with the risk of SA using the IVW method. Moreover, for omega-6 PUFAs, no association between LA (OR = 0.98, 95% CI 0.96–1.01), AA (1.00, 95% CI 0.99–1.01), and AdrA (0.93, 95% CI 0.71–1.21) with the risk of SA was found. Similar results were found in sensitivity analyses using WM and MR-PRESSO and using SNPs with a p-value of $< 5 \times 10^{-8}$ (**Supplementary Table 4**). The MR-Egger intercept suggested no evidence for directional horizontal pleiotropy (all p-values > 0.05). **Supplementary Figures 1,2** show the scatter plots of omega-3 and omega-6 PUFAs with SA in different methods. In sensitivity analysis, no associations of PUFAs with SA were found using the published GWAS from the FinnGen study (**Supplementary Tables 5,6**). There was no association of ALA and LA with SA found after adjustment for BMI (**Supplementary Table 7**).

The single-locus MR analysis (**Table 3**) showed similar results to the primary results. A little genetic association of omega-3 and omega-6 PUFAs with SA was found, with the OR ranging from 0.94 to 1.21 (p-values from 0.144 to 0.937).

Discussion

Principal findings

Our MR analyses first showed no association between lifelong exposure to plasma omega-3 PUFAs (ALA, EPA, DPA, and EHA) and omega-6 PUFAs (LA, AA, and AdrA) with the risk of SA. Protective effects reported in previous observational studies might be explained by residual confounding.

Comparison with other studies

Results of the present MR study did not support findings from previous observational studies showing that lower

1 https://shiny.cnsgenomics.com/mRnd/

TABLE 1 Mendelian randomization (MR) estimates of causality between plasma omega-3 polyunsaturated fatty acids (PUFAs) and sleep apnea (SA).

	Mendelian randomization method	No. of SNPs (mean F-statistic)	Odd ratio	95% Confidence interval	*P*-value	Cochran's Q (I^2)	MR-egger intercept (*P*-value)
α-linolenic acid (ALA)	IVW	6 (67.1)	1.09	0.67–1.78	0.738	8.14 (38.6%)	−0.003 (0.535)
	WM		1.21	0.79–1.84	0.385		
	MR Egger		1.42	0.52–3.87	0.526		
	MR-PRESSO		1.09	0.67–1.78	0.738		
Eicosapentaenoic acid (EPA)	IVW	17 (37.5)	0.94	0.88–1.01	0.095	18.8 (14.7%)	0.003 (0.467)
	WM		0.94	0.87–1.01	0.109		
	MR Egger		0.89	0.76–1.05	0.196		
	MR-PRESSO		0.94	0.88–1.01	0.095		
Docosapentaenoic acid (DPA)	IVW	12 (94.8)	0.95	0.88–1.02	0.169	11.7 (5.6%)	−0.005 (0.031)
	WM		0.96	0.88–1.06	0.447		
	MR Egger		1.06	0.94–1.20	0.375		
	MR-PRESSO		0.95	0.88–1.02	0.169		
Docosahexaenoic acid (DHA)	IVW	12 (34.4)	0.99	0.96–1.02	0.717	13.6 (19.4%)	0.003 (0.555)
	WM		0.99	0.95–1.03	0.691		
	MR Egger		0.96	0.86–1.08	0.519		
	MR-PRESSO		0.99	0.96–1.02	0.717		

IVW, inverse-variance weighted; WM, weighted median; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier.

TABLE 2 Mendelian randomization (MR) estimates of causality between plasma omega-6 polyunsaturated fatty acids and sleep apnea.

	Mendelian randomization method	No. of SNPs (mean F-statistic)	Odd ratio	95% Confidence interval	*P*-value	Cochran's Q (I^2)	MR-egger intercept (*P*-value)
Linoleic acid (LA)	IVW	23 (42.3)	0.98	0.96–1.01	0.231	48.1 (54.2%)	0.000 (0.905)
	WM		0.99	0.96–1.01	0.320		
	MR Egger		0.99	0.93–1.05	0.671		
	MR-PRESSO		0.99	0.97–1.01	0.517		
Arachidonic acid (AA)	IVW	10 (40.8)	1.00	0.99–1.01	0.580	11.4 (21.2%)	0.003 (0.512)
	WM		1.00	0.99–1.02	0.398		
	MR Egger		0.99	0.96–1.02	0.938		
	MR-PRESSO		1.00	0.99–1.01	0.580		
Adrenic acid (AdrA)	IVW	5 (145.5)	0.93	0.71–1.21	0.580	8.2 (63.6%)	−0.009 (0.009)
	WM		0.94	0.81–1.09	0.399		
	MR Egger		0.98	0.61–1.57	0.938		
	MR-PRESSO		0.93	0.71–1.21	0.580		

IVW, inverse-variance weighted; WM, weighted median; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier.

circulating omega-3 PUFAs (EPA and DHA) levels were associated with SA severity (12, 13, 30). A randomized, placebo-controlled trial showed that a daily intake of 600 mg omega-3 DHA supplements for 16 weeks improved sleep quality (less sleep disturbed breathing) (31). To date, no RCT assessed the effect of omega-6 PUFAs on SA (32), although better sleep quality was found after omega-6 PUFA supplementation in children with attention deficit hyperactivity disorder (ADHD) (33). Our study adds by clarifying that there is no causal effect of omega-3 and omega-6 PUFAs on SA, which is informative before an RCT and can make the most effective use of scarce resources.

Possible mechanisms

The underlying mechanisms explaining associations between PUFAs and SA are far from clear. One of the proposed mechanisms was an inflammatory response to obstructive sleep apnea (OSA), i.e., mediating by cytokines,

TABLE 3 Mendelian randomization (MR) estimates of causality between plasma polyunsaturated fatty acids and sleep apnea (FADS, C11orf10, and ELOVL gene loci).

Plasma polyunsaturated fatty acids		SNP (Gene)	No. of SNPs (Mean F-statistic)	Odd ratio	95% Confidence interval	*P*-value
Omega-3	α-linolenic acid(ALA)	rs174547 (FADS1)	1 (286.1)	1.21	0.76–1.90	0.419
	Eicosapentaenoic acid (EPA)	rs174538 (C11orf10)	1 (257.7)	0.94	0.86–1.02	0.144
	Docosapentaenoic acid (DPA)	rs174547 (FADS1)	1 (699.5)	0.96	0.87–1.06	0.420
	Docosahexaenoic acid (DHA)	rs174546 (FADS1)	1 (104.5)	0.98	0.93–1.04	0.478
Omega-6	Linoleic acid (LA)	rs99780 (FADS2)	1 (141.5)	1.02	0.97–1.07	0.509
	Arachidonic acid (AA)	rs472031 (FADS3)	1 (117.4)	1.00	0.97–1.02	0.937
	Adrenic acid (AdrA)	rs174550 (FADS1)	1 (635.1)	0.94	0.82–1.09	0.428

such as tumor necrosis factor (TNF-α) (15) and interleukin 6 (IL-6) (34). Animal and human studies have shown that the production of cytokines can be reduced by omega-3 PUFAs (35–37). However, the causal association between cytokines and SA was not evident. Therefore, whether the associations of PUFAs with SA are causal, a reflection of the underlying comorbidities, or merely due to chance, is yet to be confirmed, although this mechanism pathway seems reasonable.

Strengths and limitations

To our knowledge, our study is the first MR study examining the effect of omega-3 and omega-6 PUFAs on the risk of SA. The strengths of the present study included the use of MR to minimize residual confounding and reverse causality in traditional observational studies, and genetic validation of the wide range of PUFAs. Nevertheless, several limitations exist. MR is based on three stringent assumptions, i.e., the genetic instruments are strongly associated with the exposure; no confounders for the associations between the genetic instruments and the outcome; and the genetic instruments are not linked with the outcome other than *via* the exposure (no pleiotropy). To satisfy these assumptions, we only selected SNPs strongly associated with PUFAs reaching suggestive genome-wide significance, and replicated our findings using the most functionally related SNP in chromosome 11 or 6, including the well-established gene FADS (18). Population stratification might be a confounder in MR studies. However, we only used studies involving people of European descent, with genomic control. Confounding due to population stratification should be minimized. Regarding the potential pleiotropic effects, we conducted sensitivity analyses using multiple methods (i.e., WM, MR-PRESSO, and MVMR) to assess pleiotropy and found no evidence of a pleiotropic effect. Second, the estimates might be biased toward the observational associations if the exposure and outcome data came from the same sample (38). However, the sample of PUFAs GWAS had no overlap with the UK Biobank. Third, the effects of endogenous PUFAs may not be exactly the same as those of PUFAs from dietary intake. However, the essential fatty acids in omega-3 and omega-6 PUFA families, i.e., LA and ALA, cannot be synthesized directly in the human body (39). Serum LA levels are associated with dietary intake of LA (40). Moreover, the use of genetically predicted plasma PUFAs can eliminate measurement error, since the observational studies use one snapshot of measurement rather than lifetime exposure (41). Fourth, since cases of SA and snoring were identified by clinic diagnosis or self-report, misclassification was inevitable in the current study. However, the genetic correlation analyses from our upstream GWAS of SA (22) suggested different diagnostic criteria (i.e., by clinic diagnosis or self-report) have a comparable genetic architecture. After all, ascertaining SA cases using objective measures is difficult for GWAS with a large sample size. Fifth, the possible non-linear associations of PUFAs with SA could not be examined in the present study using summary-level statistics. Further studies using individual-level data are warranted to explore the potential non-linear patterns. Sixth, we could not distinguish the effects of central sleep apnea (CSA) and obstructive sleep apnea (OSA) and thus only examined SA in general, although SA likely represents the effect of OSA given the much higher prevalence of OSA (42).

Conclusion and public health implications

We first found no genetic evidence supporting the causal role of omega-3 and omega-6 PUFAs in the risk of SA. From a public health perspective, our findings refute the notion that consumption of foods rich in PUFAs or the use of PUFAs supplementation can reduce the risk of SA. Further MR studies, especially studies from other populations, providing more objective-diagnosed cases of SA and, ideally, using additional variants as genetic instruments are warranted to replicate the results.

Data availability statement

The exposure data are available on request after approval by AC and MR. The outcome data underlying this article are available in this article and in the online **Supplementary material**.

Author contributions

JW, AC, MR, and LX made substantial contributions to the conception and design and interpretation of data. JW and LX analyzed the data and drafted the article. YH, HY, ZL, AC, and MR revised it critically for important intellectual content. LX was guarantor. All authors gave their final approval for the manuscript.

Funding

This work was funded by the China Postdoctoral Science Foundation (2022M713557).

Acknowledgments

We thank the investigators from the United Kingdom (UK Biobank; UKB), Canada (Canadian Longitudinal Study of Aging; CLSA), Australia (Australian Genetics of Depression Study; AGDS), the United States (Partner's Healthcare Biobank), Finland genetic research (FinnGen), CARDIoGRAMplusC4D, MEGASTROKE, and other GWAS used in the present study.

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.2022.956900/full#supplementary-material

References

1. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. *Lancet Respir Med.* (2019) 7:687–98. doi: 10.1016/S2213-2600(19)30198-5

2. Wang X, Ouyang Y, Wang Z, Zhao G, Liu L, Bi Y. Obstructive sleep apnea and risk of cardiovascular disease and all-cause mortality: a meta-analysis of prospective cohort studies. *Int J Cardiol.* (2013) 169:207–14.

3. Huang T, Lin BM, Stampfer MJ, Tworoger SS, Hu FB, Redline S. A population-based study of the bidirectional association between obstructive sleep apnea and type 2 diabetes in three prospective U.S. Cohorts. *Diabetes Care.* (2018) 41:2111–9. doi: 10.2337/dc18-0675

4. Emamian F, Khazaie H, Tahmasian M, Leschziner GD, Morrell MJ, Hsiung G-YR, et al. The association between obstructive sleep apnea and Alzheimer's disease: a meta-analysis perspective. *Front Aging Neurosci.* (2016) 8:78. doi: 10.3389/fnagi.2016.00078

5. Shamsuzzaman AS, Winnicki M, Lanfranchi P, Wolk R, Kara T, Accurso V, et al. Elevated C-reactive protein in patients with obstructive sleep apnea. *Circulation.* (2002) 105:2462–4.

6. Cao Y, Song Y, Ning P, Zhang L, Wu S, Quan J, et al. Association between tumor necrosis factor alpha and obstructive sleep apnea in adults: a meta-analysis update. *BMC Pulm Med.* (2020) 20:215. doi: 10.1186/s12890-020-01253-0

7. Zhao JV, Schooling CM. Effect of linoleic acid on ischemic heart disease and its risk factors: a Mendelian randomization study. *BMC Med.* (2019) 17:61. doi: 10.1186/s12916-019-1293-x

8. Coelho OGL, da Silva BP, Rocha D, Lopes LL, Alfenas RCG. Polyunsaturated fatty acids and type 2 diabetes: impact on the glycemic control mechanism. *Crit Rev Food Sci Nutr.* (2017) 57:3614–9.

9. Zhao JV, Schooling CM. The role of linoleic acid in asthma and inflammatory markers: a Mendelian randomization study. *Am J Clin Nutr.* (2019) 110:685–90.

10. Alzoubi MR, Aldomi Al-Domi H. Could omega-3 fatty acids a therapeutic treatment of the immune-metabolic consequence of intermittent hypoxia in obstructive sleep apnea? *Diabetes Metab Syndr.* (2017) 11:297–304. doi: 10.1016/j.dsx.2016.06.024

11. Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, et al. Effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on immune cell function in healthy humans. *Am J Clin Nutr.* (2004) 80:1626–33.

12. Ladesich JB, Pottala JV, Romaker A, Harris WS. Membrane level of omega-3 docosahexaenoic acid is associated with severity of obstructive sleep apnea. *J Clin Sleep Med.* (2011) 7:391–6. doi: 10.5664/JCSM.1198

13. Tittus J, Huber MT, Storck K, Köhler A, Köhler JM, von Arnim T, et al. Omega-3 index and obstructive sleep apnea: a cross-sectional study. *J Clin Sleep Med.* (2017) 13:1131–6. doi: 10.5664/jcsm.6754

14. Cicero AF, Ferroni A, Ertek S. Tolerability and safety of commonly used dietary supplements and nutraceuticals with lipid-lowering effects. *Expert Opin Drug Saf.* (2012) 11:753–66.

15. Scorza FA, Cavalheiro EA, Scorza CA, Galduróz JC, Tufik S, Andersen ML. Sleep apnea and inflammation – Getting a good night's sleep with omega-3 supplementation. *Front Neurol.* (2013) 4:193. doi: 10.3389/fneur.2013.00193

16. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ.* (2018) 362:k601. doi: 10.1136/bmj.k601

17. Yuan S, Larsson SC. Association of genetic variants related to plasma fatty acids with type 2 diabetes mellitus and glycaemic traits: a Mendelian randomisation study. *Diabetologia.* (2020) 63:116–23. doi: 10.1007/s00125-019-05019-0

18. Guan W, Steffen BT, Lemaitre RN, Wu JHY, Tanaka T, Manichaikul A, et al. Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genet.* (2014) 7:321–31. doi: 10.1161/CIRCGENETICS.113.000208

19. Kettunen J, Demirkan A, Würtz P, Draisma HHM, Haller T, Rawal R, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun.* (2016) 7:11122. doi: 10.1038/ncomms11122

20. Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, et al. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet.* (2011) 7:e1002193. doi: 10.1371/journal.pgen.1002193

21. Jones HJ, Borges MC, Carnegie R, Mongan D, Rogers PJ, Lewis SJ, et al. Associations between plasma fatty acid concentrations and schizophrenia: a two-sample Mendelian randomisation study. *Lancet Psychiatry.* (2021) 8:1062–70. doi: 10.1016/S2215-0366(21)00286-8

22. Campos AI, Ingold N, Huang Y, Mitchell BL, Kho P-F, Han X, et al. Multi-trait genome-wide association study identifies new loci associated with sleep apnoea risk. *medRxiv* [Preprint]. (2022). doi: 10.1101/2020.09.29.20199893

23. Turley P, Walters RK, Maghzian O, Okbay A, Lee JJ, Fontana MA, et al. Multi-trait analysis of genome-wide association summary statistics using MTAG. *Nat Genet.* (2018) 50:229–37.

24. Strausz S, Ruotsalainen S, Ollila HM, Karjalainen J, Kiiskinen T, Reeve M, et al. Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health. *Eur Respir J.* (2021). 57:2003091. doi: 10.1183/13993003.03091-2020

25. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol.* (2016) 45:1961–74. doi: 10.1093/ije/dyw220

26. Richardson TG, Sanderson E, Palmer TM, Ala-Korpela M, Ference BA, Davey Smith G, et al. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: a multivariable Mendelian randomisation analysis. *PLoS Med.* (2020) 17:e1003062. doi: 10.1371/journal.pmed.1003062

27. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8.

28. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol.* (2015) 181:251–60.

29. Freeman G, Cowling BJ, Schooling CM. Power and sample size calculations for Mendelian randomization studies using one genetic instrument. *Int J Epidemiol.* (2013) 42:1157–63.

30. Nakabayashi K, Jujo K, Saito K, Oka T, Hagiwara N. Evaluation of the association between sleep apnea and polyunsaturated fatty acids profiles in patients after percutaneous coronary intervention. *Heart Vessels.* (2017) 32:1296–303. doi: 10.1007/s00380-017-1010-7

31. Montgomery P, Burton JR, Sewell RP, Spreckelsen TF, Richardson AJ. Fatty acids and sleep in UK children: subjective and pilot objective sleep results from the DOLAB study–a randomized controlled trial. *J Sleep Res.* (2014) 23:364–88. doi: 10.1111/jsr.12135

32. Zhao M, Tuo H, Wang S, Zhao L. The effects of dietary nutrition on sleep and sleep disorders. *Mediat Inflamm.* (2020) 2020:3142874.

33. Yehuda S, Rabinovitz-Shenkar S, Carasso RL. Effects of essential fatty acids in iron deficient and sleep-disturbed attention deficit hyperactivity disorder (ADHD) children. *Eur J Clin Nutr.* (2011) 65:1167–9. doi: 10.1038/ejcn.2011.80

34. Ünüvar Doğan F, Yosunkaya Ş, Kuzu Okur H, Can Ü. Relationships between obstructive sleep apnea syndrome, continuous positive airway pressure treatment, and inflammatory cytokines. *Sleep Disord.* (2014) 2014:518920.

35. Meydani SN. Effect of (n-3) polyunsaturated fatty acidson cytokine production and their biologic function. *Nutrition.* (1996) 12(1 Suppl.):S8–14.

36. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. *Brain Behav Immun.* (2011) 25:1725–34. doi: 10.1016/j.bbi.2011.07.229

37. Madison AA, Belury MA, Andridge R, Renna ME, Rosie Shrout M, Malarkey WB, et al. Omega-3 supplementation and stress reactivity of cellular aging biomarkers: an ancillary substudy of a randomized, controlled trial in midlife adults. *Mol Psychiatry.* (2021) 26:3034–42. doi: 10.1038/s41380-021-01077-2

38. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Genet Epidemiol.* (2016) 40:597–608.

39. Nagy K, Tiuca ID. *Importance of fatty acids in physiopathology of human body: in fatty acids.* London: IntechOpen (2017).

40. Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, et al. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney diet heart study and updated meta-analysis. *BMJ.* (2013) 346:e8707. doi: 10.1136/bmj.e8707

41. Carter AR, Gill D, Davies NM, Taylor AE, Tillmann T, Vaucher J, et al. Understanding the consequences of education inequality on cardiovascular disease: Mendelian randomisation study. *BMJ.* (2019) 365:l1855. doi: 10.1136/bmj.l1855

42. Javaheri S, Barbe F, Campos-Rodriguez F, Dempsey JA, Khayat R, Javaheri S, et al. Sleep apnea: types, mechanisms, and clinical cardiovascular consequences. *J Am Coll Cardiol.* (2017) 69:841–58.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 09 September 2022
DOI 10.3389/fnut.2022.962787

OPEN ACCESS

EDITED BY
Thea Magrone,
University of Bari Aldo Moro, Italy

REVIEWED BY
Yuan Lin,
Nanjing Medical University, China
Bryan A Wilson,
Axis Ready LLC, United States
Mustafa Öz,
Aksaray University, Turkey

*CORRESPONDENCE
Ziqin Cao
xyeyyziqincao@csu.edu.cn
Jianhuang Wu
jianhuangwu11@163.com

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 06 June 2022
ACCEPTED 22 August 2022
PUBLISHED 09 September 2022

CITATION
Li Y, Li Q, Cao Z and Wu J (2022) The causal association of polyunsaturated fatty acids with allergic disease: A two-sample Mendelian randomization study. *Front. Nutr.* 9:962787. doi: 10.3389/fnut.2022.962787

The causal association of polyunsaturated fatty acids with allergic disease: A two-sample Mendelian randomization study

Yajia Li[1,2], Qiangxiang Li[2,3,4], Ziqin Cao[2,5]* and Jianhuang Wu[2,5]*

[1]Department of Dermatology, Xiangya Hospital, Central South University, Changsha, China, [2]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China, [3]Ningxia Geriatric Disease Clinical Research Center, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan, China, [4]Hunan People's Hospital, Department of Hunan Institute of Geriatrics, Changsha, China, [5]Department of Spine Surgery and Orthopaedics, Xiangya Hospital, Central South University, Changsha, China

Objectives: Previous studies have reported a potential association of polyunsaturated fatty acids (PUFAs) levels with allergic disease risk and the possible benefit of PUFAs supplementation on allergic disease prevention. This study was performed to estimate the genetic association between PUFAs and allergic diseases using the method of both univariable and multivariable two-sample Mendelian randomization (MR).

Methods: As indicators of the PUFAs levels, we included the omega-3, omega-6, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), linoleic acid (LA), and the ratio of omega-6 to omega-3 (omega-6:3). Summarized statistics of genome-wide association studies (GWASs) for these PUFAs were obtained from the United Kingdom Biobank and the Twins United Kingdom cohort. Genetic data relating to allergic diseases, including atopic dermatitis (AD), allergic rhinitis (AR), allergic conjunctivitis (AC), allergic urticaria (AU) and asthma, were accessed from the FinnGen biobank analysis. Odds ratios and 95% CIs were used to express the impact.

Results: The MR results denoted a genetic association between the genetically determined increase in omega-3 levels and the decreased risk of some allergic diseases including AD (OR: 0.863; 95% CI: 0.785 to 0.949; p = 3.86E-03), AC (OR:0.720; 95% CI: 0.547 to 0.947; p = 1.87E-02) and AU (OR:0.821; 95% CI: 0.684 to 0.985; p = 3.42E-02), while omega-6 and DHA level was only found to have negatively correlation with risk of AC with ORs of 0.655 (95% CI: 0.445 to 0.964; p = 3.18E-02) and 0.671 (95% CI 0.490 to 0.918; p = 1.25E-02), respectively. Omega-6:3 were causally significantly associated with the increased risk of AD (OR:1.171; 95% CI: 1.045 to 1.312; p = 6.46E-03) and AC (IVW: OR:1.341; 95% CI: 1.032 to 1.743; p = 2.83E-02). After adjustment of age, economic level, BMI, smoking and alcohol behaviors in the multivariable MR analysis, a direct causal protective effect of omega-3 on AD and AC, as well as a direct causal association between DHA and AD were observed. Omega-6:3

was also found to be directly associated with an increased risk of AD and AC. No association was found of EPA or LA with allergic diseases.

Conclusion: Higher PUFA concentrations (omega-3, omega-6, DHA) and lower omega-6:3 ratios were genetically associated with a lower risk of some allergic diseases.

KEYWORDS

polyunsaturated fatty acids, omega-3, omega-6, allergic diseases, Mendelian randomization study

Introduction

Allergic diseases may involve the respiratory, digestive, skin or other systems and include common conditions such as eczema/atopic dermatitis (AD), allergic asthma, allergic rhino-conjunctivitis (AR/AC)/hay fever/seasonal allergies and allergic urticaria (AU) (1). It is also widely accepted that AD comorbidities extend beyond other allergic conditions, such as AA, AR, AC, and eosinophilic esophagitis, and that allergic diseases follow time-based sequences, suggesting both cutaneous and systemic immune activation (1–3). There has been a noticeable increase in the incidence of allergic disease, which now affects an estimated 20% of the population, making it a public health concern (2, 4, 5). Some allergic conditions with childhood-onset resolve with age, whereas others may persist throughout the lifetime (6), leading to an increased burden on families, society, and healthcare services (7). The rapid escalation of allergic diseases may not be attributed to either genetic or environmental factors (such as lifestyle and diets) alone, and mixed etiology is not fully understood (8). The association between genetic factors and allergic diseases has been extensively studied and some shared susceptibility loci have been identified (9). Large-scale genome-wide association studies (GWAS) and studies of causal roles of genetic susceptibility loci are expected to improve understanding of the prevention and treatment of atopic diseases.

Polyunsaturated fatty acids (PUFAs) of the omega-3 and omega-6 series have been identified by laboratory and epidemiological evidence as having anti-inflammatory and anti-allergy effects (10–13). Especially for omega-3, systematic reviews and meta-analyses have shown the impact of the fish oil-derived omega-3 PUFAs in the primary prevention of allergic disease (14, 15). Indeed, the omega-3 PUFA, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have been shown to have anti-inflammatory and immunoregulatory properties (13). On the contrast, linoleic acid (LA), one type of omega-6 acid, was found to be linked to increased specific IgE and pro-inflammatory responses among infants (16–18). The ratios of omega-6 to omega-3 PUFAs in some Western diets are found to arise from an equal balance of 1:1 to an unbalanced level of nearly 30:1. The significant changes in PUFAs consumption seem to be paralleled by the increase in the prevalence of atopic and allergic diseases (19), indicating a potential causal relationship between PUFA intake and allergic diseases.

PUFA supplementation has been proposed to prevent allergic disease, and genetic evidence must be considered in establishing the causal effects (20). The current study employed Mendelian randomization (MR) analysis, using instrumental variables (IVs) to explore a causal association of exposure factors with outcomes (21–23). The theory of random distribution of genetic variants within the population, which mimics the randomization process in the assortment of meiosis genetic variants, underpins the approach. An analogy between MR and RCTs may be drawn, with the former less likely to be affected by confounders and reverse causality (24). Two-sample MR analysis relies on genetic effect estimates from two independent summary sets of GWAS to the inference of causal association by comparison with one-sample MR (25). Multivariable MR (MVMR) is an extension of univariable MR and can take the pleiotropy in multiple traits into account. The assumptions of MVMR include the possible effects of genetic variants on multiple measured exposures and the extension of the exclusion restriction and exchangeability assumption (26). Therefore, MVMR can provide a consistent estimator of the direct effect of the primary exposure on the outcome that does not work via the mediator, even when a secondary exposure act as a mediator in the relationship. The current study aimed to infer causal associations between PUFAs (using genetic IVs as proxy) and with risk of atopic disease through a two-sample MR analysis (27).

Materials and methods

The overview flowchart of the hypothesis and schematic design is shown in **Figure 1**. Three principal assumptions were made (**Figure 1A**) (28): (1) genetic variants were strongly associated with exposure; (2) genetic variants were only

associated with the outcome through exposure, and (3) this association was independent of any potential confounders. Publicly available data were used, and no additional informed consent or ethical approval was required. Genetic data were obtained from two large GWAS and, after removing outliers and harmonizing alleles, MR analysis with six different methods and sensitivity analysis was applied to identify causal associations between PUFAs and allergic diseases.

Data source and selection of genetic instrumental variables

Single-nucleotide polymorphisms (SNPs) were identified and used as IVs from eligible datasets in GWAS Catalog, IEU openGWAS and NealELab. Only GWAS conducted on individuals of European ancestry were included to limit the bias resulting from ethnic confounders. Six main dietary PUFAs indexes were considered in the present study: SNPs for circulating omega-3, omega-6, DHA, EPA, and LA levels, as well as the ratio of omega-6 to omega-3 fatty acids (omega-6:3), were also obtained as instrumental variables of exposure. Genetic risk variants of exposure including omega-3, omega-6, DHA, LA, and omega-6:3 were identified from the Metabolic biomarkers in the United Kingdom Biobank (Nightingale Health 2020). Circulating omega-3 and omega-6 fatty acids, as well as DHA and LA concentrations, were measured from randomly selected EDTA plasma samples by using a targeted high-throughput nuclear magnetic resonance (NMR) metabolomics platform (Nightingale Health Ltd; biomarker quantification version 2020) (29). In total, 121,577 samples were retained for analyses after removing duplicates and observations not passing quality control in the non-fasting plasma samples collected at baseline, and 114,999 samples were retained in the final. Details for measurement technology and applications for the epidemiology of this platform have been previously reviewed (30–32). For the EPA level, it was obtained from the Twins United Kingdom cohort (33), which is an adult twin British registry composed of mostly women recruited from the general United Kingdom population through national media, and the EPA level was measurable in blood using the Metabolon platform. The detailed information was described in the previous studies (34–36). Genetic data relating to AD, AC, AR, AU, and asthma were accessed from the FinnGen biobank analysis (round 5), and diagnoses were based on ICD-10 (**Figure 1B**).

Summarized statistics of PUFA-related SNPs with genome-wide significance ($p < 5 \times 10^{-8}$) were designated as alternate IVs. Linkage disequilibrium (LD) was tested within the condition of the clumping algorithm with $r^2 = 0.001$ and kb = 10,000 to reduce the effect of strong LD. F statistics were used to assess the risk of weak instrumental bias with at least 10 being a sufficient level for MR analysis (36, 37). Based on the merged dataset of exposure-outcome, harmonization of effect alleles and subsequent analyses were conducted. Detailed information regarding IVs is presented in **Supplementary Tables 1–6**.

Two-sample Mendelian randomization

Primary MR analysis was performed using the inverse-variance weighted (IVW) model, combining Wald estimates of causality for each IV with the assumption of invalid genetic instruments (e.g., a balanced pleiotropy) (38, 39). MR-Egger regression analysis and weighted-median estimator were used to examine any violation of MR assumptions caused by directional pleiotropy (40, 41). The MR-Egger intercept estimates the effect of pleiotropy across genetic variants and provides a relatively robust estimate with the independence of IV validity and an adjusted result via the regression slope (38, 40). A consistent valid estimate could be inferred by a weighted-median estimator if over 50% of instrumental variables were valid (40, 41). The weighted mode-based method infers robust overall causal estimates on the condition that individual estimates were mostly obtained from valid IVs (42). MR-Robust Adjusted Profile Score (MRAPS) was used to derive a more accurate assessment of causal association with ideal independence of IVs (43). In addition, MR pleiotropy residual sum and outlier (MR-PRESSO) was used to detect and correct horizontal pleiotropy by the removal of outliers with $p < 0.05$ and to give a corrected causal effect (44). Cochran's Q-statistic was used to assess heterogeneity, and a random-effect model was used for subsequent analyses with $p < 0.05$ as a level of significant heterogeneity (45). In MR-PRESSO analysis, heterogeneity and pleiotropy in causal effect estimates were reduced by removing outliers and reassessing causal estimates. If heterogeneity was still significant after removing outliers, all SNPs with a p-value < 1 in the MR-PRESSO outlier test were removed. The MR analysis was re-performed with results from the random-effect IVW model being adopted. The number of distributions in the MR-PRESSO analysis was set to 1,000. Additional sensitivity analyses were performed by the exclusion of IVs one at a time (46). Other statistical tools were used to complement IVW and produced wider confidence intervals (CIs) (47). Therefore, IVW results were prioritized, and the MR-Egger was adopted for significant pleiotropy and the MR-PRESSO to detect final outliers. The flow chart of analytical methods used in this MR analysis is shown in **Supplementary Figure 1**.

In additional analyses, to investigate the direct effects of PUFAs on allergic diseases, MVMR analysis was also performed as an extension of univariable MR allowing the joint detection of causal effects of multiple risk factors (26, 48). Genetic associations between SNPs and age, average total household income, body mass index (BMI), smoking, and alcohol were obtained from a recent GWAS using a United Kingdom Biobank sample of 2,336,260 to 1,3586,591 individuals of European

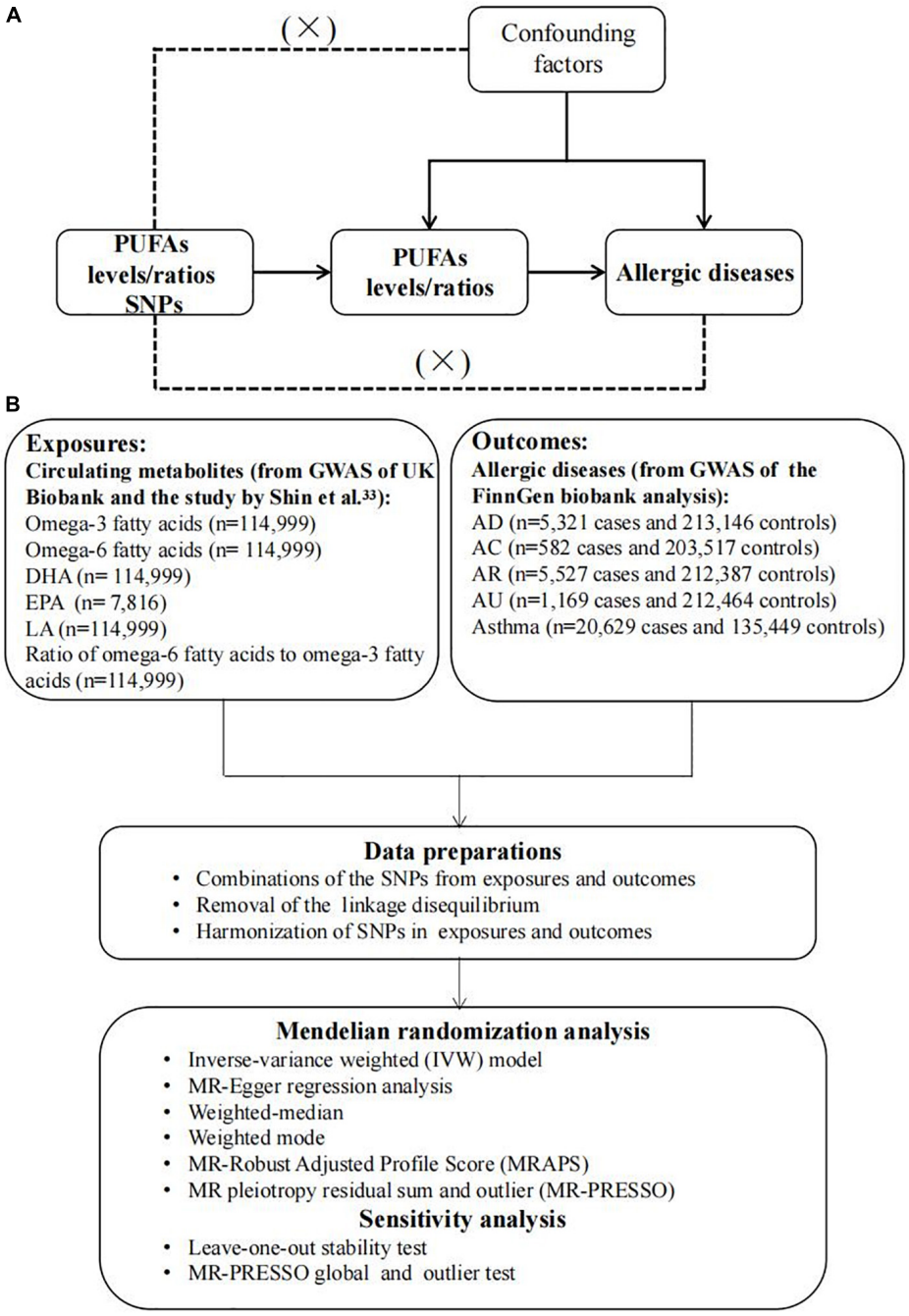


FIGURE 1
The overview flowchart of hypothesis and schematic design **(A)** Mendelian randomization key hypothesis Diagram. SNPs associated with PUFAs levels/ratios were used as the genetic instruments for investigating the causal effect of PUFA on allergic diseases. Line with arrows indicates that the genetic instruments (SNPs) are associated with the exposure and can only affect the outcome via the exposure. Dashed lines indicate that the genetic instruments (SNPs) are independent of confounders between the results. **(B)** Schematic design for the mendelian randomization analysis.

descent. MVMR takes into account the relationships among PUFAs, age, income, BMI, smoking, and alcohol drinking, and the fact that the SNPs selected in the MR analyses are often associated with several phenotypes. Therefore, MVMR was used to evaluate the direct effects of PUFAs independent of the effects of age, income, BMI, smoking, and alcohol assumptions on allergic diseases. The clumping window of r^2 = 0.001 and kb = 10,000 was also used to reduce the effect of strong LD in all

mediators. The combination of all GWAS-significant SNPs with a *P*-value less than 5×10^{-8} were extracted from each exposure and were clump for avoiding LD under a window of $r^2 = 0.001$ and kb = 10,000. Selected IVs were further analyzed in the multivariable IVW and MR-Egger models, and a *P*-value < 0.05 was considered independently significant in the MVMR analysis. It should be noted that both IVW and MR-Egger methods could reveal heterogeneity in the analysis, and the results of MR-Egger would be applied when there was pleiotropy detected (26, 49).

Statistical analysis

Odds ratios and 95% CIs were used to express the impact on allergic disease risk caused by a corresponding unit change in absolute levels of the circulating omega-3, omega-6 and DHA and the ratio omega-6:3. According to the rules of Bonferroni correction for reduction of false positives by multiple tests, a two-sided *p*-value < 0.0083 was considered statistically significant but *p*-values ≥ 0.0083 and < 0.05 were only suggestive of statistical significance. MR analyses were performed using the "TwoSampleMR." package (version 0.5.6) and Mendelian Randomization (50) (version 0.5.0) packages in R software (version 4.1.2), R Foundation for Statistical Computing, Vienna, Austria). All study results are reported according to STROBE-MR (Strengthening the Reporting of Observational Studies in Epidemiology—Mendelian Randomization) guidelines (51).

Results

Data regarding SNPs relating to omega-3, omega-6, DHA, LA, EPA, and omega-6:3 exposure are given in **Supplementary Tables 1–6**. F-statistics for all selected IVs are almost >10, indicating no weak IVs. Details of sensitivity analysis and outliers are shown in **Table 1**.

Causal effects of omega-3/omega-6 on allergic diseases

The association of omega-3 with AD risk showed no evidence of directional pleiotropy but significant heterogeneity, according to Cochran's Q test (Q = 79.029; *p* = 0.002), but the removal of 3 outliers abolished heterogeneity. The genetically determined per unit increase in circulating omega-3 was associated with decreased risk of AD (outlier-corrected: OR: 0.863; 95% CI: 0.785 to 0.949; *p* = 3.86E-03).

No directional pleiotropy or heterogeneity was found for the association of circulating omega-3 on AC, AR or AU. A genetically determined increase in plasma omega-3 levels produced a trend with suggestive significance for decreased risk of AC (IVW-fixed: OR:0.720; 95% CI: 0.547 to 0.947; *p* = 1.87E-02) and AU (IVW-fixed: OR:0.821; 95% CI: 0.684 to 0.985; *p* = 3.42E-02), but no association was found between omega-3 and AR. Pleiotropy, assessed by MR-Egger regression (intercept = −0.014; *p* = 0.005), and heterogeneity (Q = 127.849; *p* = 2.08E-09) were analyzed for the relationship between circulating omega-3 and asthma, but after removal of six outliers, there was still no significant association (**Figure 2**).

No directional pleiotropy or significant heterogeneity was found for the analysis of circulating omega-6 levels and atopic diseases. A suggestively significant association emerged between omega-6 level and AC (IVW-fixed: OR:0.655; 95% CI: 0.445 to 0.964; p = 3.18E-02), but no relationship with other allergic diseases was found (**Figure 3**).

Causal effects of docosahexaenoic acid, eicosapentaenoic acid, and linoleic acid on allergic diseases

The association between DHA and AD showed heterogeneity, detected by Cochran's Q test (Q = 65.374; *p* = 0.009), but no directional pleiotropy. The removal of 4 outliers abolished heterogeneity, allowing the adoption of a fixed-effect model. No genetic association was found between circulating DHA and AD. No heterogeneity or directional pleiotropy emerged from the analyses of DHA association with AC, AR or AU. A suggestively significant association was only revealed between the DHA level and decreased risk of AC (IVW-fixed: OR:0.671; 95% CI 0.490 to 0.918; *p* = 1.25E-02). Pleiotropy, by MR-Egger regression (intercept = −0.013; *p* = 0.004), and heterogeneity (Q = 70.079; *p* = 0.003) were assessed in the analysis of DHA and asthma but after removal of four outliers (rs2394976, rs273912, rs4860987, rs77960347), no significant association was found (**Figure 4**). There was no significant association of LA and EPA with allergic diseases (**Supplementary Figures 2**, **3**).

Causal effects of the ratio of omega-6 to omega-3 on allergic diseases

No heterogeneity or directional pleiotropy was found for the analyses of omega-6:3 on allergic diseases except for asthma and AU. Using the fixed-effect IVW model, circulating omega-6:3 was found to be significantly associated with an increased risk of AD (IVW-fixed OR:1.171; 95% CI: 1.045 to 1.312; *p* = 6.46E-03) and a suggestively significant association with increased risk of AC (IVW: OR:1.341; 95% CI: 1.032 to 1.743; *p* = 2.83E-02) was also found. There was no impact on AR. Significant heterogeneity and pleiotropy were detected respectively by Cochran's Q test (Q = 119.740; *p* = 1.76E-11) and MR-Egger regression (intercept = 0.014; *p* = 0.036) for analysis

TABLE 1 Sensitivity analyses of the raw MR analysis and the adjusted MR analysis (adjusted by excluding all outliers and heterogeneous SNPs identified by the MR-PRESSO test).

Exposure	Outcome	nIVs	Heterogeneity test		MR-Egger pleiotropy test		MR-PRESSO global test		MR-PRESSO distorted outlier test		F statistics
			Q (*P*-value)	adjusted Q (*P*-value)	Intercept (*P*-value)	adjusted Intercept (*P*-value)	RSSobs (*P*-value)	adjusted RSSobs (*P*-value)	Outlying SNPs	Heterogeneous SNPs	
Omega-3	AD	46	79.03 (0.0024)	56.32 (0.1201)	0.0026 (0.7093)	0.0067 (0.2817)	83.5102 (0.010)	54.0308 (0.268)	rs11242109	rs144018203, rs3129962	281.89
	AC	48	54.22 (0.2184)	NA	0.0135 (0.4209)	NA	55.6056 (0.280)	NA	None	None	272.84
	Asthma	42	127.85 (0.0000)	41.48 (0.4499)	−0.0135 (0.0055)	−0.0077 (0.0745)	146.0187 (< 0.001)	43.4303 (0.478)	rs11242109, rs174564	rs10184054, rs2394976, rs4860987, rs77960347	135.98
	AR	48	57.22 (0.1459)	NA	−0.0118 (0.0367)	NA	62.7635 (0.156)	NA	None	None	272.84
	AU	48	48.10 (0.4279)	NA	−0.0216 (0.0528)	NA	51.5931 (0.446)	NA	None	None	272.84
Omega-6	AD	50	53.22 (0.3152)	NA	0.0128 (0.1055)	NA	55.0880 (0.309)	NA	None	None	130.06
	AC	50	52.05 (0.3560)	NA	0.0433 (0.0521)	NA	54.6354 (0.334)	NA	None	None	130.06
	Asthma	50	55.46 (0.2444)	NA	−0.0015 (0.7368)	NA	58.3651 (0.216)	NA	None	None	130.06
	AR	50	57.63 (0.1863)	NA	−0.0001 (0.9904)	NA	59.4571 (0.196)	NA	None	None	130.06
	AU	50	39.13 (0.8424)	NA	−0.0122 (0.4298)	NA	41.1063 (0.818)	NA	None	None	130.06
RO63	AD	35	52.55(0.0220)	NA	−0.0038(0.6241)	NA	55.5381 (0.060)	NA	None	None	105.22
	AC	35	33.3804 (0.4978)	NA	0.0140 (0.4342)	NA	34.67916 (0.594)	NA	None	None	105.22
	Asthma	29	119.7397 (0.0000)	30.8934 (0.3218)	0.0135 (0.0361)	−0.0012 (0.8455)	138.0924 (0.001)	32.6248 (0.347)	rs11242109, rs11632618, rs174564	rs2394976, rs4860987, rs7222755	92.45
	AR	35	45.8239 (0.0848)	NA	0.0071 (0.3079)	NA	48.4527 (0.161)	NA	None	None	105.22
	AU	35	40.0812 (0.2185)	NA	0.0288 (0.0311)	NA	44.3980 (0.287)	NA	None	None	105.22
DHA	AD	39	65.37 (0.0091)	40.84 (0.3468)	0.0095 (0.2291)	0.0006 (0.9525)	74.2713 (0.032)	42.9112 (0.333)	rs174564	rs182611493, rs525028	95.32
	AC	42	42.95 (0.3876)	NA	–0.0138 (0.4531)	NA	44.5644 (0.451)	NA	None	None	214.17
	Asthma	37	70.08 (0.0031)	35.10 (0.5113)	−0.0125 (0.0045)	−0.0043 (0.4359)	90.4094 (0.019)	36.4349 (0.557)	rs174564	rs2394976, rs273912, rs4860987, rs77960347	97.74

(Continued)

10.3389/fnut.2022.962787

TABLE 1 (Continued)

Exposure	Outcome	nIVs	Heterogeneity test		MR-Egger pleiotropy test		MR-PRESSO global test		MR-PRESSO distorted outlier test		F statistics
			Q (*P*-value)	adjusted Q (*P*-value)	Intercept (*P*-value)	adjusted Intercept (*P*-value)	RSSobs (*P*-value)	adjusted RSSobs (*P*-value)	Outlying SNPs	Heterogeneous SNPs	
	AR	42	49.16 (0.1789)	NA	−0.0099 (0.1289)	NA	53.3817 (0.211)	NA	None	None	214.17
	AU	42	46.89 (0.2436)	NA	−0.0113 (0.4028)	NA	47.6418 (0.312)	NA	None	None	214.17
LA	AD	40	58.9374 (0.0431)	33.2374 (0.7295)	0.0138 (0.1832)	0.0168 (0.0626)	62.5178 (0.0410)	35.0954 (0.7310)	rs141469619	rs174564, rs4947302	136.62
	AC	43	48.5985 (0.2244)	NA	0.0402 (0.1340)	NA	51.8960 (0.1980)	NA	None	None	138.80
	Asthma	40	63.7698 (0.0167)	38.0106 (0.5149)	0.0017 (0.7751)	−0.0026 (0.6079)	68.9051 (0.0140)	40.7977 (0.4960)	rs693	rs77960347, rs174564	138.80
	AR	43	49.1533 (0.2084)	NA	−0.0011 (0.9023)	NA	51.2625 (0.2090)	NA	None	None	138.80
	AU	43	43.8677 (0.3923)	NA	0.0000 (0.9982)	NA	46.4538 (0.3920)	NA	None	None	138.80
EPA	AD	7	11.1523 (0.0838)	NA	0.0866 (0.0319)	NA	19.3726 (0.1340)	NA	None	None	9.91
	AC	7	3.7916 (0.7048)	NA	0.0525 (0.5606)	NA	4.6240 (0.7750)	NA	None	None	9.91
	Asthma	7	9.8296 (0.1320)	NA	−0.0211 (0.3652)	NA	21.5129 (0.1440)	NA	None	None	9.91
	AR	7	5.1788 (0.5211)	NA	0.0149 (0.6195)	NA	6.4895 (0.6070)	NA	None	None	9.65
	AU	7	4.1837 (0.6518)	NA	0.0592 (0.3644)	NA	5.2409 (0.7160)	NA	None	None	9.91

As the Supplementary Figure 1 showed, for the process of adjustment, we firstly did a raw MR analysis and got an uncorrected causal evaluation. Then, MR-PRESSO global and Outliers test was performed to find unstable SNPs, and an adjusted MR analysis was performed again after removing all unstable SNPs, and the heterogeneity, pleiotropy and causal effect values were re-evaluated. MR: Mendelian randomization analysis; nIVs: Number of instrumental variables; NA: Not applicable; AD: atopic dermatitis; AC: Atopic conjunctivitis; AR: Allergic rhinitis; AU: Allergic urticaria; Omega-3: Omega-3 fatty acids; Omega-6: Omega-6 fatty acids; DHA: Docosahexaenoic acid.

10.3389/fnut.2022.962787

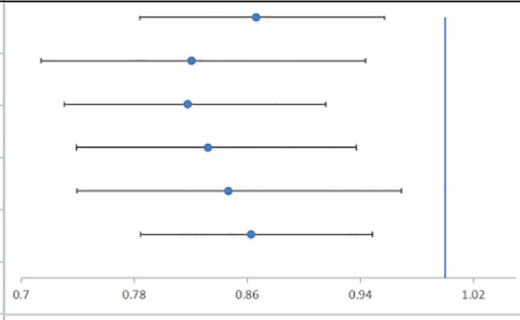
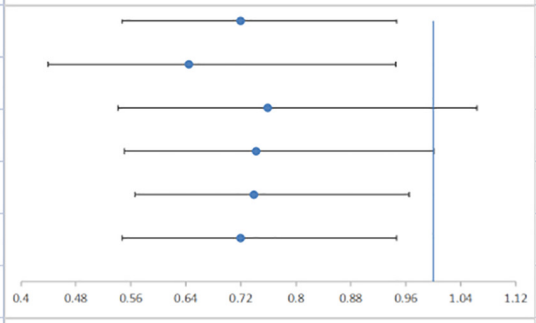
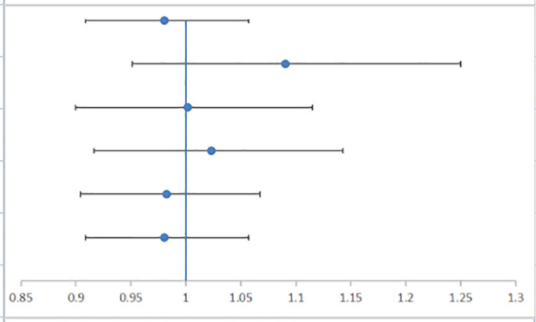
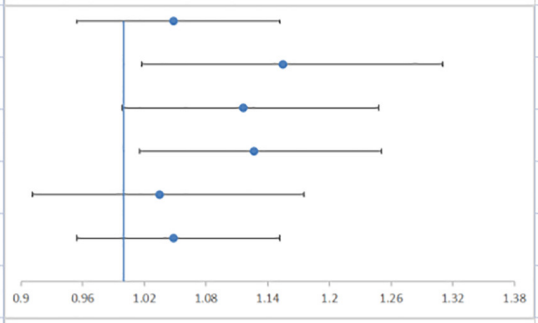
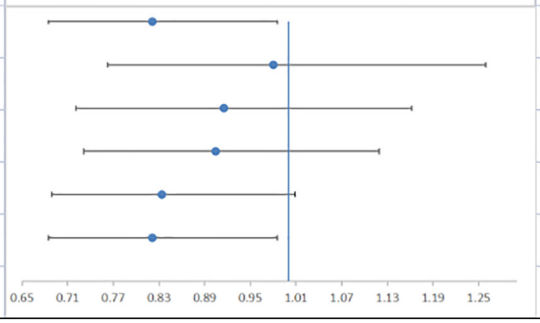
Model	Forest plots	OR	95% CIs	P-value	Outcomes
IVW		0.8663	0.7840 to 0.9573	4.87E-03	Atopic dermatitis
MR Egger		0.8207	0.7139 to 0.9434	7.97E-03	
Weighted median		0.8179	0.7305 to 0.9159	4.95E-04	
Weighted mode		0.8323	0.7393 to 0.9370	3.97E-03	
MRRAPS		0.8467	0.7398 to 0.9691	1.57E-02	
MR-PRESSO		0.8627	0.7847 to 0.9486	3.86E-03	
IVW		0.7196	0.5470 to 0.9467	1.87E-02	Atopic conjunctivitis
MR Egger		0.6444	0.4393 to 0.9452	2.94E-02	
Weighted median		0.7591	0.5416 to 1.0638	1.09E-01	
Weighted mode		0.7422	0.5506 to 1.0005	5.63E-02	
MRRAPS		0.7389	0.5659 to 0.9649	2.62E-02	
MR-PRESSO		0.7196	0.5470 to 0.9467	2.30E-02	
IVW		0.9804	0.9090 to 1.0574	6.08E-01	Asthma
MR Egger		1.0904	0.9514 to 1.2498	2.21E-01	
Weighted median		1.0017	0.8996 to 1.1153	9.76E-01	
Weighted mode		1.0232	0.9162 to 1.1426	6.87E-01	
MRRAPS		0.9825	0.9042 to 1.0675	6.76E-01	
MR-PRESSO		0.9804	0.9090 to 1.0574	6.10E-01	
IVW		1.0484	0.9542 to 1.1520	3.25E-01	Allergic rhinitis
MR Egger		1.1547	1.0176 to 1.3103	3.06E-02	
Weighted median		1.1162	0.9984 to 1.2480	5.35E-02	
Weighted mode		1.1266	1.0152 to 1.2503	2.96E-02	
MRRAPS		1.0348	0.9112 to 1.1753	5.98E-01	
MR-PRESSO		1.0484	0.9542 to 1.1520	3.30E-01	
IVW		0.8212	0.6844 to 0.9854	3.42E-02	Allergic urticaria
MR Egger		0.9802	0.7627 to 1.2597	8.77E-01	
Weighted median		0.9153	0.7208 to 1.1622	4.68E-01	
Weighted mode		0.9045	0.7312 to 1.1189	3.60E-01	
MRRAPS		0.8338	0.6894 to 1.0084	6.10E-02	
MR-PRESSO		0.8212	0.6844 to 0.9854	3.95E-02	

FIGURE 2
The forest plot of univariable Mendelian randomization analyses exploring associations between omega-3 fatty acids and risk of allergic diseases using different Mendelian randomization statistical models OR: odds ratio; CIs: confidence intervals.

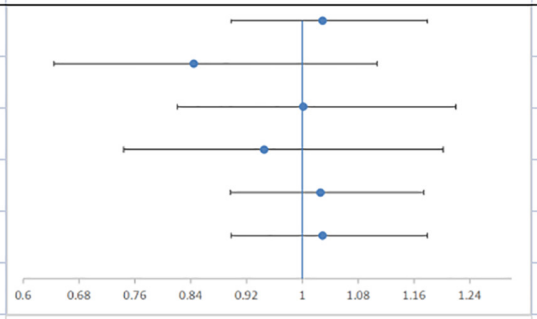
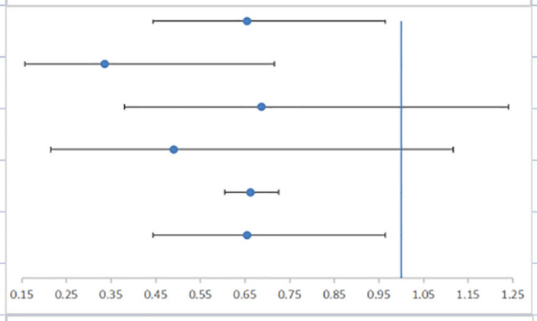
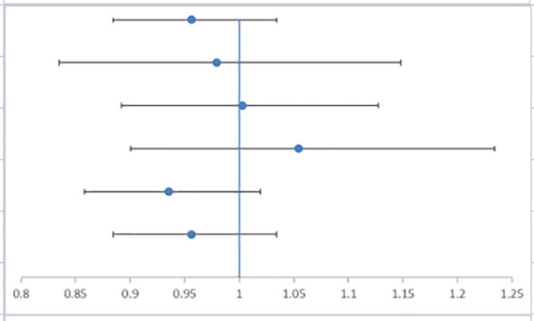
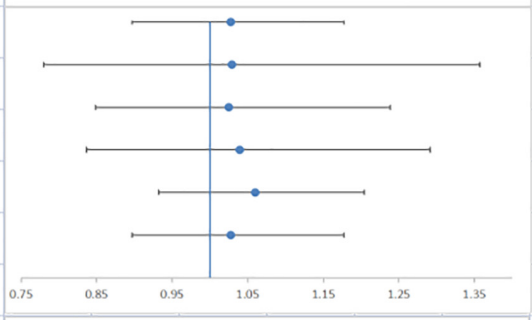
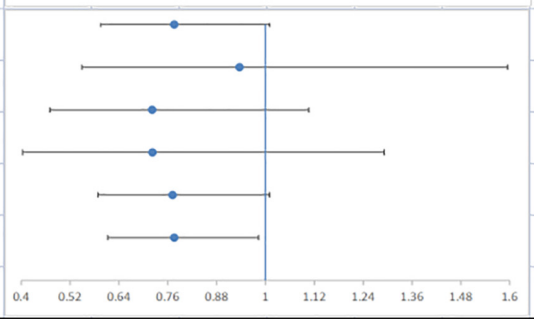
Model	Forest plots	OR	95% CIs	P-value	Outcomes
IVW		1.0292	0.8979 to 1.1796	6.80E-01	Atopic dermatitis
MR Egger		0.8446	0.6446 to 1.1068	2.27E-01	
Weighted median		1.0014	0.8217 to 1.2204	9.89E-01	
Weighted mode		0.9455	0.7438 to 1.2019	6.49E-01	
MRRAPS		1.0261	0.8969 to 1.1739	7.07E-01	
MR-PRESSO		1.0292	0.8979 to 1.1796	6.81E-01	
IVW		0.6547	0.4447 to 0.9639	3.18E-02	Atopic conjunctivitis
MR Egger		0.3357	0.1575 to 0.7157	6.84E-03	
Weighted median		0.6870	0.3805 to 1.2403	2.13E-01	
Weighted mode		0.4904	0.2154 to 1.1167	9.60E-02	
MRRAPS		0.6623	0.6044 to 0.7259	4.67E-02	
MR-PRESSO		0.6547	0.4447 to 0.9639	3.68E-02	
IVW		0.9562	0.8842 to 1.0340	2.62E-01	Asthma
MR Egger		0.9792	0.8352 to 1.1482	7.97E-01	
Weighted median		1.0028	0.8922 to 1.1271	9.62E-01	
Weighted mode		1.0543	0.9006 to 1.2342	5.14E-01	
MRRAPS		0.9353	0.8580 to 1.0195	1.29E-01	
MR-PRESSO		0.9562	0.8842 to 1.0340	2.67E-01	
IVW		1.0276	0.8968 to 1.1774	6.95E-01	Allergic rhinitis
MR Egger		1.0291	0.7800 to 1.3577	8.40E-01	
Weighted median		1.0250	0.8483 to 1.2384	7.98E-01	
Weighted mode		1.0394	0.8364 to 1.2918	7.29E-01	
MRRAPS		1.0600	0.9327 to 1.2046	3.72E-01	
MR-PRESSO		1.0276	0.8968 to 1.1774	6.97E-01	
IVW		0.7763	0.5959 to 1.0113	6.05E-02	Allergic urticaria
MR Egger		0.9367	0.5499 to 1.5955	8.11E-01	
Weighted median		0.7220	0.4709 to 1.1071	1.35E-01	
Weighted mode		0.7228	0.4046 to 1.2910	2.78E-01	
MRRAPS		0.7722	0.5895 to 1.0115	6.06E-02	
MR-PRESSO		0.7763	0.6129 to 0.9832	4.09E-02	

FIGURE 3
The forest plot of univariable Mendelian randomization analyses exploring associations between omega-6 fatty acids and risk of allergic diseases using different Mendelian randomization statistical models OR: odds ratio; CIs: confidence intervals.

10.3389/fnut.2022.962787

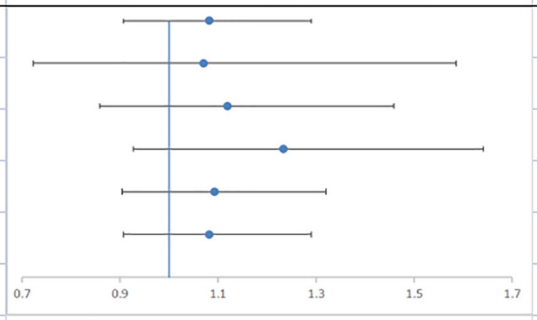
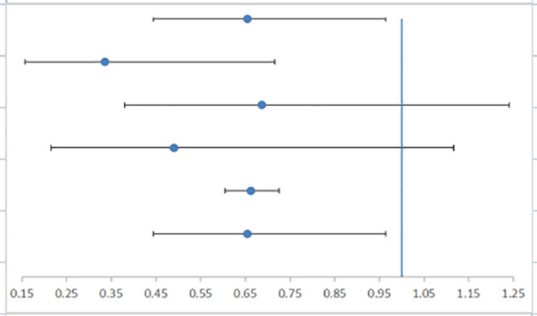
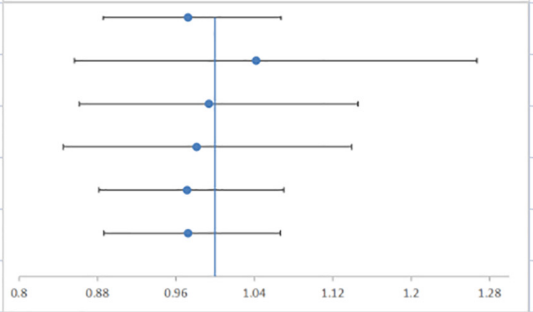
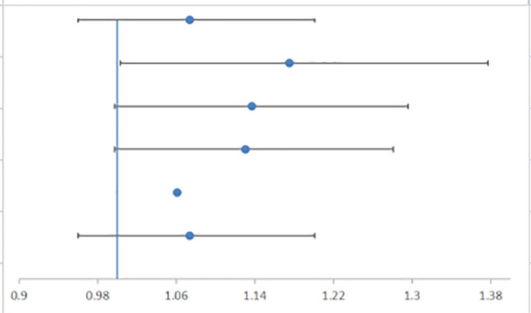
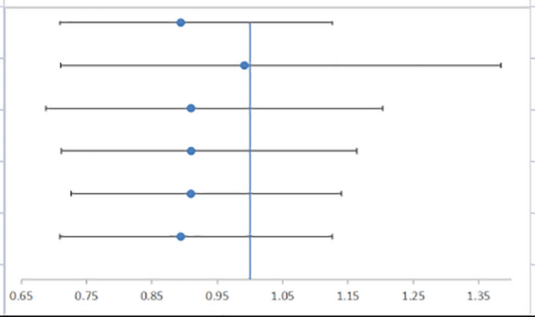
Model	Forest plots	OR	95% CIs	P-value	Outcomes
IVW		1.0817	0.9068 to 1.2902	3.83E-01	Atopic dermatitis
MR Egger		1.0701	0.7225 to 1.5850	7.37E-01	
Weighted median		1.1189	0.8586 to 1.4581	4.06E-01	
Weighted mode		1.2330	0.9262 to 1.6414	1.60E-01	
MRRAPS		1.0925	0.9041 to 1.3200	3.60E-01	
MR-PRESSO		1.0817	0.9068 to 1.2902	3.88E-01	
IVW		0.6707	0.4903 to 0.9175	1.25E-02	Atopic conjunctivitis
MR Egger		0.7607	0.4835 to 1.1968	2.44E-01	
Weighted median		0.7287	0.4892 to 1.0853	1.19E-01	
Weighted mode		0.7398	0.5058 to 1.0822	1.28E-01	
MRRAPS		0.6828	0.4979 to 0.9364	1.79E-02	
MR-PRESSO		0.6707	0.4903 to 0.9175	1.66E-02	
IVW		0.9725	0.8858 to 1.0676	5.58E-01	Asthma
MR Egger		1.0420	0.8570 to 1.2670	6.83E-01	
Weighted median		0.9938	0.8616 to 1.1462	9.32E-01	
Weighted mode		0.9812	0.8451 to 1.1393	8.05E-01	
MRRAPS		0.9715	0.8818 to 1.0703	5.59E-01	
MR-PRESSO		0.9725	0.8868 to 1.0664	5.56E-01	
IVW		1.0737	0.9598 to 1.2012	2.14E-01	Allergic rhinitis
MR Egger		1.1751	1.0027 to 1.3771	5.30E-02	
Weighted median		1.1370	0.9975 to 1.2960	5.45E-02	
Weighted mode		1.1304	0.9977 to 1.2809	6.13E-02	
MRRAPS		1.0610	1.0596 to 1.0625	5.18E-01	
MR-PRESSO		1.0737	0.9598 to 1.2012	2.21E-01	
IVW		0.8941	0.7096 to 1.1265	3.42E-01	Allergic urticaria
MR Egger		0.9916	0.7103 to 1.3842	9.61E-01	
Weighted median		0.9098	0.6877 to 1.2036	5.08E-01	
Weighted mode		0.9099	0.7114 to 1.1638	4.57E-01	
MRRAPS		0.9096	0.7260 to 1.1397	4.10E-01	
MR-PRESSO		0.8941	0.7096 to 1.1265	3.48E-01	

FIGURE 4
The forest plot of univariable Mendelian randomization analyses exploring associations between docosahexaenoic acid and allergic diseases risk using different Mendelian randomization statistical models OR: odds ratio; CIs: confidence intervals.

of omega-6:3 and asthma. However, after the removal of six outliers, no significant association remained between omega-6:3 and asthma. Significant pleiotropy was detected by MR-Egger regression (intercept = 0.029; p = 0.031) for analysis of omega-6:3 and AU but the MR-PRESSO global test reported no evident pleiotropy (RSSobs = 44.398; p = 0.287). Therefore, the negative association between omega-6:3 with AU (MR-Egger: OR: 0.967; 95% CI: 0.753 to 1.243; p = 7.96E-01) should be interpreted with caution (**Figure 5**).

A forest plot of the causal estimates of PUFAs on allergic diseases is presented in **Figures 2–5**. Overall, the consistency of effect sizes across different methods indicates that confidence may be put in the results of each analysis. The corresponding scatter plots for the MR analysis are shown in **Supplementary Figures 4–9**.

The leave-one-out stability tests conducted by excluding a single SNP at a time are detailed in the **Supplementary Figures 10–15**. Risk estimates of genetically predicted omega-6 levels and omega-6:3 ratios for allergic diseases did not change substantially after excluding one SNP at a time, indicating that it was unlikely that potential driving SNPs were causing bias to the causal association. However, the removal of rs174564 from the two analyses of omega-3 and DHA levels on risk of AR, caused a distinct change in risk estimates, indicating that this instrumental variable severely affected the outcome variable. Therefore, these particular results should be interpreted with caution.

Multivariable MR analyses

We estimated the independent effects of circulating PUFAs on allergic diseases using multivariable MR conditioned on age, income, BMI, alcohol and smoking (**Figure 6**) and observed a directly protective effect of omega-3 level on AD (IVW OR_{MVMR}: 0.841; 95% CI: 0.752 to 0.940; p = 2.00E-03) and AC (IVW OR_{MVMR}: 0.646; 95% CI: 0.482 to 0.865; p = 3.00E-03). No significant was observed for omega-6 levels and allergic diseases after adjustment of age, income, BMI, alcohol, and smoking behaviors. Genetic risk of DHA was directly associated with decreased risk of AD (IVW OR_{MVMR}: 0.851; 95% CI: 0.748 to 0.969; p = 1.50E-02). Genetic risk of circulating omega-6:3 was found to have a significant direct association with increased risk of AD (IVW OR_{MVMR}:1.192; 95% CI:1.071 to 1.328; p = 1.00E-03) and AC (IVW OR_{MVMR}:1.384; 95% CI:1.046 to 1.832; p = 2.30E-02). Similarly, there was no significant association of LA and EPA with allergic diseases according to the results of MVMR. Besides, though no significant genetic association was observed between PUFAs and asthma after adjustment of age, income, BMI, alcohol, and smoking behaviors, genetic risk of BMI was found to be associated with a higher risk of asthma. Detailed results of MVMR analyses were presented in **Supplementary Tables 7-12**.

Discussion

The current study explored the association between PUFAs and allergic disease risk using both univariable and multivariable two-sample MR. The IVs were used as proxies for PUFAs assessed both as absolute levels and as ratios to produce comparable results. In univariable MR results, Omega-3 levels were found to be likely genetic causal factors associated with decreased risk of some allergic diseases including AD, AC and AU. Genetic predisposition to high omega-6 and DHA levels was suggestively associated with reduced risk of AC. However, the genetic predisposition to high omega-6:3 showed a causal association with an increased risk of AD and AC, and this may constitute a susceptibility factor contributing to the pathogenesis of AD and AC. According to the results of MVMR, the independent protective effect of omega-3 and DHA on AD was identified in our study, as well as omega-3 for AC. Besides, omega-6:3 was independently associated with AD and AC. However, those results for asthma should be interpreted with caution as no specific GWAS data related to allergic asthma could be accessible and used in the present study.

Health benefits of PUFAs have been documented elsewhere and omega-3 (including DHA) have been associated with improvements in cardiovascular health, neurodevelopment and diabetes (52, 53) with omega-6 implicated in hair growth, lipid metabolism, and bone health (54–56). However, omega-3 and omega-6 compete for the same desaturation and elongation enzymes, and an increased ratio of omega-6 to omega-3 may reduce the benefits of omega-3 and increase the probability of inflammatory diseases (57). Inconsistencies have arisen from epidemiological studies, RCTs and meta-analyses into the effects of PUFAs intake during pregnancy (15, 58), biomarker levels (18, 59–64), maternal/individual early life PUFA supplementation, and impacts on the risk of allergic diseases in the offspring or during individual later life (12, 14, 15, 65–67). For the association between PUFAs in plasma and allergic disease, there was evidence showing that higher levels of total omega-3 fatty acid, DHA and EPA in maternal and infant plasma were associated with a lower prevalence of IgE-associated disease (such as eczema) in a dose-dependent manner (68). Reduced concentration of serum omega-3 fatty acids was also identified to characterize women with extensive eczema (69). Previous observational studies mainly provided evidence for the effects of PUFA composition of maternal and umbilical cord plasma on infants or early childhood allergic diseases, while the results in this MR study demonstrated a direct genetic association of circulating PUFAs with allergic skin diseases, especially for the protective effects of omega-3 fatty acid on AD and AC.

Multiple levels of research evidence should be considered when establishing causal effects but observational research under different conditions is susceptible to confounding factors reducing the accuracy of conclusions. Therefore, correlations

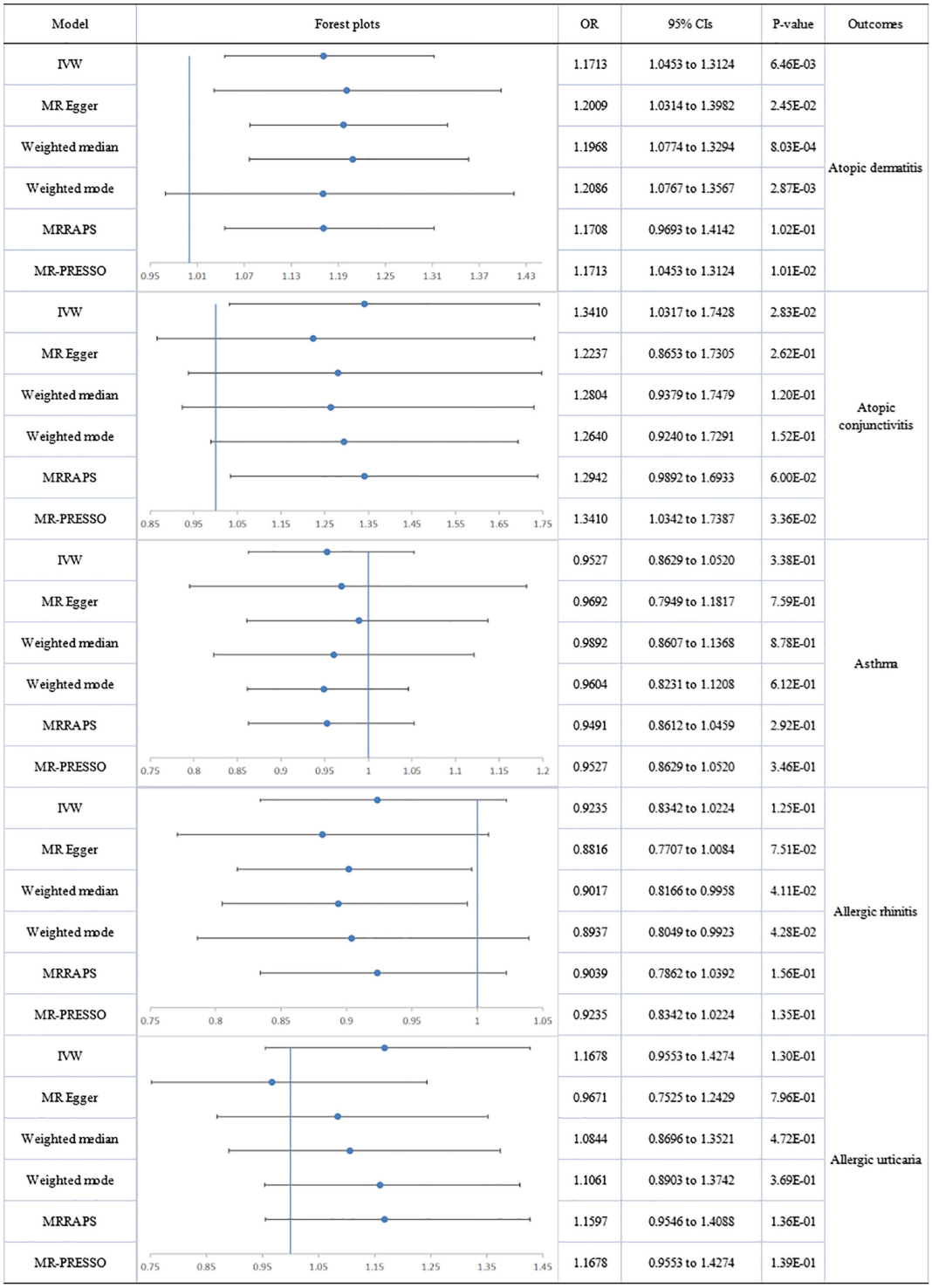


FIGURE 5
The forest plot of univariable Mendelian randomization analyses exploring associations between ratio of omega-6 fatty acids and omega-3 fatty acids to allergic diseases risk using different Mendelian randomization statistical models OR: odds ratio; CIs: confidence intervals.

reported by observational studies cannot be equated with direct causal correlation. MR avoids the influence of confounding factors through genetic instrumental variables and accurate causal assessments may be made. Caution should also be exercised in comparing RCTs with MR effects since genetic susceptibility is considered lifelong, while the effects of dietary

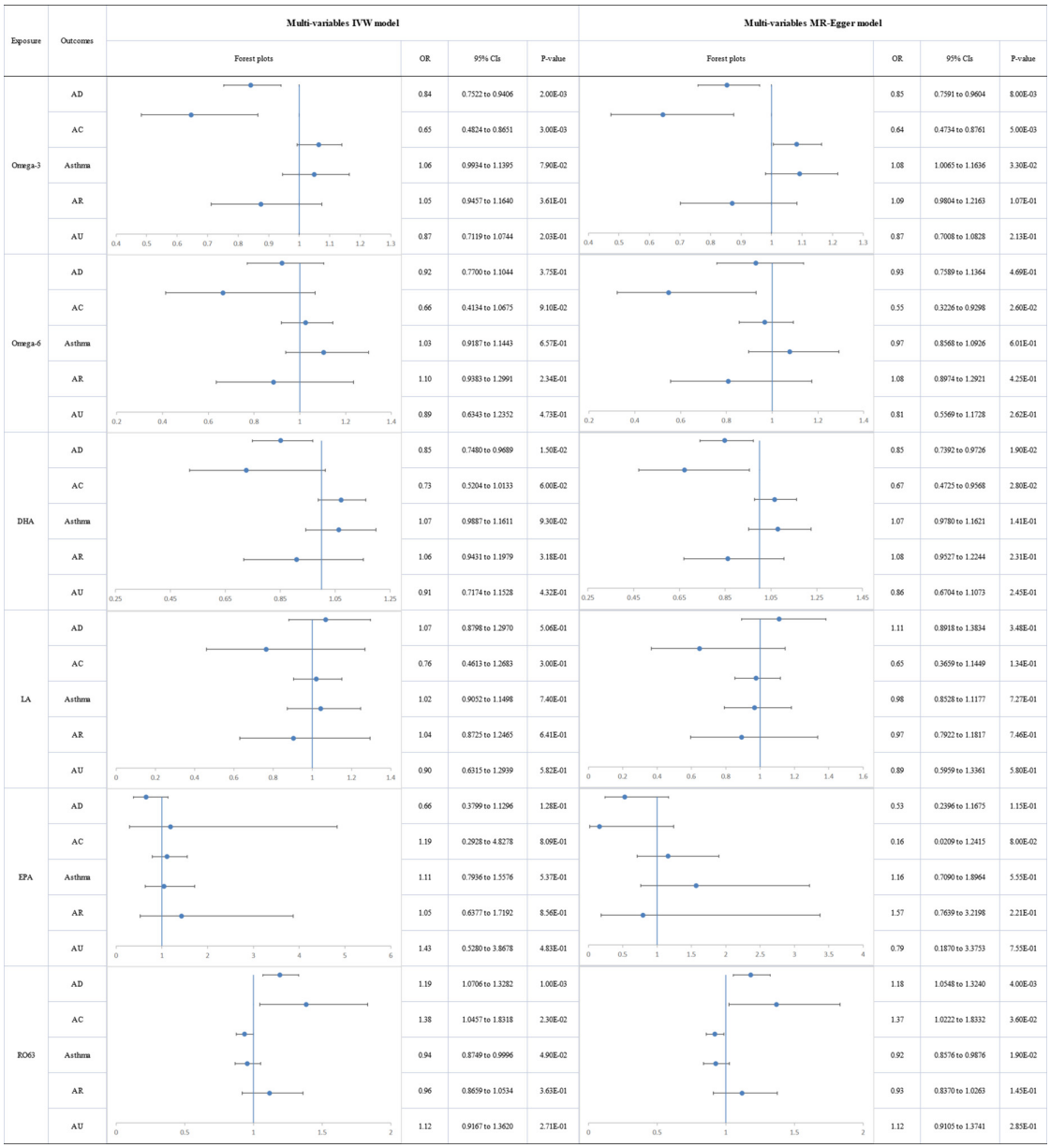


FIGURE 6
The forest plot of the multivariable Mendelian randomization exploring the associations between genetically determined polyunsaturated fatty acids and allergic diseases adjusted for confounding traits (body mass index, smoking, alcohol intake, age, and income level) OR: odds ratio; CIs: confidence intervals; Omega-3: omega-3 fatty acids; Omega-6: omega-6 fatty acids; DHA: docosahexaenoic acid; LA: linoleic acid; EPA: eicosapentaenoic acid; RO63: ratio of omega-6 fatty acids to omega-3 fatty acids; AD: atopic dermatitis; AC: Atopic conjunctivitis; AR: Allergic rhinitis; AU: Allergic urticaria.

supplementation in intervention experiments last only for the duration of the trial. Long-term exposure may be superior, given the long development period of allergic diseases.

The current study is the first MR analysis of PUFAs and allergic diseases and has several advantages. Firstly, compared with the inherent limitations of observational studies, MR studies are less likely to be affected by reverse causality and confounding. Secondly, extensive GWAS sample data, two separate sets of IVs and different methodologies were applied to causal association assessment to improve reliability. Moreover, the causal relationship was extended from single PUFA levels to include the ratio of omega-6 to omega-3. Several limitations must be acknowledged. Firstly, the present study is limited to individuals of European ancestry and may not be generalized to other races. Secondly, inconsistencies in pleiotropy detection and the occurrence of potential driving SNPs are difficult

to interpret and may cause bias. Thirdly, the GWAS effect size is based on circulatory PUFA concentration rather than membrane concentration, and membrane association may be more significant given the cell signaling of fatty acid receptors and immune responses (70, 71). Lastly, some more specific and targeted GWAS datasets, such as allergic asthma and eicosapentaenoic acid, were unavailable. However, as genetic instruments continue to improve, MR studies could shed further light on the significance of individual PUFA associations with the risk of specific allergic diseases.

Conclusion

In conclusion, through both univariable and multivariable MR analyses, our study demonstrated that higher PUFA concentrations (omega-3, DHA) and lower omega-6:3 ratios were associated with a lower risk of some allergic diseases (such as AD and AC). The strongest evidence concerned the protective effect of omega-3. This signifies the substantial clinical value of circulating PUFA levels and omega-6:3 on some allergic diseases and may assist with early diagnosis and enable more efficient targeting for prevention and therapy.

Data availability statement

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

Author contributions

ZC and JW conceived the study, participated in its design and coordination, and critically revised the manuscript. YL and ZC searched the databases. ZC, QL, and YL reviewed the GWAS datasets and finished the data collection. ZC and YL finished the data analysis. YL drafted the manuscript. YL, JW, and ZC had full access to all the data collection, analysis, and interpretation. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Sciences Foundation of China [No. 81472073], the National Key R&D Program of China [No. 2019YFA0112100], the Fundamental Research Funds for the Central Universities of Central South University [No. 2022ZZTS0824], and the Science Foundation of Hunan Province [No. 2019JJ40518]. The study funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Acknowledgments

The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for the expert linguistic services provided. The authors also would like to express their gratitude to the Special Project for Central Guidance of Local Science and Technology Development (2020YDDF0043); Changsha Clinical Research Center for Metabolic and Endocrine Diseases in the Elderly (KH2201058); Natural Science Foundation of Hunan Province, No. 2020JJ8012; Innovation Platform Project of Ningxia Clinical Research Center on Geriatric Diseases in 2020, No. 2020DPC05018; the 2021 Major RESEARCH and Development Program of Ningxia Hui Autonomous Region, No. 2021BEG01001; Ningxia Hui Autonomous Region Introduction Team (2020RXTDLX04); Education Department of Hunan Province, No. 18C0056; Project of Hunan Provincial Finance Department in 2017 (Hunan Finance Society refers to [2017] 61); and the Discipline Leader Project of "225" Project for Cultivating High-level Health Talents in Hunan Province. This manuscript is not under review with any other journal now and has not been published in any other journal previously.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.962787/full#supplementary-material

References

1. Brunner PM, Silverberg JI, Guttman-Yassky E, Paller AS, Kabashima K, Amagai M, et al. Increasing comorbidities suggest that atopic dermatitis is a systemic disorder. *J Invest Dermatol.* (2017) 137:18–25. doi: 10.1016/j.jid.2016.08.022

2. Bantz SK, Zhu Z, Zheng T. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *J Clin Cell Immunol.* (2014) 5:202.

3. Spergel. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol.* (2003) 112:S118–27.

4. Pawankar R, Caonica GW, Holgate ST. *World Allergy Organization (WAO) White Book On Allergy 2011-2012: Executive Summary[J]. Japanese Journal Of Allergology.* (2011). Available online at: http://www.worldallergy.org

5. Pawankar R, Holgate ST, Canonica GW, Lockey RF, Blaiss MS. *World Allergy Organization (WAO) White Book on Allergy*. Milwaukee, WI: World Allergy Organization (2013).

6. Spergel JM. Epidemiology of atopic dermatitis and atopic march in children. *Immunol Allergy Clin North Am.* (2010) 30:269–80. doi: 10.1016/j.iac.2010.06.003

7. Muraro A, Dubois AE, DunnGalvin A, Hourihane JO, de Jong NW, Meyer R, et al. EAACI food allergy and anaphylaxis guidelines. Food allergy health-related quality of life measures. *Allergy.* (2014) 69:845–53. doi: 10.1111/all.12405

8. Cook Q, Argenio K, Lovinsky-Desir S. The impact of environmental injustice and social determinants of health on the role of air pollution in asthma and allergic disease in the United States. *J Allergy Clin Immunol.* (2021) 148:1089.e–101.e. doi: 10.1016/j.jaci.2021.09.018

9. Ferreira MA, Vonk JM, Baurecht H, Marenholz I, Tian C, Hoffman JD, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat Genet.* (2017) 49:1752–7. doi: 10.1038/ng.3985

10. Calder PC, Krauss-Etschmann S, de Jong EC, Dupont C, Frick JS, Frokiaer H, et al. Early nutrition and immunity - progress and perspectives. *Br J Nutr.* (2006) 96:774–90.

11. Clausen M, Jonasson K, Keil T, Beyer K, Sigurdardottir ST. Fish oil in infancy protects against food allergy in Iceland-results from a birth cohort study. *Allergy.* (2018) 73:1305–12. doi: 10.1111/all.13385

12. Hansen S, Strøm M, Maslova E, Dahl R, Hoffmann HJ, Rytter D, et al. Fish oil supplementation during pregnancy and allergic respiratory disease in the adult offspring. *J Allergy Clin Immunol.* (2017) 139:104.e–11.e. doi: 10.1016/j.jaci.2016.02.042

13. Kumar A, Mastana SS, Lindley MR. n-3 Fatty acids and asthma. *Nutr Res Rev.* (2016) 29:1–16. doi: 10.1017/s0954422415000116

14. Anandan C, Nurmatov U, Sheikh A. Omega 3 and 6 oils for primary prevention of allergic disease: systematic review and meta-analysis. *Allergy.* (2009) 64:840–8. doi: 10.1111/j.1398-9995.2009.02042.x

15. Best KP, Gold M, Kennedy D, Martin J, Makrides M. Omega-3 long-chain PUFA intake during pregnancy and allergic disease outcomes in the offspring: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Am J Clin Nutr.* (2016) 103:128–43. doi: 10.3945/ajcn.115.111104

16. Reichardt P, Müller D, Posselt U, Vorberg B, Diez U, Schlink U, et al. Fatty acids in colostrum from mothers of children at high risk of atopy in relation to clinical and laboratory signs of allergy in the first year of life. *Allergy.* (2004) 59:394–400. doi: 10.1111/j.1398-9995.2003.00429.x

17. Rosenlund H, Fagerstedt S, Alm J, Mie A. Breastmilk fatty acids in relation to sensitization - the ALADDIN birth cohort. *Allergy.* (2016) 71:1444–52. doi: 10.1111/all.12896

18. Yu YM, Chan YH, Calder PC, Hardjojo A, Soh SE, Lim AL, et al. Maternal PUFA status and offspring allergic diseases up to the age of 18 months. *Br J Nutr.* (2015) 113:975–83. doi: 10.1017/s000711451500001x

19. Simopoulos AP. Fatty Acids | Omega-3 polyunsaturated. In: Caballero B Editor. *Encyclopedia of Human Nutrition*, 2nd Edn. Oxford: Elsevier (2005). p. 205–19. doi: 10.1016/B0-12-226694-3/00120-4

20. Zhang GQ, Liu B, Li J, Luo CQ, Zhang Q, Chen JL, et al. Fish intake during pregnancy or infancy and allergic outcomes in children: a systematic review and meta-analysis. *Pediatr Allergy Immunol.* (2017) 28:152–61. doi: 10.1111/pai.12648

21. Wang P, Liu L, Lei SF. Causal effects of homocysteine levels on the changes of bone mineral density and risk for bone fracture: a two-sample mendelian randomization study. *Clin Nutr.* (2021) 40:1588–95. doi: 10.1016/j.clnu.2021.02.045

22. Bennett DA, Holmes MV. Mendelian randomisation in cardiovascular research: an introduction for clinicians. *Heart.* (2017) 103:1400–7. doi: 10.1136/heartjnl-2016-310605

23. Liao LZ, Zhang SZ, Li WD, Liu Y, Li JP, Zhuang XD, et al. Serum albumin and atrial fibrillation: insights from epidemiological and mendelian randomization studies. *Eur J Epidemiol.* (2020) 35:113–22. doi: 10.1007/s10654-019-00583-6

24. Evans DM, Davey Smith G. Mendelian randomization: new applications in the coming age of hypothesis-free causality. *Annu Rev Genomics Hum Genet.* (2015) 16:327–50. doi: 10.1146/annurev-genom-090314-050016

25. Pierce BL, Burgess S. Efficient design for mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol.* (2013) 178:1177–84. doi: 10.1093/aje/kwt084

26. Burgess S, Thompson SG. Multivariable mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol.* (2015) 181:251–60. doi: 10.1093/aje/kwu283

27. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published data in mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol.* (2015) 30:543–52. doi: 10.1007/s10654-015-0011-z

28. Davies NM, Holmes MV, Davey Smith G. Reading mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ.* (2018) 362:k601. doi: 10.1136/bmj.k601

29. Julkunen H, Cichońska A, Slagboom PE, Würtz P. Metabolic biomarker profiling for identification of susceptibility to severe pneumonia and COVID-19 in the general population. *Elife.* (2021) 10:e63033. doi: 10.7554/eLife.63033

30. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet.* (2015) 8:192–206. doi: 10.1161/circgenetics.114.000216

31. Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst.* (2009) 134:1781–5. doi: 10.1039/b910205a

32. Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: a primer on -omic technologies. *Am J Epidemiol.* (2017) 186:1084–96. doi: 10.1093/aje/kwx016

33. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet.* (2014) 46:543–50. doi: 10.1038/ng.2982

34. Krumsiek J, Suhre K, Evans AM, Mitchell MW, Mohney RP, Milburn MV, et al. Mining the unknown: a systems approach to metabolite identification combining genetic and metabolic information. *PLoS Genet.* (2012) 8:e1003005. doi: 10.1371/journal.pgen.1003005

35. Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wägele B, et al. Human metabolic individuality in biomedical and pharmaceutical research. *Nature.* (2011) 477:54–60. doi: 10.1038/nature10354

36. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol.* (2016) 45:1961–74. doi: 10.1093/ije/dyw220

37. Sanderson E, Windmeijer F. A weak instrument [Formula: see text]-test in linear IV models with multiple endogenous variables. *J Econo.* (2016) 190:212–21. doi: 10.1016/j.jeconom.2015.06.004

38. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol.* (2013) 37:658–65. doi: 10.1002/gepi.21758

39. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol.* (2017) 41:341–52. doi: 10.1002/gepi.22041

40. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080

41. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965

42. 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

43. Zhao Q, Wang J, Hemani G, Bowden J, Small DS. *Statistical Inference In Two-Sample Summary-Data Mendelian Randomization Using Robust Adjusted Profile*

Score. arXiv: 1801.09652. (2018). Available online at: https://ui.adsabs.harvard.edu/abs/2018arXiv180109652Z (accessed January 1, 2018).

44. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

45. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in mendelian randomisation studies with summary data and a continuous outcome. *Stat Med.* (2015) 34:2926–40. doi: 10.1002/sim.6522

46. Burgess S, Thompson SG. Interpreting findings from mendelian randomization using the MR-Egger method. *Eur J Epidemiol.* (2017) 32:377–89. doi: 10.1007/s10654-017-0255-x

47. Slob EAW, Burgess S. A comparison of robust mendelian randomization methods using summary data. *Genet Epidemiol.* (2020) 44:313–29. doi: 10.1002/gepi.22295

48. 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. doi: 10.1093/ije/dyy262

49. Rees JMB, Wood AM, Burgess S. Extending the MR-egger method for multivariable mendelian randomization to correct for both measured and unmeasured pleiotropy. *Stat Med.* (2017) 36:4705–18. doi: 10.1002/sim.7492

50. Yavorska OO, Burgess S. Mendelian randomization: an R package for performing mendelian randomization analyses using summarized data. *Int J Epidemiol.* (2017) 46:1734–9. doi: 10.1093/ije/dyx034

51. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomization: the STROBE-MR statement. *JAMA.* (2021) 326:1614–21. doi: 10.1001/jama.2021.18236

52. Shahidi F, Ambigaipalan P. Omega-3 polyunsaturated fatty acids and their health benefits. *Annu Rev Food Sci Technol.* (2018) 9:345–81. doi: 10.1146/annurev-food-111317-095850

53. Elagizi A, Lavie CJ, O'Keefe E, Marshall K, O'Keefe JH, Milani RV. An update on omega-3 polyunsaturated fatty acids and cardiovascular health. *Nutrients.* (2021) 13:204. doi: 10.3390/nu13010204

54. Le Floc'h C, Cheniti A, Connétable S, Piccardi N, Vincenzi C, Tosti A. Effect of a nutritional supplement on hair loss in women. *J Cosmet Dermatol.* (2015) 14:76–82. doi: 10.1111/jocd.12127

55. Bjørklund G, Dadar M, Doşa MD, Chirumbolo S, Pen JJ. Insights into the effects of dietary omega-6/omega-3 polyunsaturated fatty acid (PUFA) ratio on oxidative metabolic pathways of oncological bone disease and global health. *Curr Med Chem.* (2021) 28:1672–82. doi: 10.2174/0929867327666200427095331

56. Hooper L, Al-Khudairy L, Abdelhamid AS, Rees K, Brainard JS, Brown TJ, et al. Omega-6 fats for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* (2018) 7:Cd011094. doi: 10.1002/14651858.CD011094.pub3

57. Klemens CM, Berman DR, Mozurkewich EL. The effect of perinatal omega-3 fatty acid supplementation on inflammatory markers and allergic diseases: a systematic review. *Bjog.* (2011) 118:916–25. doi: 10.1111/j.1471-0528.2010.02846.x

58. Venter C, Agostoni C, Arshad SH, Ben-Abdallah M, Du Toit G, Fleischer DM, et al. Dietary factors during pregnancy and atopic outcomes in childhood: a systematic review from the European academy of allergy and clinical immunology. *Pediatr Allergy Immunol.* (2020) 31:889–912. doi: 10.1111/pai.13303

59. Standl M, Demmelmair H, Koletzko B, Heinrich J. Cord blood LC-PUFA composition and allergic diseases during the first 10 yr. Results from the LISAplus study. *Pediatr Allergy Immunol.* (2014) 25:344–50. doi: 10.1111/pai.12212

60. Barman M, Johansson S, Hesselmar B, Wold AE, Sandberg AS, Sandin A. High levels of both n-3 and n-6 long-chain polyunsaturated fatty acids in cord serum phospholipids predict allergy development. *PLoS One.* (2013) 8:e67920. doi: 10.1371/journal.pone.0067920

61. Rucci E, den Dekker HT, de Jongste JC, Steenweg-de-Graaff J, Gaillard R, Pasmans SG, et al. Maternal fatty acid levels during pregnancy, childhood lung function and atopic diseases. The Generation R Study. *Clin Exp Allergy.* (2016) 46:461–71. doi: 10.1111/cea.12613

62. Notenboom ML, Mommers M, Jansen EH, Penders J, Thijs C. Maternal fatty acid status in pregnancy and childhood atopic manifestations: KOALA birth cohort study. *Clin Exp Allergy.* (2011) 41:407–16. doi: 10.1111/j.1365-2222.2010.03672.x

63. Pike KC, Calder PC, Inskip HM, Robinson SM, Roberts GC, Cooper C, et al. Maternal plasma phosphatidylcholine fatty acids and atopy and wheeze in the offspring at age of 6 years. *Clin Dev Immunol.* (2012) 2012:474613. doi: 10.1155/2012/474613

64. Newson RB, Shaheen SO, Henderson AJ, Emmett PM, Sherriff A, Calder PC. Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema. *J Allergy Clin Immunol.* (2004) 114:531–7. doi: 10.1016/j.jaci.2004.05.010

65. Zhang Y, Lin J, Zhou R, Zheng X, Dai J. Effect of omega-3 fatty acids supplementation during childhood in preventing allergic disease: a systematic review and meta-analysis. *J Asthma.* (2021) 58:523–36.

66. Best KP, Sullivan T, Palmer D, Gold M, Kennedy DJ, Martin J, et al. Prenatal fish oil supplementation and allergy: 6-year follow-up of a randomized controlled trial. *Pediatrics.* (2016) 137:e20154443. doi: 10.1542/peds.2015-4443

67. Vahdaninia M, Mackenzie H, Dean T, Helps S. ω-3 LCPUFA supplementation during pregnancy and risk of allergic outcomes or sensitization in offspring: a systematic review and meta-analysis. *Ann Allergy Asthma Immunol.* (2019) 122:302.e–13.e. doi: 10.1016/j.anai.2018.12.008

68. Furuhjelm C, Warstedt K, Fagerås M, Fälth-Magnusson K, Larsson J, Fredriksson M, et al. Allergic disease in infants up to 2 years of age in relation to plasma omega-3 fatty acids and maternal fish oil supplementation in pregnancy and lactation. *Pediatr Allergy Immunol.* (2011) 22:505–14. doi: 10.1111/j.1399-3038.2010.01096.x

69. Johansson S, Wold AE, Sandberg AS. Low breast milk levels of long-chain n-3 fatty acids in allergic women, despite frequent fish intake. *Clin Exp Allergy.* (2011) 41:505–15. doi: 10.1111/j.1365-2222.2010.03678.x

70. Son SE, Koh JM, Im DS. Activation of free fatty acid receptor 4 (FFA4) ameliorates ovalbumin-induced allergic asthma by suppressing activation of dendritic and mast cells in mice. *Int J Mol Sci.* (2022) 23:5270. doi: 10.3390/ijms23095270

71. Patel D, Newell M, Goruk S, Richard C, Field CJ. Long chain polyunsaturated fatty acids docosahexaenoic acid and arachidonic acid supplementation in the suckling and the post-weaning diet influences the immune system development of T helper type-2 bias brown norway rat offspring. *Front Nutr.* (2021) 8:769293. doi: 10.3389/fnut.2021.769293

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 23 September 2022
DOI 10.3389/fnut.2022.964805

OPEN ACCESS

EDITED BY
Li Cai,
Sun Yat-sen University, China

REVIEWED BY
Xiaoqin Luo,
Xi'an Jiaotong University, China
Jingjing Jiao,
Zhejiang University, China

*CORRESPONDENCE
Weiliang Xia
xiaweiliang@zju.edu.cn

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

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 09 June 2022
ACCEPTED 07 September 2022
PUBLISHED 23 September 2022

CITATION
Sun Q, Gao N and Xia W (2022) Association between omega-3/6 fatty acids and cholelithiasis: A mendelian randomization study. *Front. Nutr.* 9:964805. doi: 10.3389/fnut.2022.964805

Association between omega-3/6 fatty acids and cholelithiasis: A mendelian randomization study

Qi Sun[1,2†], Ning Gao[3†] and Weiliang Xia[1,2*]

[1]Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, [2]Key Laboratory of Combined Multi-organ Transplantation, Ministry of Public Health, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, [3]Department of Cardiovascular Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Background: Omega-3 and omega-6 may be protective factors for cholelithiasis. However, this relationship has not yet been demonstrated clearly. Therefore, we attempted to identify these causal relationships.

Materials and methods: The omega-3/6 fatty acid discovery dataset was obtained from UK Biobank and contained 114,999 individuals. The validation set was derived from an independent genome-wide association study (GWAS) and contained 13,544 individuals. The cholelithiasis dataset was derived from FinnGen and contained 19,023 cases and 195,144 controls. The inverse variance weighting (IVW) method was used as the main method of analysis in this study. Multiple methods of analysis were also used in the repeated methods, including the MR-Egger, weighted median, MR-pleiotropic residual sum (MR-PRESSO), outliers, and maximum likelihood methods. In addition, we used multiple sensitivity analyses to identify the potential pleiotropy.

Result: In the discovery stage, the results of the random effect IVW analysis showed that higher omega-3 levels were correlated inversely with the risk of cholelithiasis (β = −0.22, 95% CI [−0.32 to −0.12], $P = 1.49 \times 10^{-5}$). When the replication analysis was performed using another set of instrumental variables (IVs), the causal relationship between omega-3 fatty acids and cholelithiasis remained stable (β = −0.42, 95% CI [−0.66 to −0.18], $P = 5.49 \times 10^{-4}$), except for the results obtained using the MR-Egger method, which were not significant. The results of the IVW approach showed that each SD increase in omega-6 levels was associated negatively with the risk of cholelithiasis, both in the discovery (β = −0.21, 95% CI [−0.35 to −0.06], $P = 4.37 \times 10^{-3}$) and the validation phases (β = −0.21, 95% CI [−0.40 to −0.02], $P = 3.44 \times 10^{-2}$).

Conclusion: The results of our MR study suggest that omega-3/6 is associated with cholelithiasis risk. Attention to the risk of cholelithiasis in individuals with low serum omega-3/6 levels is necessary.

KEYWORDS

cholelithiasis, polyunsaturated acids, omega-3 (ω-3) and omega-6 (ω-6) fatty acids, mendelian randomisation, causal relationship

Introduction

Cholelithiasis is an increasingly common hepatobiliary disease. Approximately 10–20% of adults have had cholelithiasis (1, 2). In addition to biliary malignancy, cholelithiasis is associated strongly with small intestinal, prostate, and kidney cancers (3). This is a public health concern on which greater emphasis should be placed.

In general, cholelithiasis can be classified as cholesterol and pigment gallstones according to the composition, with cholesterol gallstones accounting for approximately 80–90% of all the gallstones in most western countries (1, 4). Hepatic cholesterol hypersecretion, supersaturated bile juice, and gallbladder hypomotility contribute to the pathophysiology of cholesterol gallstones. These factors work collaboratively and cause the failure of biliary cholesterol solubility homeostasis, which subsequently results in cholesterol crystallization in bile juice and eventually biliary stone formation (1).

Among the polyunsaturated fatty acids (PUFAs), omega-3 (ω-3) and omega-6 (ω-6) are the two main families that have been shown to be relevant to human health (5, 6). In animal studies, it has been confirmed that high intake of PUFAs can decrease the risk of cholelithiasis by reducing the cholesterol saturation index (CSI) and suppressing the production of gallbladder mucin which is regarded as a trigger for gallstone formation (7, 8). Furthermore, it was reported that PUFAs combined with ursodeoxycholic acid can dissolve cholesterol stones in mice (9). However, the beneficial effects of PUFAs in humans remain debatable. While a prospective cohort study linked high intakes to a reduced prevalence of cholelithiasis in men (4), an epidemiologic study demonstrated that PUFA intake had no effect on cholelithiasis development (10).

Therefore, it is necessary to understand the causal relationship between PUFAs and cholelithiasis. Mendelian randomisation (MR) is an emerging epidemiological method that uses genetic variation as an instrumental variable (IV) to assess the causal association between exposure and outcome (11). Genetic variation is passed randomly to offspring during meiosis, and thus, its estimates of causal effects are consistent with the time order in which they should be. More importantly, the use of MR minimizes the interference of confounding variables between exposure and outcome by avoiding confounding factors to the greatest extent possible (12). Therefore, to examine the potential causal relationship between PUFAs and cholelithiasis, we performed an MR analysis of two samples using summary-level genome-wide association study (GWAS) data and validated them using additional datasets.

Materials and methods

Study design

Similar to most MR analyses, our study rested on the following three assumptions: genetic variation is linked strongly to exposure, genetic variants should not be considered confounders, and genetic variants should be related to outcomes only *via* exposure (13). We used two exposure datasets from different sources for the analysis: the discovery and validation sets. **Figures 1**, **2** show the overview of the study's design. Ethical review approval and informed consent were obtained for the original study.

Selection of instrumental variables

The genetic instrumental variables for ω-3/6 were derived from the UK Biobank (UKB) and included 114,999 participants (**Table 1**). The data were adjusted for age, age squared, and sex. Since this GWAS study included more participants and analyzed more single nucleotide polymorphisms (SNPs), it was used as the discovery set. We screened for SNPs with genome-wide significance ($P < 5 \times 10^{-8}$). Subsequently, to ensure that the SNPs were valid and independent, we removed the linkage disequilibrium (LD) between the SNPs at $r^2 < 0.001$. Furthermore, the secondary phenotype of each SNP was retrieved to ensure that it was not associated with cholelithiasis. The F-statistic was performed to assess the strength of the IVs. The threshold of the F statistic > 10 indicated a relatively strong estimated effect of IVs (14).

Another group of ω-3/6 IVs was derived from a GWAS containing 13,544 European participants (15). The screening criteria were the same as those described previously. This set of IVs was used as the validation set.

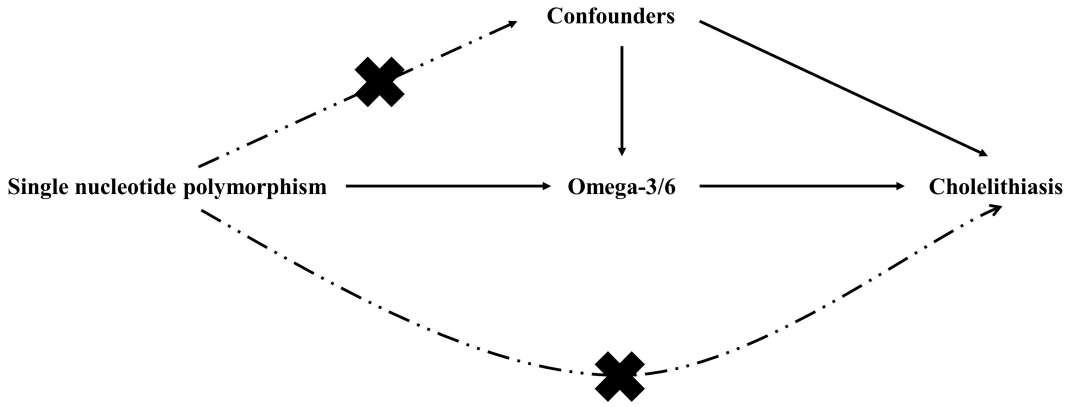


FIGURE 1
Basic assumptions of mendelian randomization.

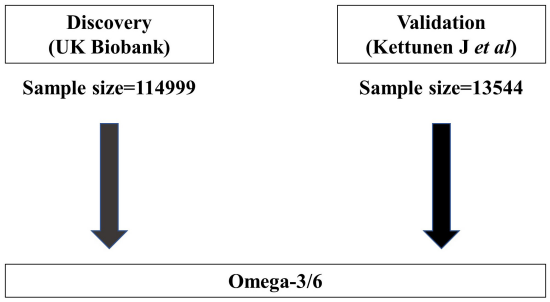


FIGURE 2
Main design of this study.

Outcome data source

GWAS summary statistics for gallstone disease were obtained from the FinnGen Consortium[1] (Table 1). This was a large cohort study analyzing more than 16,000,000 SNPs, adjusted for sex, age, and genotyping batches. The definition of cholelithiasis in this study was based on the K80 type in ICD-10, and strict SNP inclusion criteria (MAF > 1%) were used. Including 19,023 cholelithiasis cases and 195,144 controls were included in this study.

Statistical analysis

The random-effects model inverse variance weighting method was used as the main method of analysis in this study (16). Multiple analysis methods have also been used for repeated analysis, including the MR-Egger (17), weighted median (18), MR-pleiotropic residual sum and outliers (19) and maximum likelihood methods (20). Each approach employs different hypothetical models to assess causal effects, which are then used to check the robustness of the results. The MR-Egger provides calculation after adjusting for pleiotropy (17). Median weighting allowed estimation of causal effects when 50% of SNPs were invalid (18). The median weighting method allows for the estimation of causal effects when 50% of the SNPs are invalid (18). The MR-PRESSO method detects and corrects outliers, providing MR calculation that are robust in terms of heterogeneity after removing the identified outliers (19).

An MR analysis is often confounded by horizontal pleiotropy, which can lead to biased results. Therefore, we used multiple sensitivity analyses to identify potential pleiotropy. First, Cochran's Q statistic was used to assess heterogeneity among the SNPs. Cochran's Q-derived $P < 0.05$ was considered an indicator of heterogeneity in the IVs, at which point the multiplicative random effects IVW method was considered the gold standard (17). Second, an intercept test of the MR-Egger method was performed to measure horizontal multiplicity (17). Third, a leave-one-out analysis was performed to assess whether the association was driven by a single SNP (17).

The correlations with a P-value < 0.05 were considered to be statistically significant. All the analyses were performed using R software (version 4.1.2). The MR analyses were performed using the R packages "TwoSampleMR" and "MendelianRandomization."

Results

The instrumental variable strength analysis showed that the general F statistic was greater than the empirical threshold of 10 (Table 1), indicating that a weak instrumental bias was unlikely to affect the estimation of the causal effects. Using PhenoScanner 2, we did not

1 https://r5.finngen.fi/

TABLE 1 Demographic overview and strength assessment.

Traits	Data sources	Sample size (case/control)	Ancestry	R^2 (%)	*F*-statistic (total)
Exposure					
Omega-3 Discovery	UK Biobank	114,999	European	4.10	103.15
Omega-6 Discovery	UK Biobank	114,999	European	0.98	26.70
Omega-3 Validation	Kettunen J. et al.	13,544	European	1.55	40.11
Omega-6 Validation	Kettunen J. et al.	13,544	European	1.08	16.43
Outcome					
Cholelithiasis	FinnGen	19,023/195,144	European		

find any IVs of ω-3/6 that were associated with potential confounding factors.

Genetic liability to omega-3 with cholelithiasis

In the discovery stage, the random effect IVW analysis showed that higher ω-3 levels were correlated inversely with the risk of cholelithiasis (β = −0.22, 95% CI [−0.32 to −0.12], $P = 1.49 \times 10^{-5}$) (Figure 3). The Cochran's Q statistic suggested heterogeneity; therefore, we adopted the results of the random-effect IVW analysis. The MR-Egger intercept method revealed no evidence of horizontal pleiotropy (Table 2). The remaining analyses showed that the significant results were not driven by any single SNP (Supplementary Figure 3). The MR-Egger, weighted median, and maximum likelihood methods produced the same results as the IVW method (Figure 3). The MR-PRESSO method detected outliers; however, the results did not change after the outliers were removed, which further demonstrated the reliability of our results. Forest plots and funnel plots are presented in Supplementary Figures 2, 3. Detailed information on the SNPs involved is in Supplementary Table 1.

When the replication analysis was performed using another set of IVs, the causal relationship between ω-3 and cholelithiasis remained stable (β = −0.42, 95% CI [−0.66 to −0.18], $P = 5.49 \times 10^{-4}$), except for the findings that were obtained using the MR-Egger method, which were not significant (Figure 4). Further sensitivity analyses showed no evidence of horizontal pleiotropy despite the heterogeneity of the IVs. The results of the leave-one-out analysis were consistent with the discovery phase and the results were not caused by any single SNP (Supplementary Figure 3). The results of the MR-PRESSO method remained significant after removal of the outliers. Forest plots and funnel plots are presented in Supplementary Figures 2, 3. Detailed information on the SNPs involved is in Supplementary Table 2.

Genetic liability to omega-6 with cholelithiasis

The results of the IVW approach showed that each SD increase in ω-6 was associated negatively with the risk of cholelithiasis, both in the discovery (β = −0.21, 95% CI [−0.35 to −0.06], $P = 4.37 \times 10^{-3}$) and in the validation phases (β = −0.21, 95% CI [−0.40 to −0.02], $P = 3.44 \times 10^{-2}$) (Figures 5, 6). The sensitivity analysis showed no evidence of horizontal pleiotropy, although heterogeneity was observed among the IVs (Table 2). Furthermore, the ω-6 association with cholelithiasis was not driven by any SNP (Supplementary Figure 3). In the discovery phase, the results of the MR-PRESSO method were consistent with the original values, after the removal of the outliers. However, the results were inconsistent after removing the outliers during the validation phase. One possible explanation is the heterogeneity of the IVs. However, since the validation set contained fewer IVs, the excessive elimination of SNPs would result in a loss of statistical power. Forest plots and funnel plots are presented in Supplementary Figures 2, 3. Detailed information on the SNPs involved is in Supplementary Tables 3, 4.

Discussion

In this study, both the discovery phase and the validation phase suggest that higher serum omega-3/6 fatty acid concentrations may be associated with a lower risk of cholelithiasis. At the same time, sensitivity analysis found that horizontal pleiotropy did not significantly interfere with the results of this study.

Epidemiological or clinical studies on the relationship between PUFA consumption and the risk of cholelithiasis have been conflicting and sparse. In a prospective cohort study, the high consumption of PUFAs was correlated inversely with the risk of cholelithiasis in men (4). This was supported by a cross-sectional study which indicated that high consumption of PUFAs played a protective role in cholelithiasis (21). However, PUFA intake did not seem to be associated with cholelithiasis in a observational study that was conducted in Argentina (22).

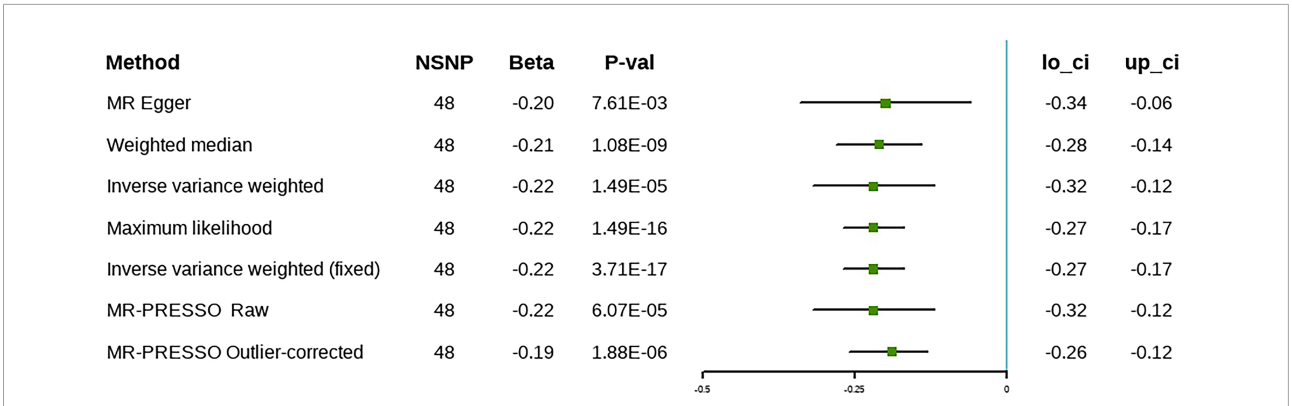


FIGURE 3
The causal relationship between omega-3 and cholelithiasis (discovery).

TABLE 2 Pleiotropy and heterogeneity test of the omega-3/6 IVs from cholelithiasis GWAS.

		Pleiotropy test			Heterogeneity test			
		MR-Egger			MR-Egger		IVW	
		Intercept	SE	P	Q	Q_pval	Q	Q_pval
Omega-3	Discovery	−0.003	0.006	6.78E-01	177.075	2.86E-17	177.746	4.42E-17
	Validation	−0.025	0.059	6.95E-01	19.572	2.08E-04	20.788	3.49E-04
Omega-6	Discovery	0.001	0.009	8.87E-01	147.322	2.41E-13	147.392	4.41E-13
	Validation	−0.030	0.037	4.52E-01	43.603	2.55E-07	47.555	1.20E-07

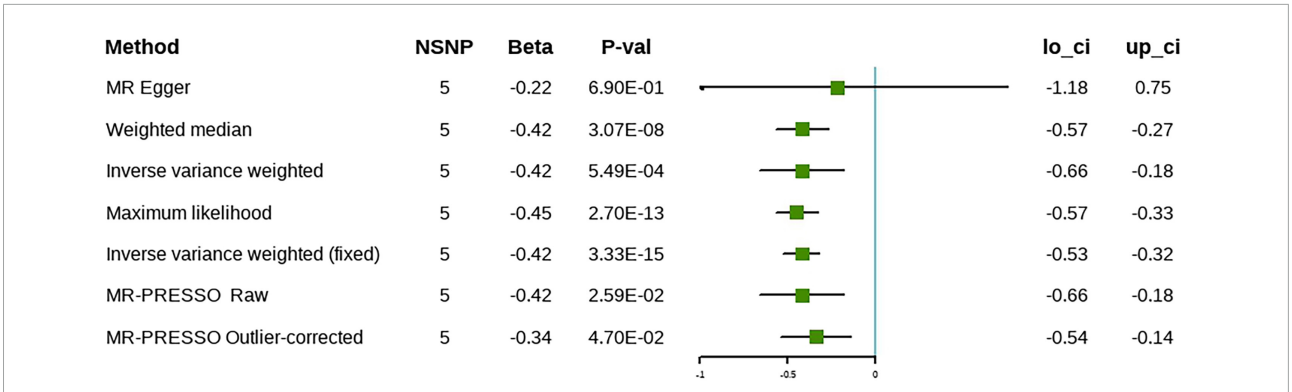


FIGURE 4
The causal relationship between omega-3 and cholelithiasis (validation).

Furthermore, the intake of PUFAs in patients with gallstones was higher in a retrospective study that was conducted in Spain (23). These results may have been due to the use of small sample sizes or the lack of long-term dietary information.

Our results may be explained by several possible underlying mechanisms. It has been reported previously that ω-3 PUFA supplementation may modify the composition of biliary phosphatidylcholine (24). This change may stabilize the cholesterol-phospholipid vesicles, which play a significant role in preventing cholesterol nucleation and gallstone formation (25). The reason for this change may be the fact that supplementation with ω-3 PUFA down-regulates the expression of canalicular transporters ABC, which has a major role in cholesterol secretion. Second, the antinucleating effect of ω-3 PUFA may also be explained by the arachidonic acid (AA) hypothesis. According to a study on the African Green Monkey, a high intake of ω-3 PUFA may reduce the percentage of AA in biliary phospholipids (26). In addition, the presence of AA in biliary phospholipids causes the hypersecretion of gallbladder mucins which has been considered to trigger the

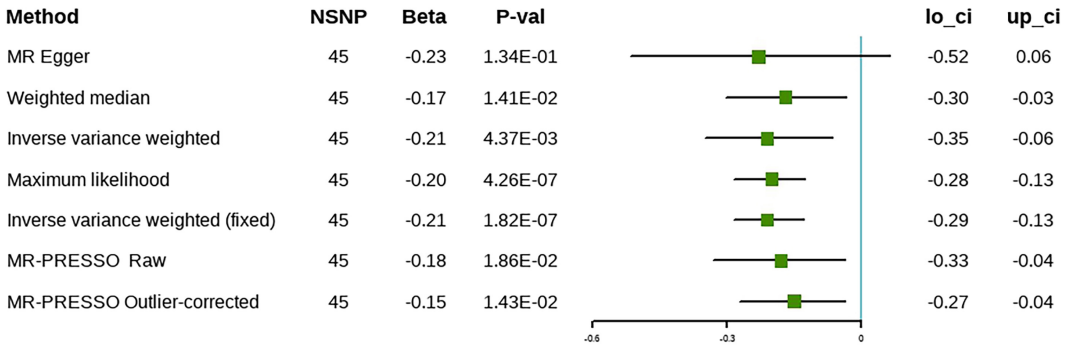


FIGURE 5
The causal relationship between omega-6 and cholelithiasis (discovery).

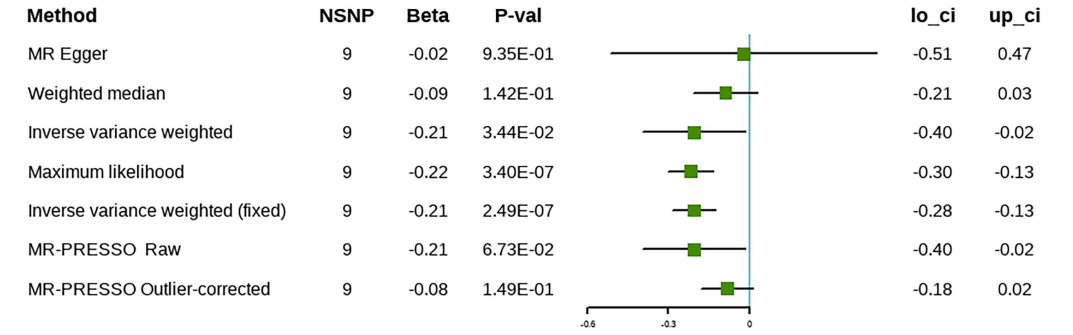


FIGURE 6
The causal relationship between omega-6 and cholelithiasis (validation).

formation of gallstones by serving as a nidus of gallstones (27, 28). Besides, a high intake of ω-3 PUFA can also decrease mucins secretion by reducing expression of mucin gene expression such as Muc2, Muc5ac, Muc5b. Third, previous studies have demonstrated that dietary supplementation with ω-3 PUFA promoted the secretion of hepatic phospholipids by reducing the hydrophobicity of phospholipids (26, 29). Enhanced hepatic phospholipid secretion may increase the bile phospholipid concentration and reduce CSI. Finally, the effects of ω-3 PUFA may also be explained by increased insulin sensitivity. Metabolic studies have suggested that an increased intake of ω-3 PUFA may improve insulin sensitivity by changing the fatty acid composition of the adipocyte plasma membrane (30, 31). In addition, there is an increased synthesis of cholesterol and hypersecretion of biliary cholesterol in patients with insulin resistance (32–34). Previous studies have also speculated that insulin resistance may participate in the pathogenesis of cholelithiasis by promoting the release of proinflammatory cytokines that are related to gallbladder inflammation (35, 36).

Recent evidence has shown that ω-6 PUFA is inversely associated with type 2 diabetes mellitus (T2DM) (37). T2DM has been proved to be a high-risk factor for cholelithiasis. Furthermore, ω-6 PUFA may significantly decrease triglycerides and increase high-density lipoprotein (HDL) cholesterol levels (38). High triglyceride and low HDL cholesterol levels are established risk factors for cholelithiasis. In addition, ω-6 PUFA can promote the production and secretion of bile acids by inducing the synthesis of cholesterol 7α-hydroxylase (39). This may be related to the reduced expression of sterol 27-hydroxylase. Therefore, this suggested that ω-6 PUFA may also reduce CSI and prevent cholelithiasis.

Although laparoscopic cholecystectomy has become the most common minimally surgical procedure performed worldwide, it may be suboptimal in the long term (9, 40). As a surgical procedure, laparoscopic cholecystectomy is inevitably associated with surgical complications and even patient death (41, 42). In addition, cholelithiasis is considered to be one of the highest medical burdens among digestive diseases. In the future, more attention should be paid to preventing cholelithiasis. Our study may promote a paradigm shift from the diagnosis and treatment of gallstones to prevention. Patients with a strong susceptibility to gallstones may benefit from preventive PUFA supplementation.

Our study had several strengths. Firstly, for the first time, the causal association between omega fatty acids and

cholelithiasis was explored using MR analysis; secondly, this study consisted of two parts: discovery and validation, which made the results more reliable, and there was no overlap in the population between different data sets. Thirdly, a series of replicate and sensitivity analyses were applied to improve the credibility of our results.

Our study also had several limitations. First, the participants involved in this study were of European origin; therefore, this result should be interpreted with caution in other populations. Second, there was heterogeneity among the IVs used in this study; however, the absence of pleiotropy in the MR-Egger test suggested balanced pleiotropy, which was unlikely to bias the results. Third, although we used several approaches to remove confounders and minimize the possibility of bias, the potential pleiotropy could not be removed completely. However, the sensitivity analyses suggested that horizontal pleiotropy was unlikely to have an impact on our results.

Conclusion

In summary, our findings indicate that individuals with lower omega-3/6 fatty acid levels have a higher risk of cholelithiasis. Given the greater disease burden of cholelithiasis, ω-3/6 fatty acid supplementation may be a promising adjunct treatment modality. Standardized randomized controlled trials should be designed to further explore the benefits of PUFAs in cholelithiasis.

Data availability statement

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

Author contributions

QS and WX designed the study and drafted the article. QS, NG, and WX conducted the data acquisition and performed the data analysis and manuscript revision. All authors have read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (81970552).

Acknowledgments

We thank all the participants and researchers for their participation in this MR study. European Bioinformatics Institute GWAS Catalog provided summary data for the analyses.

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 JJ declared a shared parent affiliation with the authors to the handling editor at the time of the review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.964805/full#supplementary-material

References

1. Lammert F, Gurusamy K, Ko CW, Miquel JF, Mendez-Sanchez N, Portincasa P, et al. Gallstones. *Nat Rev Dis Primers.* (2016) 2:16024. doi: 10.1038/nrdp.2016.24

2. Wirth J, Joshi AD, Song M, Lee DH, Tabung FK, Fung TT, et al. A healthy lifestyle pattern and the risk of symptomatic gallstone disease: results from 2 prospective cohort studies. *Am J Clin Nutr.* (2020) 112:586–94. doi: 10.1093/ajcn/nqaa154

3. Tavani A, Rosato V, Di Palma F, Bosetti C, Talamini R, Dal Maso L, et al. History of cholelithiasis and cancer risk in a network of case-control studies. *Ann Oncol.* (2012) 23:2173–8. doi: 10.1093/annonc/mdr581

4. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. The effect of long-term intake of cis unsaturated fats on the risk for gallstone disease in men: a prospective

cohort study. *Ann Int Med.* (2004) 141:514–22. doi: 10.7326/0003-4819-141-7-200410050-00007

5. Downie LE, Ng SM, Lindsley KB, Akpek EK. Omega-3 and omega-6 polyunsaturated fatty acids for dry eye disease. *Cochrane Database Syst Rev.* (2019) 12:Cd011016. doi: 10.1002/14651858.CD011016.pub2

6. Brown TJ, Brainard J, Song F, Wang X, Abdelhamid A, Hooper L. Omega-3, omega-6, and total dietary polyunsaturated fat for prevention and treatment of type 2 diabetes mellitus: systematic review and meta-analysis of randomised controlled trials. *BMJ.* (2019) 366:l4697. doi: 10.1136/bmj.l4697

7. Kim JK, Cho SM, Kang SH, Kim E, Yi H, Yun ES, et al. N-3 polyunsaturated fatty acid attenuates cholesterol gallstones by suppressing mucin production with a high cholesterol diet in mice. *J Gastroenterol Hepatol.* (2012) 27:1745–51. doi: 10.1111/j.1440-1746.2012.07227.x

8. Cohen BI, Mosbach EH, Ayyad N, Miki S, McSherry CK. Dietary fat and fatty acids modulate cholesterol cholelithiasis in the hamster. *Lipids.* (1992) 27:526–32. doi: 10.1007/bf02536135

9. Jang SI, Fang S, Kim KP, Ko Y, Kim H, Oh J, et al. combination treatment with N-3 polyunsaturated fatty acids and ursodeoxycholic acid dissolves cholesterol gallstones in mice. *Sci Rep.* (2019) 9:12740. doi: 10.1038/s41598-019-49095-z

10. Misciagna G, Centonze S, Leoci C, Guerra V, Cisternino AM, Ceo R, et al. Diet, physical activity, and gallstones–a population-based, case-control study in southern Italy. *Am J Clin Nutr.* (1999) 69:120–6. doi: 10.1093/ajcn/69.1.120

11. 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

12. 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

13. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA.* (2017) 318:1925–6. doi: 10.1001/jama.2017.17219

14. Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol.* (2011) 40:755–64. doi: 10.1093/ije/dyr036

15. Kettunen J, Demirkan A, Würtz P, Draisma HH, Haller T, Rawal R, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun.* (2016) 7:11122. doi: 10.1038/ncomms11122

16. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med.* (2008) 27:1133–63. doi: 10.1002/sim.3034

17. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080

18. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965

19. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

20. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* (2015) 32:268–74. doi: 10.1093/molbev/msu300

21. Gilat T, Horwitz C, Halpern Z, Bar Itzhak A, Feldman C. Gallstones and diet in Tel Aviv and gaza. *Am J Clin Nutr.* (1985) 41:336–42. doi: 10.1093/ajcn/41.2.336

22. Compagnucci AB, Perroud HA, Batalles SM, Villavicencio R, Brasca A, Berli D, et al. A nested case-control study on dietary fat consumption and the risk for gallstone disease. *J Hum Nutr Diet.* (2016) 29:338–44. doi: 10.1111/jhn.12332

23. Ortega RM, Fernandez-Azuela M, Encinas-Sotillos A, Andres P, Lopez-Sobaler AM. Differences in diet and food habits between patients with gallstones and controls. *J Am Coll Nutr.* (1997) 16:88–95. doi: 10.1080/07315724.1997.10718655

24. Peled Y, Gilat T. Effect of dietary phospholipids and their constituents on bile composition in rats and hamsters. *Hepatology.* (1994) 19:708–13. doi: 10.1002/hep.1840190324

25. Ayyad N, Cohen BI, Ohshima A, Mosbach EH. Prevention of cholesterol cholelithiasis by dietary unsaturated fats in hormone-treated female hamsters. *Lipids.* (1996) 31:721–7. doi: 10.1007/bf02522888

26. Scobey MW, Johnson FL, Parks JS, Rudel LL. Dietary fish oil effects on biliary lipid secretion and cholesterol gallstone formation in the African green monkey. *Hepatology.* (1991) 14(4 Pt 1):679–84. doi: 10.1016/0270-9139(91)90057-3

27. LaMont JT, Smith BF, Moore JR. Role of gallbladder mucin in pathophysiology of gallstones. *Hepatology.* (1984) 4(5 Suppl.):51s–6s. doi: 10.1002/hep.1840040809

28. Wang DQ, Cohen DE, Lammert F, Carey MC. No Pathophysiologic relationship of soluble biliary proteins to cholesterol crystallization in human bile. *J Lipid Res.* (1999) 40:415–25.

29. LaMorte WW, O'Leary DP, Booker ML, Scott TE. Increased dietary fat content accelerates cholesterol gallstone formation in the cholesterol-fed prairie dog. *Hepatology.* (1993) 18:1498–503.

30. Rivellese AA, De Natale C, Lilli SC. Type of dietary fat and insulin resistance. *Ann N Y Acad Sci.* (2002) 967:329–35. doi: 10.1111/j.1749-6632.2002.tb04288.x

31. Clandinin MT, Cheema S, Field CJ, Baracos VE. Dietary lipids influence insulin action. *Ann N Y Acad Sci.* (1993) 683:151–63. doi: 10.1111/j.1749-6632.1993.tb35701.x

32. Bennion LJ, Grundy SM. Risk factors for the development of cholelithiasis in man (first of two parts). *N Engl J Med.* (1978) 299:1161–7. doi: 10.1056/nejm197811232992104

33. Bennion LJ, Grundy SM. Risk factors for the development of cholelithiasis in man (second of two parts). *N Engl J Med.* (1978) 299:1221–7. doi: 10.1056/nejm197811302992205

34. Attili AF, Capocaccia R, Carulli N, Festi D, Roda E, Barbara L, et al. Factors associated with gallstone disease in the micol experience. Multicenter Italian study on epidemiology of cholelithiasis. *Hepatology.* (1997) 26:809–18. doi: 10.1002/hep.510260401

35. Grimble RF. Inflammatory status and insulin resistance. *Curr Opin Clin Nutr Metab Care.* (2002) 5:551–9. doi: 10.1097/00075197-200209000-00015

36. Lee YH, Pratley RE. The evolving role of inflammation in obesity and the metabolic syndrome. *Curr Diabetes Rep.* (2005) 5:70–5. doi: 10.1007/s11892-005-0071-7

37. Forouhi NG, Imamura F, Sharp SJ, Koulman A, Schulze MB, Zheng J, et al. Association of plasma phospholipid N-3 and N-6 polyunsaturated fatty acids with type 2 diabetes: the epic-interact case-cohort study. *PLoS Med.* (2016) 13:e1002094. doi: 10.1371/journal.pmed.1002094

38. Vanhala M, Saltevo J, Soininen P, Kautiainen H, Kangas AJ, Ala-Korpela M, et al. Serum omega-6 polyunsaturated fatty acids and the metabolic syndrome: a longitudinal population-based cohort study. *Am J Epidemiol.* (2012) 176:253–60. doi: 10.1093/aje/kwr504

39. Sato M, Yoshida S, Nagao K, Imaizumi K. Superiority of dietary safflower oil over olive oil in lowering serum cholesterol and increasing hepatic mRnas for the LDL receptor and cholesterol 7alpha-hydroxylase in exogenously hypercholesterolemic (exHC) rats. *Biosci Biotechnol Biochem.* (2000) 64:1111–7. doi: 10.1271/bbb.64.1111

40. Kao LS, Ball CG, Chaudhury PK, for Members of the Evidence Based Reviews in Surgery Group. Evidence-based reviews in surgery: early cholecystectomy for cholecystitis. *Ann Surg.* (2018) 268:940–2. doi: 10.1097/SLA.0000000000002867

41. Deziel DJ, Millikan KW, Economou SG, Doolas A, Ko ST, Airan MC. Complications of laparoscopic cholecystectomy: a national survey of 4,292 hospitals and an analysis of 77,604 cases. *Am J Surg.* (1993) 165:9–14. doi: 10.1016/s0002-9610(05)80397-6

42. Jensen SAS, Fonnes S, Gram-Hanssen A, Andresen K, Rosenberg J. Long-term mortality and intestinal obstruction after laparoscopic cholecystectomy: a systematic review and meta-analysis. *Int J Surg.* (2022) 105:106841. doi: 10.1016/j.ijsu.2022.106841

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 29 September 2022
DOI 10.3389/fnut.2022.978679

OPEN ACCESS

EDITED BY
Zhiyong Zou,
Peking University, China

REVIEWED BY
Kaixiong Ye,
University of Georgia, United States
Hans Demmelmair,
Ludwig Maximilian University of Munich, Germany

*CORRESPONDENCE
Li Cai
caili5@mail.sysu.edu.cn

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 26 June 2022
ACCEPTED 30 August 2022
PUBLISHED 29 September 2022

CITATION
Wu S, Zhao F, He Y, He T, Duan S, Feng G, Chen Y, Wang X, Szeto IM-Y, Lin L and Cai L (2022) Association between maternal erythrocyte polyunsaturated fatty acid levels during pregnancy and offspring weight status: A birth cohort study. *Front. Nutr.* 9:978679. doi: 10.3389/fnut.2022.978679

Association between maternal erythrocyte polyunsaturated fatty acid levels during pregnancy and offspring weight status: A birth cohort study

Shengchi Wu[1], Feng Zhao[2], Yannan He[2], Tingchao He[3,4], Sufang Duan[4,5], Gang Feng[3,4], Yujing Chen[1], Xin Wang[1], Ignatius Man-Yau Szeto[3,5], Lizi Lin[6] and Li Cai[1,7]*

[1]Department of Maternal and Child Health, School of Public Health, Sun Yat-sen University, Guangzhou, China, [2]Institute of Nutrition & Health, Qingdao University, Qingdao, China, [3]Yili Maternal and Infant Nutrition Institute, Inner Mongolia Yili Industrial Group Co., Ltd., Hohhot, China, [4]Inner Mongolia Dairy Technology Research Institute Co., Ltd., Hohhot, China, [5]Nutrition and Health Research Center, National Center of Technology Innovation for Dairy, Hohhot, China, [6]Department of Occupational and Environmental Health, School of Public Health, Sun Yat-sen University, Guangzhou, China, [7]Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou, China

Background: The findings of the association between maternal polyunsaturated fatty acid (PUFA) levels during pregnancy and offspring weight status are controversial. Furthermore, few studies have focused on Asian populations or used erythrocyte membranes as biological markers. We aimed to examine the associations between maternal erythrocyte PUFA and offspring weight status within the first 2 years among the Chinese population.

Materials and methods: A total of 607 mother-child pairs were recruited from a birth cohort. Maternal erythrocyte n-3 and n-6 PUFA during pregnancy were measured by gas chromatography, and the ratio of PUFA was calculated. Weight- and body mass index (BMI)-for-age z (WAZ and BAZ) scores were calculated for offspring at 1, 3, 6, 8, 12, 18, and 24 months of age. The risk of overweight and obesity was defined by the WHO criterion. The Generalized Estimating Equation (GEE) model was carried out for repeated anthropometric data within 2 years of age.

Results: Maternal erythrocyte docosapentaenoic acid (DPA, n-3) was inversely associated with offspring BAZ score [tertile 2 vs. tertile 1, β: −0.18 (−0.29, −0.00)]. Higher maternal erythrocyte arachidonic acid (AA) was inversely associated with lower offspring WAZ and BAZ [tertile 3 vs. tertile 1, β: −0.18 (−0.35, −0.02), −0.22 (−0.38, −0.06), respectively]. Furthermore, higher maternal erythrocyte AA [tertile 3 vs. tertile 1, odds ratio [OR]: 0.52 (0.36, 0.75),

p_{trend} < 0.001] and total n-6 PUFA [tertile 3 vs. tertile 1, OR: 0.56 (0.39, 0.81), p_{trend} = 0.002] were associated with decreased risk of overweight and obesity in offspring. Maternal erythrocyte n-6/n-3 PUFA and AA/eicosapentaenoic acid (EPA) ratios were not associated with offspring weight status.

Conclusion: Maternal erythrocyte PUFA might influence offspring weight status within 2 years of age in the Chinese population. Further Asian studies are still needed.

KEYWORDS

polyunsaturated fatty acid, pregnancy, offspring, weight status, birth cohort

Introduction

The increasing prevalence of childhood overweight and obesity remains a serious health concern (1). Public prevention of childhood obesity is urgently needed, and the first 1,000 days of life are a unique window (2). The intrauterine nutritional environment might affect fetal growth and the risk of disease later in life by affecting developmental programming (3), among which polyunsaturated fatty acids (PUFA) are critical nutrients for fetal growth and development (4). It was suggested that the maternal PUFA levels during pregnancy have a long-term effect on offspring weight status.

Several *in vitro* and animal studies have suggested that PUFA could affect fetal adipose tissue accretion. However, the effect may vary by the type and ratio of PUFA (5, 6), especially the composition and ratio of n-3 and n-6 PUFAs. It was suggested that the maternal n-6/n-3 PUFA ratio during pregnancy was more closely associated with fetal metabolic programming (7). Prospective cohort studies also showed conflicting results. The studies from America showed that higher maternal n-3 PUFA was associated with lower total subcutaneous fat mass and lower odds of obesity in children aged 3 years (8). Similarly, another Netherlands study found that lower maternal levels of n-3 PUFA and higher levels of n-6 PUFA were associated with higher total body fat and abdominal fat levels in childhood (9). However, another American study showed that maternal PUFA was not associated with childhood body mass index (BMI) z scores aged 8 years (10). Similarly, results from randomized controlled trials (RCTs) showed inconclusive effects of n-3 PUFA supplement during pregnancy on offspring BMI or body fat percentage (11), because of differences in design, especially the amounts, balance, and type of PUFA given.

Notably, previous studies are almost exclusively from American and European populations, but evidence from Asian populations has been limited. As the Asian population has different genetic variations and dietary patterns when compared with American or European populations (12), more investigation into Asian populations is highly warranted. Moreover, the majority of these studies used plasma as biomarkers or non-objective food frequency questionnaires as exposure measures. Fatty acid composition in erythrocytes could reflect dietary fatty acid intake over the past month or 2 (13), which may be a more stable biomarker than plasma phospholipid fraction. Therefore, we explored the associations between maternal erythrocyte PUFA during pregnancy with offspring weight status within the first 2 years in a Chinese birth cohort.

Materials and methods

Study design and participants

Mothers and infants were from a prospective birth cohort study (registration number: NCT03023293), which was carried out at a hospital in Guangzhou, China. We enrolled pregnant women who were aged 20–45 years and at 20–28 weeks of gestation during 2017 and 2018 and then followed up their offspring for 2 years postpartum. Mothers with pre-existing diabetes mellitus, cardiovascular disease, thyroid disease, hematopathy, polycystic ovary syndrome, pregnancy infection, mental disorder, or multiple pregnancies and infants who did not have follow-up data were excluded from the study.

A total of 691 mother-offspring pairs were enrolled. We further excluded women whose erythrocyte PUFA information was missing (n = 84). Overall, 607 mother-child pairs were finally included in our analysis. This study was approved by the institutional review boards of Sun Yat-sen University. Informed consent forms were obtained from all participants.

Maternal erythrocyte fatty acid analysis

Maternal blood samples were collected during 20–28 weeks of gestation. The venous blood samples were collected by professional nurses in the morning after an overnight fast of at

least 10 h. The samples were centrifuged at 841 × *g* for 15 min to obtain agglutinated blood cells. Blood cell samples were kept at −80°C until later laboratory analysis.

Laboratory analysis of erythrocyte fatty acid was as follows. First, removed the blood samples from the refrigerator and thawed them. In total, 100 μl of blood cells per sample was dropped on the test paper (Omegabandz Inc., China) and shipped to the laboratory after drying. Second, the tris–HCl buffer was added to the sample. After the red blood cells were hemolyzed, we centrifuged them to obtain the bottom layer of milky red blood cell fragments. And then, the lipid component of erythrocyte fragments was extracted with a chloroformmethanol (2:1, v/v) solvent system containing 10 mg/L of butylated-hydroxytoluene (BHT, Sigma Chemical Co., St. Louis, MO, USA) (14). Third, the methyl esters of the fatty acids from the lipid extract were transesterified with H_2SO_4 in methanol (5%, v/v), together with toluene, in sealed tubes at 70°C for 2 h. The methanol layer was transferred to a new test tube, blown by nitrogen, and then dissolved in hexane. Fourth, the derived fatty acid methyl esters were analyzed by using Agilent 7820 Gas Chromatograph (Agilent Corporation,USA) equipped with a 60 m × 0.25 mm × 0.25 μm fused silica-bonded phase column (DB-23, Agilent Corporation, USA) and a flame ionization detector. The column temperature was firstly programmed from 150 to 180°C at a rate of 10°C/min, with an initial hold time of 2 min; then it was increased to 215 at 2.5°C/min and held for 6 min; and finally, it was increased to 230 at 10°C/min and held for another 5 min. Fatty acids were identified by comparison of retention time with standard mixtures of fatty acid methyl ester (Nu-Chek Prep, Inc., Waterville, MN, USA). Quantification of the fatty acid compositions was achieved by the comparison of peak areas with the internal standard (tricosanoic acid, Nu-Chek Prep, Inc., Waterville, MN, USA), which was added to the samples (1 mg of internal standard in 500 mg sample) prior to extraction.

Polyunsaturated fatty acid levels were expressed as a proportion of the total fatty acids. Based on findings from previous studies, selected PUFAs were total n-3 PUFAs, which included α-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3), and total n-6 PUFA, which included linoleic acid (LA, C18:2 n-6), γ-linolenic acid (GLA, C18:3 n-6), dihomo-gamma-linolenic acid (DGLA, C20:3 n-6), and arachidonic acid (AA, C20:4 n-6). We also calculated the ratio of total n-6/n-3 PUFA and the ratio of AA/EPA.

Offspring anthropometric measurements

At the age of 1, 3, 6, 8, 12, 18, and 24 months, offspring length and weight without shoes and heavy clothing were measured by the trained nurses. The length was measured to the nearest 0.1 cm by a stadiometer, and weight was measured to the nearest 0.01 kg using an electronic scale. All measurements were performed by trained professionals, and standardized tools were used. BMI was calculated as weight (kg)/height in meters square.

According to the criterion from the World Health Organization Child Growth Standards 2006 (15), offspring weight-for-age z (WAZ) score, length-for-age z (LAZ) score, and BMI-for-age z (BAZ) score were calculated. Furthermore, offspring's weight status was defined as a normal, possible risk of overweight, or overweight and obesity based on the BAZ score.

Covariates

Information on maternal age, pre-pregnancy weight and height, educational level (high school or below/junior college/college or above), monthly household income (<4,000/4,000–6,000/6,000–10,000/≥10,000 RMB), and passive smoking during pregnancy were obtained by a face-to-face questionnaire survey in the baseline investigation. Maternal pre-pregnancy BMI was calculated from weight (kg) divided by height squared (m^2). In addition, all pregnant women were scheduled for a 75-g oral glucose tolerance test between 20 and 28 weeks of gestation. Women were diagnosed with gestational diabetes (GDM) when meeting the criteria of the International Association of Diabetes and Pregnancy Study Groups (16). Offspring sex, birth weight, and other delivery information were obtained from the hospital birth records. Offspring feeding status at 6 months (breastfeeding/formula feeding/mixed feeding) was obtained at the age of 6 months by a structured questionnaire.

Statistical analysis

All statistical analyses were carried out using SAS statistical software package (version 9.4; SAS Institute Inc., Cary, NC, USA). PUFA levels were divided into three levels based on the tertiles of each PUFA. Continuous variables were reported as the mean ± standard deviation (SD) or median (25th–75th percentile), and categorical variables were expressed as percentages.

Considering the within-subject correlation due to repeated measures, the Generalized Estimating Equation (GEE) (17) was selected to correct for children's repeated anthropometric measurements at 1, 3, 6, 8, 12, 18, and 24 months of age because the method takes this within-subject correlation into account. After comparing the quasi-likelihood under the independence model criterion (QIC) value, the GEE with six dependent correlation matrices was used to estimate the relationship between the maternal PUFA and offspring WAS score, LAZ score, BAZ score (continuous), and risk of overweight and

obesity (ordered categorical) within the first 2 years. The model was adjusted for confounding factors from mothers and infants, which included maternal age, educational level, family income, GDM, pre-pregnancy BMI, passive smoking during pregnancy, infant age, sex, and breastfeeding. $p < 0.05$ was considered significant.

Results

Subject characteristics

Table 1 and Supplementary Table 1 show the characteristics of pregnant women and their offsprings. A total of 607 mother-offspring pairs were included in our analyses. The median age of the women was 30.51 years and the median pre-pregnancy BMI was 20.0 kg/m^2.

Table 2 shows the maternal erythrocyte PUFA levels in mid-pregnancy. The median maternal erythrocyte total n-3 and n-6 PUFA levels were 9.69 and 36.33%, respectively. Among the individual PUFAs, the content of AA was the highest (17.48%), followed by LA, DHA, DGLA, and DPA (15.32, 7.29, 2.43, and 1.25%, respectively). In addition, the ratios of total n-6/n-3 PUFA and AA/EPA were 3.61, and 18.41, respectively.

TABLE 1 Characteristics of pregnant women and offspring (n = 607)[a].

Characteristic	n (%) or median (25th–75th percentile)
Maternal characteristics	
Age, median (year)	30.51 (26.96, 33.74)
Pre-pregnancy body mass index (kg/m^2)	20.00 (18.44, 22.19)
Education, n (%)	
High school or below	229 (33.78)
Junior college	219 (32.30)
College or above	230 (33.92)
Monthly household income n (%)	
<4000 (RMB)	119 (17.76)
4000∼6000 (RMB)	163 (24.33)
6000∼10000 (RMB)	172 (25.67)
≥10000 (RMB)	216 (32.24)
Passive smoking during pregnancy, yes (%)	352 (51.69)
Gestational diabetes, yes (%)	131 (18.96)
Offspring characteristics	
Males, yes (%)	349 (50.51)
Birth weight (kg)	3.17 (2.95, 3.45)
Birth length (cm)	50.00 (49.00, 50.00)
Feeding status at 6 months, n (%)	
Breastfeeding	243 (35.2)
Formula feeding	71 (10.3)
Mixed feeding	351 (50.8)

[a]Values represent the median (the 25th, the 75th percentile) or number of subjects (valid %).

Maternal erythrocyte polyunsaturated fatty acid ratios and offspring weight status

Table 3 shows that there was an inverse association between medium-level maternal erythrocyte DPA and offspring BAZ scores [tertile 2 vs. tertile 1, β (95% CI): −0.18 (−0.29, −0.00)]. Higher maternal AA levels were associated with lower offspring WAZ score in the adjusted models [tertile 3 vs. tertile 1, β (95% CI): −0.18 (−0.35, −0.02), p_{trend} = 0.028]. Moreover, higher maternal erythrocyte AA was associated with lower offspring BAZ score [tertile 3 vs. tertile 1, β (95% CI): −0.22 (−0.38, −0.06), p_{trend} = 0.006]. We did not find associations between other n-3 PUFA or n-6 PUFA with offspring's WAS score or BAZ score. In addition, Supplementary Tables 2, 3 show the associations of maternal erythrocyte PUFA with the risk of low birth weight and LAZ score in offspring, respectively.

Table 4 shows that higher maternal erythrocyte total n-6 PUFA and AA were associated with decreased risk of overweight and obesity in offspring (tertile 3 vs. tertile 1, OR (95% CI): 0.56 (0.39, 0.81), p_{trend} = 0.002; tertile 3 vs. tertile 1, OR (95% CI): 0.52 (0.36, 0.75), p_{trend} < 0.001, respectively). Non-significant associations were observed between other maternal PUFAs with offspring's risk of overweight and obesity.

Maternal erythrocyte polyunsaturated fatty acid ratios and offspring weight status

Figure 1 shows that maternal total n-6/n-3 ratios and AA/EPA ratios were not associated with offspring WAZ score,

TABLE 2 Maternal erythrocyte PUFA levels during pregnancy (n = 607).

	Percentage by weight of total sum of fatty acids (%)[a]
Total n-3 PUFA (%)	9.69 (7.86, 11.52)
ALA (C18:3 n-3)	0.25 (0.18, 0.34)
EPA (C20:5 n-3)	0.89 (0.63, 1.36)
DPA (C22:5 n-3)	1.25 (0.99, 1.53)
DHA (C22:6 n-3)	7.29 (5.41, 9.01)
Total n-6 PUFA (%)	36.33 (33.42, 38.12)
LA (C18:2 n-6)	15.32 (13.83, 16.69)
GLA (C18:3 n-6)	0.23 (0.15, 0.31)
DGLA (C20:3 n-6)	2.43 (2.08, 2.77)
AA (C20:4 n-6)	17.48 (14.69, 19.05)
Ratio	
Total n-6/n-3 PUFA	3.61 (3.09, 4.20)
AA/EPA	18.41 (11.93, 26.66)

[a]Values represent the median (25th–75th percentile).
PUFA, polyunsaturated fatty acids; ALA, α-linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, γ-linolenic acid; α-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid.

TABLE 3 Association of maternal erythrocyte PUFA during pregnancy with offspring weight status[a,b].

	Weight for age z score				BMI for age z score			
	T1	T2	T3	P_{trend}	T1	T2	T3	P_{trend}
		β (95% *CI*)	β (95% *CI*)			β (95% *CI*)	β (95% *CI*)	
Total n-3 PUFA	*Ref.*	−0.06 (−0.22, 0.09)	−0.04 (−0.20, 0.11)	0.597	*Ref.*	0.00 (−0.15, 0.15)	0.05 (−0.10, 0.20)	0.515
ALA (C18:3 n-3)	*Ref.*	0.07 (−0.07, 0.22)	0.05 (−0.11, 0.21)	0.524	*Ref.*	0.05 (−0.10, 0.19)	−0.03 (−0.19, 0.13)	0.690
EPA (C20:5 n-3)	*Ref.*	0.01 (−0.15, 0.16)	−0.05 (−0.2, 0.11)	0.550	*Ref.*	0.10 (−0.05, 0.25)	0.01 (−0.14, 0.17)	0.842
DPA (C22:5 n-3)	*Ref.*	−0.11 (−0.27, 0.04)	−0.06 (−0.22, 0.10)	0.419	*Ref.*	**−0.15 (−0.29, -0.00)**	−0.14 (−0.29, 0.02)	0.071
DHA (C22:6 n-3)	*Ref.*	−0.02 (−0.19, 0.14)	−0.1 (−0.27, 0.07)	0.220	*Ref.*	−0.09 (−0.25, 0.08)	−0.08 (−0.25, 0.1)	0.408
Total n-6 PUFA	*Ref.*	−0.10 (−0.25, 0.05)	−0.01 (−0.17, 0.16)	0.930	*Ref.*	−0.11 (−0.26, 0.04)	−0.13 (−0.3, 0.03)	0.104
LA (C18:2 n-6)	*Ref.*	0.12 (−0.03, 0.27)	0.12 (−0.04, 0.27)	0.131	*Ref.*	0.10 (−0.06, 0.25)	0.01 (−0.14, 0.16)	0.858
GLA (C18:3 n-6)	*Ref.*	−0.02 (−0.18, 0.13)	0.00 (−0.16, 0.16)	0.955	*Ref.*	0.020 (−0.14, 0.18)	0.04 (−0.11, 0.20)	0.577
DGLA (C20:3 n-6)	*Ref.*	−0.07 (−0.23, 0.09)	0.03 (−0.12, 0.18)	0.699	*Ref.*	−0.06 (−0.21, 0.10)	0.00 (−0.16, 0.16)	0.986
AA (C20:4 n-6)	*Ref.*	−0.02 (−0.18, 0.14)	**−0.18 (−0.35,−0.02)**	**0.028**	*Ref.*	−0.01 (−0.17, 0.14)	**−0.22 (−0.38,−0.06)**	**0.006**

[a]Model was adjusted for pregnancy factors and infant factors, which included maternal age, educational level, family income, gestational diabetes, pre-pregnancy body mass index, passive smoking during pregnancy, infant age and sex, and feeding status at 6 months.
[b]Statistically significant results are in bold ($p < 0.05$).
PUFA, polyunsaturated fatty acids; ALA, α-linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, γ-linolenic acid; α-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; T3, tertile 3; T2, tertile 2; T1, tertile 1.

BAZ score, or the risk of overweight and obesity in the adjusted GEE model.

Discussion

To the best of our knowledge, this is the first prospective study to explore the association between maternal erythrocyte PUFA during pregnancy with offspring weight status in the Chinese population. We found that higher maternal erythrocyte DPA during pregnancy was associated with lower offspring BAZ score within 2 years old. Higher maternal erythrocyte AA was associated with lower offspring WAZ and BAZ scores. Similarly, maternal erythrocyte AA and total n-6 PUFAs were associated with decreased risk of overweight and obesity.

An appropriate supply of n-3 PUFA during pregnancy has an effect on optimal fetal development (4), but whether the effect can persist into childhood is not clear. DPA is one of the n-3 PUFAs, which can play an independent role. We found a negative association between maternal DPA and offspring BAZ score. Consistent with our results, Vdakovic et al. also observed that higher maternal plasma DPA levels were associated with lower childhood total body fat percentage in the Netherlands (9). The underlying possible mechanisms were as followed. Firstly, it is known that overweight and obesity are characterized by chronic low-grade inflammation, while DPA is the precursor of a large panel of lipid mediators (protectins and resolvins) principally implicated in the pro-resolution of the inflammation, with specific effects (18). Secondly, it has been confirmed that DPA inhibits the process of adipocyte differentiation through the inhibition of the activity of the cyclooxygenase enzymes (19), leading the decreased fat accumulation and expression of inflammatory markers (20). Thus, it is possible that maternal DPA may have a long-lasting effect on offspring growth and development through multiple potential mechanism pathways. It is necessary to further advance the critical window of opportunity for the prevention of lifelong obesity (21).

It is worth mention that we found no association of maternal DHA or EPA with offspring weight or BAZ score. In line with our results, Moon et al. found a non-significant relationship between maternal plasma DHA or EPA and offspring growth (22). Moreover, a recent meta-analysis suggested a null correlation between maternal DHA and EPA supplementation in pregnancy and offspring BMI z score at 0–4 years of age (23). However, in contrast with our results, Donahue et al. found that an enhanced maternal DHA and EPA status was associated with lower childhood adiposity in the American population (8). These discrepancies might be partly explained by differences in the n-3 PUFA levels in various populations. DHA and EPA levels of our population were higher than that of American women in whom differences were found (24) but close to the reference interval for healthy Norwegian pregnant women (25). This indicated that the variation of DHA or EPA in our population might not be large enough, which makes it more difficult to find significant associations. Furthermore, the possible interactions of environmental pollutants with DHA and EPA have complex effects on offspring growth and development (26). Therefore, a larger sample size and diverse research designs may be needed for the Chinese population to further confirm the association of maternal DHA and EPA with offspring WAZ or BAZ scores.

TABLE 4 Association of maternal erythrocyte PUFA during pregnancy with the risk of overweight and obesity in offspring[a,b].

	T1	T2	T3	P_{trend}
		OR (95% *CI*)	OR (95% *CI*)	
Total n-3 PUFA	*Ref.*	0.78 (0.54, 1.14)	1.06 (0.72, 1.54)	0.755
ALA (C18:3 n-3)	*Ref.*	0.95 (0.66, 1.36)	0.97 (0.66, 1.43)	0.888
EPA (C20:5 n-3)	*Ref.*	0.99 (0.68, 1.44)	1.01 (0.67, 1.53)	0.955
DPA (C22:5 n-3)	*Ref.*	0.76 (0.53, 1.10)	0.75 (0.52, 1.09)	0.119
DHA (C22:6 n-3)	*Ref.*	0.74 (0.49, 1.11)	0.69 (0.45, 1.04)	0.089
Total n-6 PUFA	*Ref.*	**0.68 (0.46, 0.99)**	**0.56 (0.39, 0.81)**	**0.002**
LA (C18:2 n-6)	*Ref.*	1.26 (0.87, 1.82)	0.84 (0.57, 1.22)	0.408
GLA (C18:3 n-6)	*Ref.*	1.02 (0.70, 1.48)	1.07 (0.7, 1.65)	0.746
DGLA (C20:3 n-6)	*Ref.*	0.84 (0.58, 1.22)	0.76 (0.51, 1.11)	0.152
AA (C20:4 n-6)	*Ref.*	**0.62 (0.42, 0.91)**	**0.52 (0.36, 0.75)**	**<0.001**

[a]Model was adjusted for pregnancy factors and infant factors, which included maternal age, educational level, family income, gestational diabetes, pre-pregnancy body mass index, passive smoking during pregnancy, infant age and sex, and feeding status at 6 months.
[b]Statistically significant results are in bold ($p < 0.05$).
PUFA, polyunsaturated fatty acids; ALA, α-linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, γ-linolenic acid; α-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; T3, tertile 3; T2, tertile 2; T1, tertile 1.

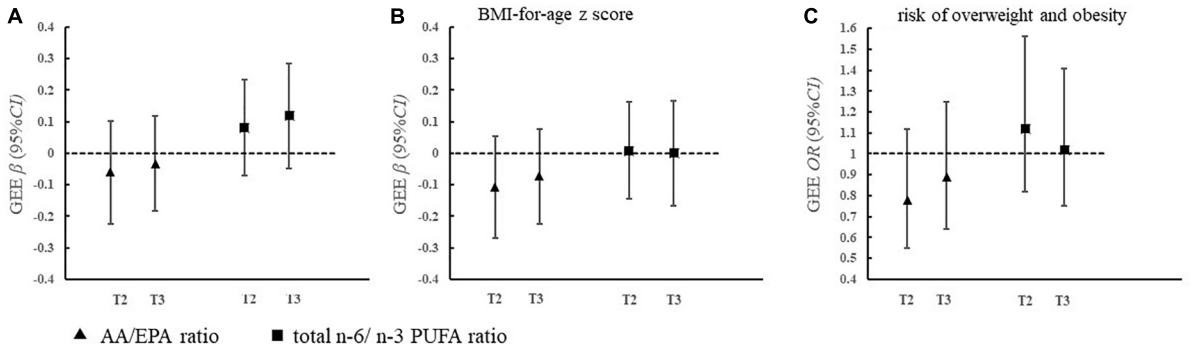


FIGURE 1
Association of maternal erythrocyte PUFA ratio during pregnancy with offspring weight status **(A–C)**. **(A)**▲ AA/EPA ratio ■ total n-6/n-3 PUFA ratio. **(B)** Model was adjusted for pregnancy factors and infant factors, which included maternal age, educational level, family income, gestational diabetes, pre-pregnancy body mass index, passive smoking during pregnancy, infant age and sex, and feeding status at 6 months. PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; AA, arachidonic acid; T3, tertile 3; T2, tertile 2; T1, tertile 1.

Interestingly, we observed that maternal erythrocyte AA, an n-6 PUFA, was related to lower offspring WAZ score, BAZ score, and decreased risk of overweight and obesity. Consistent with our results (9), Much et al. found that higher maternal AA during pregnancy was associated with lower BMI in offspring at 1 year of age in the German population (27). Similarly, Al-Hinai et al. suggested that Mexican maternal intake of AA during mid-pregnancy was inversely associated with offspring linear growth (25). Nevertheless, the hypothesis that higher maternal AA during pregnancy promotes offspring adiposity was confirmed in some European and American mother-offspring pairs (9, 22). The main reason for the inconsistent results might be that AA played different roles at different levels. Although excess AA can serve as a substrate for the production of many pro-inflammatory mediators, (28) optimal AA during pregnancy is beneficial for fetal brain and immune system development (29). Moreover, AA-derived metabolites also have roles in the resolution of inflammation (30). Compared with the American and European populations, the absolute intake of AA in the Chinese population was lower than the Chinese Recommended Nutrient Intakes (RNIS) and North American recommendations for fat intake. (31). Therefore, it might be possible that appropriate AA during pregnancy promotes offspring growth rather than fat accumulation.

Previous studies have shown that an increase in the n-6/n-3 ratio has accelerated the risk for obesity (32). Over the past few decades, the intake of n-6 PUFA is increased while n-3 PUFA is decreased in the modern Western diet, which has pushed the n-6/n-3 ratios from 1:1 to 15:1 (33). Donahue et al. have found that a higher ratio of cord plasma n-6/n-3 PUFA was

associated with a higher risk of obesity in American children (8). However, we did not find the association of maternal n-6/n-3 PUFA ratio or AA/EPA ratio with offspring weight status, consistent with the results of the study conducted in Germany (34). A potential explanation for the discrepant findings was the various levels of n-6/n-3 PUFA ratio in the participants. The ratio of maternal erythrocyte n-6/n-3 PUFAs in our population was 3.61, within the reference interval for pregnant women (25). Studies also suggested that a ratio of n-6/n-3 PUFA lower than 5 could reduce the risk of adverse inflammation (35). The n-6/n-3 PUFA ratio in our study was at an appropriate level and might not cause pathological inflammation in the fetus, which helps us to understand the null association between maternal n-6/n-3 PUFA and offspring weight gain or obesity. Based on the above evidence, maternal n-6 PUFAs, especially AA, during pregnancy may benefit offspring growth and development within 2 years of age in the Chinese population when the ratio of n-6/n-3 PUFAs falls within the appropriate range. However, further studies are still needed to explore whether the association of maternal PUFA ratio with offspring weight status varies by different n-6/n-3 PUFA levels in other populations.

Our study has several limitations. First, although the observational study could not establish an exact causal relationship, our study was a prospective cohort study and we have performed an extensive adjustment for the potential maternal and childhood confounders. Second, due to the difficulty in measuring body composition in young children, we have only measured offspring anthropometric index as the outcome but did not provide information on body composition, which can reflect the fat distribution (36). Nevertheless, the BAZ score of children can still predict obesity in childhood and even in adulthood accurately. Third, the sample size of our study was moderate when compared to previous literature. It was possible that the statistical power for individual fatty acids might be insufficient. Therefore, more future studies are still needed. Finally, the levels of PUFA in pregnant women might change with the prolongation of pregnancy. Our study only measured the maternal PUFA in the second trimester, lacking information about the third trimester. However, the second trimester is a critical period for fetal adipocyte development (37), and the level of PUFA in the third trimester was shown to be similar to those in the second trimester (38). Therefore, it is theoretically appropriate to select the second trimester as the exposure window for our study.

Conclusion

The maternal erythrocyte DPA, AA, and total n-6 PUFA might influence offspring weight status within 2 years old in the Chinese population. No significant associations were found between maternal n-6/n-3 PUFA or AA/EPA ratio and offspring weight status. Further Asian studies are still needed to assess the effects of maternal PUFA on offspring weight status throughout childhood.

Data availability statement

The datasets presented in this article are not readily available because they are from an ongoing cohort. Requests to access the datasets should be directed to LC, caili5@mail.sysu.edu.cn.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the School of Public Health of Sun Yat-sen University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LC conceived the study. SW performed data curation, statistical analysis, and prepared the manuscript draft. FZ and YH provided guidance on the process of fatty acid detection. YC performed the investigation and carried out quality control. LC, XW, and LL revised the initial manuscript. TH, SD, and IS critically reviewed this manuscript. All authors contributed to the manuscript revision and approved the submitted version.

Funding

This work was supported by the Key-Area Research and Development Program of Guangdong Province (2019B030335001) and Huhhot Science & Technology Plan (No. 2021-National Center of Technology innovation for Dairy-4).

Acknowledgments

We thank all the participating families and the research assistants involved in our cohort study.

Conflict of interest

Authors TH, GF, and IS were employed by Inner Mongolia Yili Industrial Group Co., Ltd. Authors TH, SD, and GF were employed by Inner Mongolia Dairy Technology Research Institute Co., Ltd.

The remaining 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.2022.978679/full#supplementary-material

References

1. Pan XF, Wang L, Pan A. Epidemiology and determinants of obesity in China. *Lancet Diabetes Endocrinol.* (2021) 9:373–92.

2. Pietrobelli A, Agosti M, MeNu G. Nutrition in the first 1000 days: ten practices to minimize obesity emerging from published science. *Int J Environ Res Public Health.* (2017) 14:1491. doi: 10.3390/ijerph14121491

3. Barker DJ. The origins of the developmental origins theory. *J Int Med.* (2007) 261:412–7.

4. Collins CT, Gibson RA, McPhee AJ, Makrides M. The role of long chain polyunsaturated fatty acids in perinatal nutrition. *Semin Perinatol.* (2019) 43:151156.

5. Oosting A, Kegler D, Boehm G, Jansen HT, van de Heijning BJ, van der Beek EM. N-3 long-chain polyunsaturated fatty acids prevent excessive fat deposition in adulthood in a mouse model of postnatal nutritional programming. *Pediatr Res.* (2010) 68:494–9. doi: 10.1203/PDR.0b013e3181f74940

6. Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog Lipid Res.* (2006) 45:203–36. doi: 10.1016/j.plipres.2006.01.003

7. Heerwagen MJ, Stewart MS, de la Houssaye BA, Janssen RC, Friedman JE. Transgenic increase in N-3/n-6 fatty acid ratio reduces maternal obesity-associated inflammation and limits adverse developmental programming in mice. *PLoS One.* (2013) 8:e67791. doi: 10.1371/journal.pone.0067791

8. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. *Am J Clin Nutr.* (2011) 93:780–8. doi: 10.3945/ajcn.110.005801

9. Vidakovic AJ, Gishti O, Voortman T, Felix JF, Williams MA, Hofman A, et al. Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the generation R study. *Am J Clin Nutr.* (2016) 103:1017–25. doi: 10.3945/ajcn.115.112847

10. Maslova E, Rifas-Shiman SL, Olsen SF, Gillman MW, Oken E. Prenatal n-3 long-chain fatty acid status and offspring metabolic health in early and mid-childhood: results from project viva. *Nutr Diabetes.* (2018) 8:29. doi: 10.1038/s41387-018-0040-2

11. Vahdaninia M, Mackenzie H, Dean T, Helps S. The effectiveness of omega-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. *Eur J Nutr.* (2019) 58:2597–613. doi: 10.1007/s00394-018-1824-9

12. Mathias RA, Pani V, Chilton FH. Genetic variants in the FADS gene: implications for dietary recommendations for fatty acid intake. *Curr Nutr Rep.* (2014) 3:139–48. doi: 10.1007/s13668-014-0079-1

13. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res.* (2008) 47:348–80.

14. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* (1957) 226:497–509.

15. WHO. WHO child growth standards based on length/height, weight and age. *Acta Paediatr.* (2006) 450:76–85.

16. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care.* (2010) 33:676–82.

17. Schwinger C, Fadnes LT, Van den Broeck J. Using growth velocity to predict child mortality. *Am J Clin Nutr.* (2016) 103:801–7.

18. Drouin G, Rioux V, Legrand P. The n-3 docosapentaenoic acid (DPA): a new player in the n-3 long chain polyunsaturated fatty acid family. *Biochimie.* (2019) 159:36–48. doi: 10.1016/j.biochi.2019.01.022

19. Kim HK, Della-Fera M, Lin J, Baile CA. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. *J Nutr.* (2006) 136:2965–9.

20. Wojcik C, Lohe K, Kuang C, Xiao Y, Jouni Z, Poels E. Modulation of adipocyte differentiation by omega-3 polyunsaturated fatty acids involves the ubiquitin-proteasome system. *J Cell Mol Med.* (2014) 18:590–9. doi: 10.1111/jcmm.12194

21. Simeoni U, Armengaud JB, Siddeek B, Tolsa JF. Perinatal origins of adult disease. *Neonatology.* (2018) 113:393–9.

22. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, et al. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. *J Clin Endocrinol Metab.* (2013) 98:299–307. doi: 10.1210/jc.2012-2482

23. Ren X, Vilhjalmsdottir BL, Rohde JF, Walker KC, Runstedt SE, Lauritzen L, et al. Systematic literature review and meta-analysis of the relationship between polyunsaturated and trans fatty acids during pregnancy and offspring weight development. *Front Nutr.* (2021) 8:625596. doi: 10.3389/fnut.2021.625596

24. Williams MA, Frederick IO, Qiu C, Meryman LJ, King IB, Walsh SW, et al. Maternal erythrocyte omega-3 and omega-6 fatty acids, and plasma lipid concentrations, are associated with habitual dietary fish consumption in early pregnancy. *Clin Biochem.* (2006) 39:1063–70. doi: 10.1016/j.clinbiochem.2006.09.008

25. Araujo P, Kjellevold M, Nerhus I, Dahl L, Aakre I, Moe V, et al. Fatty acid reference intervals in red blood cells among pregnant women in Norway-cross sectional data from the 'little in Norway' cohort. *Nutrients.* (2020) 12:2950. doi: 10.3390/nu12102950

26. Cano-Sancho G, Casas M. Interactions between environmental pollutants and dietary nutrients: current evidence and implications in epidemiological research. *J Epidemiol Commun Health.* (2021) 75:108–13. doi: 10.1136/jech-2020-213789

27. Much D, Brunner S, Vollhardt C, Schmid D, Sedlmeier EM, Brüderl M, et al. Effect of dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal and fetal fatty acid profile and its relation to offspring growth and body composition at 1 year of age. *Eur J Clin Nutr.* (2013) 67:282–8. doi: 10.1038/ejcn.2013.2

28. Djuricic I, Calder PC. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: an update for 2021. *Nutrients.* (2021) 13:2421. doi: 10.3390/nu13072421

29. Innis SM. Essential fatty acid transfer and fetal development. *Placenta.* (2005) 26(Suppl. A):S70–5.

30. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: novel pro-resolving lipid mediators in resolution. *Semin Immunol.* (2015) 27:200–15. doi: 10.1016/j.smim.2015.03.004

31. Barbarich BN, Willows ND, Wang L, Clandinin MT. Polyunsaturated fatty acids and anthropometric indices of children in rural China. *Eur J Clin Nutr.* (2006) 60:1100–7. doi: 10.1038/sj.ejcn.1602424

32. Simopoulos AP. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients.* (2016) 8:128.

33. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother.* (2002) 56:365–79.

34. Brei C, Stecher L, Much D, Karla MT, Amann-Gassner U, Shen J, et al. Reduction of the n-6: n-3 long-chain PUFA ratio during pregnancy and lactation on offspring body composition: follow-up results from a randomized controlled trial up to 5 y of age. *Am J Clin Nutr.* (2016) 103:1472–81. doi: 10.3945/ajcn.115.128520

35. Wei Y, Meng Y, Li N, Wang Q, Chen L. The effects of low-ratio n-6/n-3 PUFA on biomarkers of inflammation: a systematic review and meta-analysis. *Food Funct.* (2021) 12:30–40. doi: 10.1039/d0fo01976c

36. Shah NR, Braverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. *PLoS One.* (2012) 7:e33308. doi: 10.1371/journal.pone.0033308

37. Poissonnet CM, Burdi AR, Bookstein FL. Growth and development of human adipose tissue during early gestation. *Early Hum Dev.* (1983) 8:1–11.

38. Kawabata T, Kagawa Y, Kimura F, Miyazawa T, Saito S, Arima T, et al. Polyunsaturated fatty acid levels in maternal erythrocytes of japanese women during pregnancy and after childbirth. *Nutrients.* (2017) 9:245. doi: 10.3390/nu9030245

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 05 October 2022
DOI 10.3389/fnut.2022.1016943

OPEN ACCESS

EDITED BY
Zheqing Zhang,
Southern Medical University, China

REVIEWED BY
Jun Liu,
Zunyi Medical University, China
Sui Zhu,
Jinan University, China

*CORRESPONDENCE
Ling Lu
selca941@126.com

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

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 11 August 2022
ACCEPTED 07 September 2022
PUBLISHED 05 October 2022

CITATION
Lu L, Li X, Lv L, Xu Y, Wu B and Huang C (2022) Associations between omega-3 fatty acids and insulin resistance and body composition in women with polycystic ovary syndrome. *Front. Nutr.* 9:1016943. doi: 10.3389/fnut.2022.1016943

Associations between omega-3 fatty acids and insulin resistance and body composition in women with polycystic ovary syndrome

Ling Lu[1]*†, Xiaoqin Li[2†], Lin Lv[1], Yao Xu[1], Baohua Wu[1] and Chaolin Huang[1]

[1]Department of Gynecology, First Affiliated Hospital of Chengdu Medical College, Chengdu, China, [2]Department of Oncology, Guangdong Second People's Hospital, Guangzhou, China

Background: Polycystic ovary syndrome (PCOS) is strongly associated with abdominal obesity and insulin resistance and effective approaches to nutrition (e.g., omega-3 fatty acids intake) might improve the cardiometabolic risk profile. This study aimed to examine the associations of dietary and serum omega-3 fatty acids with insulin resistance (IR) and body composition among PCOS patients.

Methods: A total of 185 patients with PCOS were included in our analysis. Dietary information was collected through face-to-face interviews using a 102-item food frequency questionnaire (FFQ). Serum omega-3 fatty acid levels were measured with the gas chromatography method. Body composition was measured by both dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance (BIA) methods. The multivariable linear regression model was applied to analyze the associations of dietary and serum omega-3 fatty acids with the levels of Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and body composition parameters among PCOS patients.

Results: Our results indicated that the dietary long-chain omega-3 polyunsaturated fatty acids (PUFA) intakes were negatively associated with HOMA-IR ($\beta = -0.089$, $P = 0.040$), fat mass ($\beta = -0.022$, $P = 0.047$), and body fat percentage ($\beta = -0.026$, $P = 0.032$). For serum biomarkers, higher total omega-3 PUFAs levels ($\beta = -0.158$, $P = 0.021$) and long-chain omega-3 PUFAs levels ($\beta = -0.187$, $P < 0.001$), particularly eicosapentaenoic acid (EPA) ($\beta = -164$, $P = 0.011$) and docosahexaenoic acid (DHA) ($\beta = -0.158$, $P = 0.001$) were also associated with decreased HOMA-IR. In addition, generally, dietary and serum long-chain omega-3 PUFA levels, DPA, and DHA levels were both positively associated with muscle mass measured by DXA; whereas serum total, long-chain and individual omega-3 PUFA levels (e.g., DPA, EPA, and DHA) were all negatively associated with fat mass and body fat percentage. These findings were further confirmed by the findings for body composition measured by the BIA method.

Conclusion: Higher levels of dietary and serum omega-3 PUFAs, particularly long-chain omega PUFAs (DPA and DHA), might have beneficial effects on metabolic parameters and body composition among PCOS patients.

KEYWORDS

polycystic ovary syndrome, omega-3 polyunsaturated fatty acids, case-control studies, insulin resistance, body composition

Introduction

Characterized by a series of manifestations of hyperandrogenemia, persistent anovulation, and ovarian polycystic changes (1), polycystic ovary syndrome (PCOS) is one of the most common endocrine-metabolic disorders that affects 5 to 10% of women of reproductive age (2). PCOS entails diseases such as type 2 diabetes, hypertension, cardiovascular disease, and endometrial cancer (3, 4), and insulin resistance (IR) and hyperandrogenemia might be closely associated with the pathogenesis of PCOS (5). Excessive insulin level is not only a potential cause of hyperandrogenemia but also one of the high-risk factors leading to metabolic syndromes among those with PCOS (6). PCOS can also be accompanied by obesity because central obesity could exaggerate IR and lead to hyperinsulinemia (7). Seventy percentage of overweight PCOS patients were accompanied by IR, with the majority of overweight PCOS patients also being accompanied by hyperinsulinemia (8), suggesting potential relationships between body fat with PCOS.

The etiology of PCOS is not yet clear, but genetic, environmental and lifestyle factors may contribute to its development (9, 10). Lifestyle intervention can effectively alleviate the pathological manifestations of abnormal reproduction and metabolism in patients with PCOS, and dietary intervention may also exert important effects on the prevention and control of PCOS and its complications (11, 12). Ensuring a certain intake level of dietary polyunsaturated fatty acids (PUFAs) might help improve the clinical manifestations of dyslipidemia, impaired vascular endothelial function, and IR in PCOS patients (11). The omega-3 fatty acids and omega-6 fatty acids are mainly dietary sources of the essential PUFAs (6), but the dietary sources of omega-3 fatty acids are relatively less abundant than omega-6 fatty acids, only found in plant foods (e.g., green leafy vegetables, vegetable oils, and seeds) and deep-sea fish (6). Particularly, the role of long-chain omega-3 PUFAs (eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) in the primary prevention of cardiovascular diseases (13), metabolic syndrome (adiposity, dyslipidemia, insulin resistance, and diabetes, etc.) have been reported before (14).

A growing number of evidence have shown that omega-3 PUFAs have been used to improve lipid and insulin profiles in inflammation and obesity (15–17). Omega-3 fatty acids are involved in the regulatory process of arachidonic acid derivatives in the human body, thereby down-regulating the expression levels of transcription factors related to inflammation in cells (18). An omega-3 fatty acids treatment could effectively enhance systemic insulin sensitivity in mice by up-regulating the function of G protein-coupled receptor 120 (GPR120) (19). A meta-analysis indicated that omega-3 fatty acids supplements have also been found to exert an anti-obesity effect on humans by reducing body fat (weight, waist circumference) (20). The chemical structures and effects of omega-3 PUFAs might vary between dietary and serum sources, but rare studies investigated the effects of dietary and serum omega-3 PUFA levels on IR and body composition in PCOS patients, respectively.

Hence, the purpose of this study is to evaluate whether dietary and serum of different types of omega-3 PUFAs are associated with IR and body composition among patients with PCOS.

Materials and methods

Assessment of dietary omega-3 fatty acid intakes

The assessment of omega-3 fatty acids in diet was conducted using a quantitative food frequency questionnaire (FFQ), which was adapted from the 2002 China National Nutrition and Health Survey capturing the usual intake of nutrients and major foods from Chinese (21). Based on the dietary habits of the Sichuan population, a total of 102 items were included in our FFQ. The main food items included cereals and their products, beans, vegetables, fruits, fungi and algae, nuts, livestock meat, poultry, dairy products, eggs, fish and seafood, and condiments. The Spearman correlation coefficients between the FFQ and 3-d dietary records for different fatty acid intakes were calculated among 24 local participants, and the results ranged from moderate to good ($r = 0.28$–0.59). The participants were interviewed through a face-to-face interview by trained researchers to recall their food consumption over the past year before PCOS diagnosis. The participants were asked to report the frequency (none, daily, weekly, monthly, and yearly) and portion size of consumption for each food item. Dietary intakes of individual omega-3 fatty acids included ALA (18:3

omega-3), EPA (20:5 omega-3), docosapentaenoic acid (DPA, 22:5 omega-3), and DHA (22:6 omega-3) were measured in this study.

Measurement of serum omega-3 fatty acids

According to a previously published approach (22), blood fatty acid concentrations were determined using gas chromatography. Lipid extraction and subsequent gas chromatography analysis were used to assess the amounts of serum individual fatty acid levels. The Bligh et al. (23) method was used to extract and purify the total lipid from serum. An Agilent 6890A gas chromatograph (Agilent, Palo Alto, USA) and a capillary column (SP2380, 0.25 mm × 30 m, 0.25 μm film, Supelco, Bellefonte, PA) were used to separate fatty acid methyl esters. 1, 2-dinonadecanoyl-sn -glycerol-3-phosphocholine (C19:0) was regarded as an internal standard. A total of 28 fatty acids were quantified and the percentage composition of fatty acid methyl esters was calculated and normalized to 100%. The intra-assay coefficients of variation (CV) for the total omega-3 PUFA and individual omega-3 PUFA were 7.8 and 5.2∼9.7%, respectively. All assays were performed at the laboratory of the First Affiliated Hospital of Chengdu Medical College.

Measurement of body composition

In this study, we have body composition measured by both dual-energy X-ray absorptiometry and a body composition analyzer.

Dual-energy X-ray absorptiometry (DXA; GE-lunar Prodigy, Wisconsin, USA) scans were used to measure body composition. Total lean mass, total fat mass (FM), and body fat percentage (FM%) were automatically analyzed using the software V.enCORE10.50.086. According to the manufacturer's instructions, daily quality assurance scans were conducted by scanning the spine phantom. During the test, the subjects should keep quiet and prohibit strenuous or confrontational movements. The coefficient of variation was <2% for LM, FM, and FM% by duplicate scans with 18 volunteer subjects.

A body composition analyzer (InBodyS10) was based on the principle of BIA. The analyzer is an eight-electrode, multi-frequency (1, 50, 100, 250, and 500 kHz), 800 μA alternating current, which measures the human body in sections and measures the resistance and reactance at the same time to obtain the proportions of muscle and fat tissues of the limbs and trunk. Inter-individual coefficients of variations were 2.8% for muscle mass and 2.1% for fat mass among 35 randomly selected duplicates.

Participant recruitment

A cross-sectional study was conducted from July 15, 2016, to July 28, 2019. Potentially eligible patients who suffered PCOS were consecutively recruited from the Department of Gynecology, the First Affiliated Hospital of Chengdu Medical College in China. The detailed information on the study design has been described in a previous publication (24).

Based on the diagnostic criteria of the Rotterdam conference in 2003 (25), those would be diagnosed with PCOS if they meet at least two of the following items: (i) oligo menorrhea (interval between two menstrual periods more than 35 days) and/or chronic anovulation (no vaginal bleeding for at least 6 months); (ii) clinical signs (hirsutism by elevated Ferriman-Gallwey score) and/or biochemical (elevated testosterone or free androgen index) hyperandrogenism; (iii) and/or polycystic ovaries on an ultrasound exam (≥12 follicles measuring 2–9 mm in diameter, or ovarian volume >10 mL in at least one ovary). We excluded participants who (i) were pregnant; (ii) had other genetic or endocrine disorders; (iii) had significant changes in dietary habits in the past 1 year; (iv) had mental or other diseases that might affect the accurate answer of the questionnaire survey; (v) reported a daily energy intake <600 or >3,500 kcal/d (26).

The demographic characteristics and biochemical parameters of PCOS patients were obtained from medical records. We collected the demographic characteristics (age, body mass index (BMI), waist-hip ratio (WHR), age at menarche, education level), living habits (smoking, drinking, fish oil supplementation, and physical activity frequency), the biochemical parameters (systolic blood pressure (SBP), diastolic blood pressure (DPB), fasting glucose, fasting insulin, total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) levels and PCOS phenotypes). The Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index was calculated using the formula: HOMA-IR= fasting glucose (mg/dL) × fasting insulin [μUI/mL (mg/dL)]/405 (27).

A total of 351 eligible patients were identified and 325 (92.6% response rate) patients were successfully interviewed for dietary habits and biological sample collection. All subjects signed informed written consent forms for inclusion before they participated in the study. The protocol was approved by the ethical committee of the First Affiliated Hospital of Chengdu Medical College (2016CYFYHEC025).

Statistical analysis

Data were expressed as means and standard deviations (SDs) for normal disquieted continuous variables, or medians and interquartile ranges (IQRs) for skewed variables. To assess the associations of the omega-3 PUFAs with HOMI-IR and body composition (LM, FM, and FM%), the multivariable

linear regression test was performed and the standardized beta-coefficients and corresponding standard errors (SEs) were computed. All statistical analyses were performed using SPSS22.0. All statistical tests were two-tailed, and statistical significance was considered at P-values <0.05.

Results

Table 1 summarizes the demographic characteristic and biochemical parameters of the study patients. After completing the FFQ, the serum omega-3 fatty acids assessment, and DIX measurements, the sample only included 185 PCOS patients with a mean age of 29.3± 6.45 years. Mean values of BMI, WHR, and age at menarche were 21.5 ± 3.36 kg/m^2, 0.88 ± 0.72, and 13.4 ± 1.21 years, respectively. For biochemical parameters, the mean levels of SBP, DBP, fasting glucose, fasting insulin, and HOMA-IR were 116.8 ± 10.9 mmHg, 79.7 ± 7.6 mmHg, 5.21 ± 0.79 mmol/L, 12.2 ± 5.14 mIU/ml, and 1.44 ± 0.72 respectively. The majority of participants were non-smokers (95.7%) and non-drinkers (95.1%). The percentage of fish oil supplement users was only 11.4%. Most patients reported that exercising <1 time per week (56.2%). For PCOS-related parameters, the mediums and IQRs of FSH and LH were 6.77 (3.16, 8.75) U/L and 10.41 (6.32, 17.05) U/L, respectively. For PCOS phenotypes, 33.0, 29.2, 22.7 and 15.1% of PCOS patients suffered from polycystic ovarian morphology (PCOM) + hyperandrogenism (HA) + ovulatory dysfunction (OD), PCOM + HA, PCOM + OD and HA + OD, respectively. For body composition measured by DXA, the mean levels were 30.23 ± 3.12, 18.62 ± 3.66, and 33.01 ± 3.56 for muscle mass, fat mass, and body fat percentage, respectively.

As shown in Table 2, for dietary omega-3 PUFAs, the average daily dietary intake of total omega-3 PUFAs was 1.05 ± 0.42 g/d, and long-chain omega-3 PUFAs was 44.3 ± 17.1 g/d. Among them, the daily intake of EPA (19.8 ± 9.3 mg/d) was the highest, whereas the level of ALA was the lowest (1.02 ± 0.39). With regard to serum phospholipid omega-3 PUFAs, the total serum- and the long-chain omega-3 PUFA levels were 4.84 ± 1.96 and 4.81 ± 1.93, respectively. Among the categories of omega-3 PUFAs, the level of DHA (3.45 ± 1.63) was the highest, while its serum level in ALA was the lowest (0.07 ± 0.04).

By using the multivariable linear regression with covariates entered, Table 3 shows the associations between omega-3 PUFAs and HOMA-IR level in PCOS patients. As for dietary intake of omega-3 PUFAs, the long-chain omega-3 PUFAs were significantly negatively related to HOMA-IR ($ß$ = –0.089, P = 0.040), whereas total omega-3 PUFAs, as well as each category of long-chain omega-3 PUFAs, was not related with HOMA-IR (all P-values > 0.05). In terms of the serum omega-3 PUFAs, total levels of omega-3 PUFAs ($ß$=-0.158, P = 0.021), long-chain omega-3 PUFAs ($ß$ = –0.187, P < 0.001), DHA ($ß$ = –0.158, P = 0.001) and EPA ($ß$ = –0.164, P = 0.011) were all negatively

TABLE 1 Demographic characteristics and biochemical parameters among PCOS cases.

	Cases (n = 185)
Age, years	29.3 ± 6.45
BMI, kg/m^2	21.5 ± 3.36
WHR	0.88 ± 0.72
Age at menarche, year	13.4 ± 1.21
Education, n (%)	
Middle school or below	50 (27.0)
High school	72 (38.9)
College or above	63 (34.1)
Current smokers, n (%)	
Yes	8 (4.3)
No	177 (95.7)
Current alcohol drinkers, n (%)	
Yes	9 (4.9)
No	176 (95.1)
Users of fish oil supplements, n (%)	
Yes	21 (11.4)
No	164 (88.6)
Physical activity [n (%)]	
<1 time/week	104 (56.2)
1∼3 times/week	57 (30.8)
>3 times/week	24 (13.0)
SBP, mmHg	116.8 ± 10.9
DBP, mmHg	79.7 ± 7.6
Fasting glucose, mmol/L	5.21 ± 0.79
Fasting insulin (mIU/mL)	12.2 ± 5.14
HOMA-IR	1.44 ± 0.72
Total energy intake, kcal/day	1,724.1 ± 609.7
Total testosterone, nmol/L[a]	1.51 (0.50, 2.98)
FSH, U/L [a]	6.77 (3.16, 8.75)
LH, U/L [a]	10.41 (6.32, 17.05)
Insulin resistance, n (%)	
Yes	42 (22.50%)
No	143 (77.50%)
Obesity, n (%)	
Yes	85 (46.20%)
No	100 (53.80%)
Usage of drugs, n (%)[b]	
Metformin	32 (17.30%)
Spironolactone	10 (5.20%)
Rosiglitazone	1 (0.42%)
Statins	36 (19.50%)
Hormones	39 (21.30%)
Phenotypes, n (%)	
PCO+HA+OD	61 (33.0)
PCO+HA	54 (29.2)

(Continued)

TABLE 1 (Continued)

	Cases ($n = 185$)
PCO +OD	42 (22.7)
HA+OD	28 (15.1)
Body composition by DXA	
MM	30.23 ± 3.12
FM	18.62 ± 3.66
Body fat percentage (FM%)	33.01 ± 3.56
Body composition by BIA	
MM	31.76 ± 6.12
FM	18.21 ± 6.91
Body fat percentage (FM%)	32.45 ± 6.21

PCOS, polycystic ovary syndrome; BMI, body mass index; WHR, Waist Hip Ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCO, polycystic ovarian morphology; HA, hyperandrogenism; OD, ovulatory dysfunction; MM, muscle mass; FM, fat mass.
[a]Data was expressed as median and interquartile ranges (IQRs).
[b]Numbers (percentages) of participants who used specific drugs.

TABLE 2 Dietary and serum phospholipid omega-3 polyunsaturated fatty acids among PCOS cases ($n = 185$).

	Mean ± SD	Median (25th, 75th)
Dietary omega-3 PUFA intakes		
Total omega-3 PUFAs (g/day)	1.05 ± 0.42	1.04 (0.53, 1.42)
Long-chain omega-3 PUFAs (mg/day) [a]	44.3 ± 17.1	37.1 (14.6, 65.7)
ALA (g/day)	1.02 ± 0.39	1.00 (0.51, 1.30)
DPA (mg/day)	6.39 ± 3.51	4.30 (1.57, 9.23)
EPA (mg/day)	19.8 ± 9.3	17.5 (5.9, 30.7)
DHA (mg/day)	18.1 ± 10.7	14.2 (6.0, 28.1)
Serum phospholipid omega-3 PUFAs, %		
Total omega-3 PUFAs	4.84 ± 1.96	4.63 (2.15, 6.71)
Long-chain omega-3 PUFAs [a]	4.81 ± 1.93	4.65 (2.12, 6.67)
18:3 omega-3	0.07 ± 0.04	0.06 (0.03, 0.08)
22:5 omega-3 (DPA)	1.01 ± 0.49	0.93 (0.41, 1.62)
20:5 omega-3 (EPA)	0.34 ± 0.15	0.31 (0.10, 0.55)
22:6 omega-3 (DHA)	3.45 ± 1.63	3.34 (1.35, 5.24)

PCOS, polycystic ovary syndrome; SD, standard deviation; PUFA, polyunsaturated fatty acids; ALA, alpha-linolenic acid (18:3 omega-3); DPA, docosapentaenoic acid (22:5 omega-3); EPA, eicosapentaenoic acid (20:5 omega-3); DHA, docosahexaenoic acid (22:6 omega-3).
[a]Long-chain omega-3 PUFAs=DPA+EPA+DHA.

TABLE 3 The multivariable analysis between Omega-3 fatty acids and the level of HOMA-IR among PCOS patients.

	Standardized coefficients β	SE	*P*-value[a]
Dietary omega-3 PUFA intakes			
Total omega-3 PUFAs (g/day)	−0.051	0.036	0.158
Long-chain omega-3 PUFAs (mg/day)[b]	−0.089	0.043	**0.040**
ALA (g/day)	−0.004	0.013	0.759
DPA (mg/day)	−0.035	0.056	0.533
EPA (mg/day)	−0.054	0.032	0.093
DHA (mg/day)	−0.064	0.034	0.061
Serum phospholipid omega-3 PUFAs, %			
Total omega-3 PUFAs	−0.158	0.068	**0.021**
Long-chain omega-3 PUFAs [b]	−0.187	0.051	**<0.001**
18:3 omega-3	−0.045	0.078	0.565
22:5 omega-3 (DPA)	−0.091	0.052	0.082
20:5 omega-3 (EPA)	−0.164	0.064	**0.011**
22:6 omega-3 (DHA)	−0.158	0.047	**0.001**

PCOS, polycystic ovary syndrome; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; β, standardized linear regression coefficients; SE, standard error; PUFA, polyunsaturated fatty acids; ALA, alpha-linolenic acid (18:3 omega-3); DPA, docosapentaenoic acid (22:5 omega-3); EPA, eicosapentaenoic acid (20:5 omega-3); DHA, docosahexaenoic acid (22:6 omega-3).
[a]Statistically significant P-value < 0.05 in bold.
[b]Long-chain omega-3 PUFAs=DPA+EPA+DHA.

associated with the HOMA-IR, while blood levels of ALA ($P = 0.565$) and DPA ($P = 0.082$) were not.

The associations between omega-3 PUFAs and body composition measured by DXA were presented in Table 4. For dietary intakes of omega-3 PUFAs, the total level of long-chain omega-3 PUFAs and DHA was negatively associated with fat mass and body fat percentage but positively associated with muscle mass (all $p < 0.05$). For the serum levels of omega-3 PUFAs, both total long-chain omega-3 PUFAs ($\beta = 0.024$, $P = 0.017$), DPA ($\beta = 0.019$, $P = 0.036$), and DHA ($\beta = 0.022$, $P = 0.029$) were positively correlated with muscle mass. On the contrary, the levels of total omega-3 PUFAs ($\beta = -0.025$, $P = 0.037$), long-chain omega-3 PUFAs ($\beta = -0.043$, $P < 0.001$), DPA ($\beta = -0.025$, $P = 0.024$), EPA ($\beta = -0.031$, $P = 0.005$) and DHA ($\beta = -0.042$, $P < 0.001$) were all significantly negatively correlated with fat mass, with the same associations were found between the serum levels of total omega-3 PUFAs ($\beta = -0.041$), long-chain omega-3 PUFAs ($\beta = -0.061$), DPA ($\beta = -0.031$), EPA ($\beta = -0.045$) and DHA ($\beta = -0.051$) and body fat percentage (all P-values <0.05). The findings for composition measured by DXA were generally confirmed by those measured by BIA (Table 1).

Discussion

This cross-sectional study in Chinese women revealed reverse correlations between dietary or serum phospholipid long-chain omega-3 PUFAs (total- and long-chain- omega-3 PUFAs, EPA, DHA) and HOMA-IR in PCOS patients. Similarly, we found that, generally, dietary and serum phospholipid

TABLE 4 Associations between Omega-3 fatty acids and body composition measured by DXA method among PCOS patients.

	Muscle mass			Fat mass			Body fat percentage		
	Standardized coefficients β	SE	*P* value[a]	Standardized coefficients β	SE	*P* value[a]	Standardized coefficients β	SE	*P* value[a]
Dietary omega-3 PUFA intakes									
Total omega-3 PUFAs (g/day)	0.007	0.005	0.163	−0.012	0.010	0.232	−0.015	0.010	0.135
Long-chain omega-3 PUFAs (mg/day)[b]	0.019	0.006	**0.002**	−0.021	0.010	**0.037**	−0.028	0.012	**0.021**
ALA (g/day)	0.001	0.009	0.912	−0.003	0.006	0.618	−0.004	0.008	0.618
DPA (mg/day)	0.011	0.005	**0.029**	−0.017	0.010	0.091	−0.010	0.012	0.406
EPA (mg/day)	0.010	0.008	0.213	−0.013	0.011	0.239	−0.009	0.012	0.454
DHA (mg/day)	0.021	0.008	**0.009**	−0.020	0.008	**0.013**	−0.023	0.010	**0.023**
Serum phospholipid omega-3 PUFAs, %									
Total omega-3 PUFAs	0.010	0.009	0.268	−0.023	0.012	0.057	−0.041	0.013	**0.002**
Long-chain omega-3 PUFAs[b]	0.024	0.010	**0.017**	−0.043	0.011	**<0.001**	−0.061	0.012	**<0.001**
18:3 omega-3	0.003	0.007	0.669	−0.017	0.013	0.193	−0.016	0.015	0.288
22:5 omega-3 (DPA)	0.019	0.009	**0.036**	−0.025	0.011	**0.024**	−0.031	0.012	**0.011**
20:5 omega-3 (EPA)	0.021	0.011	0.058	−0.031	0.011	**0.005**	−0.045	0.013	**0.001**
22:5 omega-3 (DHA)	0.022	0.010	**0.029**	−0.042	0.010	**<0.001**	−0.051	0.011	**<0.001**

PCOS, polycystic ovary syndrome; SE, standard error; PUFA, polyunsaturated fatty acids; ALA, alpha-linolenic acid (18:3 omega-3); DPA, docosapentaenoic acid (22:5 omega-3); EPA, eicosapentaenoic acid (20:5 omega-3); DHA, docosahexaenoic acid (22:6 omega-3).
[a]Statistically significant P-value < 0.05 in bold.
[b]Long-chain omega-3 PUFAs=DPA+EPA+DHA.

omega-3 PUFAs (total- and long-chain- omega-3 PUFAs, DPA, EPA, and DHA) were negatively associated with fat mass and body fat percentage, but positively correlated with muscle mass.

Prior evidence suggested that IR in ovarian tissue might appear to damage metabolic signaling but intact mitogenic and steroidogenic activity, which could further stimulate the production of ovarian androgens and aggravate hyperandrogenemia (28). Independent of BMI, hyperinsulinemia, which is closely related to personal lifestyle including inadequate nutrition accompanied by a lack of physical exercise, is a major component of PCOS pathogenesis (7). Diet is an effective, acceptable, and safe intervention for relieving IR (29). Regular consumption of fish and/or oral supplementation of omega-3 PUFAs are both key sources of omega-3 PUFAs in diet (30).

Prior studies showed a relationship between some omega-3 PUFAs and PCOS indices, though the effects of omega-3 PUFAs on insulin metabolism remain inconclusive. For instance, a double-blinded randomized placebo-controlled trial (RCT) among 30 pairs of PCOS patients and controls aged 18–40 years old suggested that a 12-week linseed oil omega-3 PUFAs supplementation (rich in DHA + EPA) significantly reduced insulin values and HOMA-IR, but increased insulin sensitivity check index (all *P*-values <0.05) (17). Another RCT suggested that a combination of caloric restriction (1,200–1,500 kcal/day) and long-chain omega-3 PUFAs supplementation (DHA + EPA) significantly decreased the levels of HOMA-IR, insulin, and glucose-dependent insulinotropic polypeptide (GIP) secretion during the oral glucose tolerance test (OGTT) in obese subjects, but these effects were not seen with caloric restriction alone (31).

Yet, prior studies have yielded inconsistent effects of omega-3 PUFAs on body composition. An animal model indicated that treatment with flaxseed oil (rich in EPA and DHA) significantly reduced blood glucose and the amount of liver fat in rats fed with a high-fat diet (32). Of note, the present study did not observe the beneficial effect of ALA, but a prior cross-sectional study (33) involving 554 women aged 65–72 years found significant associations between dietary ALA and lower fat mass ($\beta = -0.081$, $P = 0.034$). This inconsistent result might be partly ascribed to the different portion size estimation or other biases related to subjective consumption assessment via different food record methods (3-day food record vs. 102 items food frequency questionnaire). Furthermore, given that there is no consistent evidence that omega-3 PUFA supplementation could be related to weight loss or reduced body fat mass in adults, (34) further researches are necessary to demonstrate the beneficial effect of dietary omega-3 PUFAs on body composition.

Although the exact etiology remains elusive, the beneficial effects of omega-3 PUFAs on insulin could be partly attributable to its role in insulin signaling and gene expression. Omega-3 PUFAs could protect glucose tolerance and avoid the accumulation of bioactive lipid mediators by up-regulating the

mRNA expression of insulin-stimulated glucose transporter-4 (GLUT4), insulin receptor substrate-1 (IRS1), and glycogen synthase-1 (GYS1) (35). Additionally, by reducing endoplasmic reticulum stress, increasing β- oxidation of mitochondrial fatty acids and mitochondrial uncoupling, as well as limiting lipid deposits and reactive oxygen species generation, omega-3 PUFAs could further improve insulin sensitivity (16). DHA has been found to alleviate IR by regulating the pathway of the silent information regulator 1 (SIRT 1), a member of a protein family that could play a role in glucose homeostasis and IR reduction through lowering mitochondrial dysfunction (36). Additionally, IR and obesity are characteristics of chronic macrophage-mediated inflammation. Through signaling G-protein-coupled receptor 120 (GPR120), an omega-3 fatty acid receptor/sensor, omega-3 PUFAs could block both toll-like receptor (TLR) and TNF-inflammatory signaling pathways, then mediate M1–M2 macrophage polarization *via* down-regulating the expression of inflammatory genes (e.g., IL-6, TNF-a, MCP-1) but up-regulating the expression of anti-inflammatory genes (e.g., IL-10, MGL1, YM-1) in adipose tissue (37). Particularly, DHA has been found to decrease macrophage-derived inflammation and angiogenesis in adipose tissues, then reduce adipocyte size and body fat composition in middle-aged rats fed a high-fat diet (HFD) (36). Considering that most studies have been undertaken using animal and cellular models, whereas limited works have been conducted among humans, more high-quality studies are warranted to further explore the potential impact of omega-3 PUFAs on metabolic function in humans.

Concerning serum omega-3 PUFAs, we found that the HOMA-IR, fat mass and body fat percentages decreased as the circulating levels of total-, and long-chain omega-3 PUFAs (total-, DPA, EPA, and DHA) increased, but did not relate to ALA. However, the present study found a null association of total dietary omega-3 PUFA and its subtypes (EPA and DHA) with muscle mass, in accord with a study by Masoud Isanejad et al. (33). The inconsistent results between dietary and circulating omega-3 PUFAs may be attributable to the difference in concentration in omega-3 PUFA concentrations in the dietary and serum phospholipids (38). Because gastrointestinal digestion and the absorption process of foods could affect the bioavailability and clinical efficacy of dietary omega-3 PUFAs, leading to the underestimation of their beneficial effects (24). Furthermore, not only genetic factors could influence the molecular response to different sources of omega-3 PUFAs, but also epigenetic modifications could obscure the clinical benefits of omega-3 PUFAs (24).

The protective roles of omega-3 PUFAs on muscle cells are under-reported. A placebo-controlled, double-blind RCT among 28 boys with Duchenne muscular dystrophy (DMD) demonstrated that the blood levels of EPA and DHA in erythrocytes in patients who were supplemented with 2.9 g/d of long-chain omega-3 PUFAs for 6 months were both significantly higher than that of the placebo group ($P < 0.05$), and the supplementation could significantly slow the progression of muscle loss, lowered the fat mass and IR in DMD patients (39). An RCT conducted among 124 participants found that 16 weeks of omega-3 PUFA supplementation (containing 1,650 mg/day of DHA and 150 mg/day of EPA) significantly increased the quality of muscle in lower limbs in overweight/obese postmenopausal women (40).

The n-3 PUFAs could also counter pro-atrophic mediators in the muscle cells, such as maintaining the insulin receptor number, IRS-1 tyrosine phosphorylation, phosphatidylinositol (PI) 3′-kinase activity, and GLUT-4 content in rat muscle (41). A vitro study by Vigdis Aas et al. (42) found that glucose transport and oxidation (CO_2) in muscle cells were increased by 2-folds and the glucose transporter-1 (GLUT1) expression was increased by 2.5-folds after preincubation of myotubes with 0.6 mM EPA for 24 h, indicating that exposure to EPA could stimulate the uptake and oxidation of glucose in skeletal muscle cells from young and healthy subjects. The supplementation of omega-3 PUFAs might improve muscle function, including increasing protein synthesis, improving metabolic function, and suppressing atrophy in muscle cells (43).

Limitations

Even though this is the first study to comprehensively show that dietary and serum n-3 PUFAs might exert a positive effect on metabolism indexes and body composition among PCOS patients, several limitations need to be taken into consideration when interpreting our study. Firstly, the nature of cross-sectional epidemic research does not allow to draw of a causal relationship. Secondly, dietary intakes were self-reported, and therefore prone to some degree of misclassification. However, our trained investigators explained the dietary questions with common food pictures to help them estimate their dietary consumption. Thirdly, recall bias could not be ruled out as the patients were asked to recall their dietary patterns and other living habits over the past years. Nevertheless, to minimize the recall bias, face-to-face interviews were conducted with PCOS patients at the time when they were diagnosed. Fourthly, we cannot rule out selection bias because this is a hospital-based study. Finally, the conclusions were drawn from PCOS patients in Chengdu and Sichuan province in China, so our results may not be generalizable to other populations.

Conclusion

In summary, this study found that higher levels of both dietary and serum omega-3 PUFAs, particularly long-chain omega PUFAs (DPA and DHA), might exert positive effects on metabolic parameters and body composition among PCOS patients. These findings may have important practical implications because they suggest that PCOS patients might

gain health benefits from dietary supplementation of omega-3 PUFAs.

Data availability statement

The datasets generated or analyzed during this study are available from the corresponding author on reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by First Affiliated Hospital of Chengdu Medical College. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Formulating the research question(s): LLu. Designing the study: LLu, XL, and LLv. Carrying out the study: XL and LLv. Analyzing the data: YX, BW, and CH. Interpreting the findings: YX and CH. Writing the article: LLu, XL, and BW. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Chengdu Medical Scientific Research project (No. 2021410), General Project of Natural Science Foundation of Xinjiang Uygur Autonomous Region (No. 2018D01C256), and Talent Research Startup Fund of Guangdong Second People's Hospital.

Acknowledgments

The authors acknowledge all the participants and administrators of this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1016943/full#supplementary-material

References

1. Laven JS, Imani B, Eijkemans MJ. Fauser BC. New approach to polycystic ovary syndrome and other forms of anovulatory infertility. *Obstet Gynecol Surv.* (2002) 57:755–67. doi: 10.1097/00006254-200211000-00022

2. Banaszewska B, Pawelczyk L. Spaczynski R. Current and future aspects of several adjunctive treatment strategies in polycystic ovary syndrome. *Reprod Biol.* (2019) 19:309–15. doi: 10.1016/j.repbio.2019.09.006

3. Gao H, Meng J, Xu M, Zhang S, Ghose B, Liu J, et al. Serum Heat Shock Protein 70 Concentration in Relation to Polycystic Ovary Syndrome in a Non-Obese Chinese Population. *PLoS ONE.* (2013) 8:e67727. doi: 10.1371/journal.pone.0067727

4. Galazis N, Afxentiou T, Xenophontos M, Diamanti-Kandarakis E. Atiomo W. Proteomic biomarkers of type 2 diabetes mellitus risk in women with polycystic ovary syndrome. *Eur J Endocrinol.* (2013) 168:R33–43. doi: 10.1530/EJE-12-0718

5. Farrell K. Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. *Fertil Steril.* (2010) 94:1565–74. doi: 10.1016/j.fertnstert.2010.03.081

6. Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-McComb J, et al. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. *J Nutr Biochem.* (2018) 58:1–16. doi: 10.1016/j.jnutbio.2018.02.012

7. Regidor PA, Mueller A, Sailer M, Gonzalez Santos F, Rizo JM, Egea FM.Chronic Inflammation in PCOS: The Potential Benefits of Specialized Pro-Resolving Lipid Mediators (SPMs) in the Improvement of the Resolutive Response. *Int J Mol Sci.* (2020) 22:384. doi: 10.3390/ijms22010384

8. Lee H, Oh JY, Sung YA. Chung H. Is insulin resistance an intrinsic defect in Asian polycystic ovary syndrome? *Yonsei Med J.* (2013) 54:609–14. doi: 10.3349/ymj.2013.54.3.609

9. Merkin SS, Phy JL, Sites CK. Yang D. Environmental determinants of polycystic ovary syndrome. *Fertil Steril.* (2016) 106:16–24. doi: 10.1016/j.fertnstert.2016.05.011

10. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS. Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev.* (2015) 36:487–525. doi: 10.1210/er.2015-1018

11. Faghfoori Z, Fazelian S, Shadnoush M. Goodarzi R. Nutritional management in women with polycystic ovary syndrome: a review study Diabetes. *Metab Syndr Obes.* (2017) 11:S429–32. doi: 10.1016/j.dsx.2017.03.030

12. Nardo LG, Patchava S. Laing I. Polycystic ovary syndrome: pathophysiology, molecular aspects and clinical implications. *Panminerva Med.* (2008) 50:267–78. doi: 10.17179/excli2019-1534

13. Schulze MB, Minihane AM, Saleh RNM. Risérus U. Intake and metabolism of omega-3 and omega-6 polyunsaturated fatty acids: nutritional

implications for cardiometabolic diseases. *Lancet Diabetes Endocrinol.* (2020) 8:915–30. doi: 10.1016/S2213-8587(20)30148-0

14. Poudyal H, Panchal SK, Diwan V. Brown L. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. *Prog Lipid Res.* (2011) 50:372–87. doi: 10.1016/j.plipres.2011.06.003

15. Sadeghi A, Djafarian K, Mohammadi H. Shab-Bidar S. Effect of omega-3 fatty acids supplementation on insulin resistance in women with polycystic ovary syndrome: Meta-analysis of randomized controlled trials. *Diabetes Metab Syndr Obes.* (2017) 11:157–62. doi: 10.1016/j.dsx.2016.06.025

16. Salek M, Clark CCT, Taghizadeh M. Jafarnejad S. N-3 fatty acids as preventive and therapeutic agents in attenuating PCOS complications. *EXCLI J.* (2019) 18:558–75.

17. Mirmasoumi G, Fazilati M, Foroozanfard F, Vahedpoor Z, Mahmoodi S, Taghizadeh M, et al. The effects of flaxseed oil omega-3 fatty acids supplementation on metabolic status of patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Exp Clin Endocrinol Diabetes.* (2018) 126:222–8. doi: 10.1055/s-0043-119751

18. Schweitzer GRB, Rios I, Gonçalves VSS, Magalhães KG. Pizato N. Effect of N-3 long-chain polyunsaturated fatty acid intake on the eicosanoid profile in individuals with obesity and overweight: a systematic review and meta-analysis of clinical trials. *J Nutr Sci.* (2021) 10:e53. doi: 10.1017/jns.2021.46

19. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* (2010) 142:687–98. doi: 10.1016/j.cell.2010.07.041

20. Bender N, Portmann M, Heg Z, Hofmann K, Zwahlen M. Egger M. Fish or n3-PUFA intake and body composition: a systematic review and meta-analysis. *Int J Obes.* (2014) 15:657–65. doi: 10.1111/obr.12189

21. Li LM, Rao KQ, Kong LZ, Yao CH, Xiang HD, Zhai FY, et al. [A description on the Chinese national nutrition and health survey in 2002]. *Zhonghua Liu Xing Bing Xue Za Zhi.* (2005) 26:478–84.

22. Yaemsiri S, Sen S, Tinker LF, Robinson WR, Evans RW, Rosamond W, et al. Serum fatty acids and incidence of ischemic stroke among postmenopausal women. *Stroke.* (2013) 44:2710–7. doi: 10.1161/STROKEAHA.111.000834

23. Bligh EG. Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* (1959) 37:911–7. doi: 10.1139/o59-099

24. Lu L, Li X, Lv L, Xu Y, Wu B, Huang C.Dietary and serum n-3 PUFA and polycystic ovary syndrome: a matched case-control study. *Br J Nutr.* (2021) 124:1–10. doi: 10.1017/S0007114521003007

25. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril.* (2004); 81:19-25. doi: 10.1016/j.fertnstert.2003.10.004

26. Nimptsch K, Zhang X, Cassidy A, Song M, O'Reilly É J, Lin JH, et al. Habitual intake of flavonoid subclasses and risk of colorectal cancer in 2 large prospective cohorts. *Am J Clin Nutr.* (2016) 103:184–91. doi: 10.3945/ajcn.115.117507

27. Jensterle M, Weber M, Pfeifer M, Prezelj J, Pfutzner A. Janez A. Assessment of insulin resistance in young women with polycystic ovary syndrome. *Int J Gynaecol Obstet.* (2008) 102:137–40. doi: 10.1016/j.ijgo.2008.03.017

28. Rojas J, Chávez M, Olivar L, Rojas M, Morillo J, Mejías J, et al. Polycystic ovary syndrome, insulin resistance, and obesity: navigating the pathophysiologic labyrinth. *Int J Reprod Med.* (2014) 2014:719050. doi: 10.1155/2014/719050

29. Shang Y, Zhou H, Hu M, Feng H. Effect of diet on insulin resistance in polycystic ovary syndrome. *J Clin Endocrinol Metab.* (2020) 105:3346–60. doi: 10.1210/clinem/dgaa425

30. Todorčević M, Hodson L. The effect of marine derived n-3 fatty acids on adipose tissue metabolism and function. *J Clin Med.* (2015) 5:3. doi: 10.3390/jcm5010003

31. Razny U, Kiec-Wilk B, Polus A, Goralska J, Malczewska-Malec M, Wnek D, et al. Effect of caloric restriction with or without n-3 polyunsaturated fatty acids on insulin sensitivity in obese subjects: a randomized placebo controlled trial. *BBA Clin.* (2015) 4:7–13. doi: 10.1016/j.bbacli.2015.05.001

32. Dias BV, Gomes SV, Castro M, Carvalho LCF, Breguez GS, de Souza DMS, et al. EPA/DHA and linseed oil have different effects on liver and adipose tissue in rats fed with a high-fat diet. *Prostaglandins Other Lipid Mediat.* (2022) 159:106622. doi: 10.1016/j.prostaglandins.2022.106622

33. Isanejad M, Tajik B, McArdle A, Tuppurainen M, Sirola J, Kröger H, et al. Dietary omega-3 polyunsaturated fatty acid and alpha-linolenic acid are associated with physical capacity measure but not muscle mass in older women 65-72 years. *Eur J Clin Nutr.* (2022) 61:1813–21. doi: 10.1007/s00394-021-02773-z

34. Delpino FM, Figueiredo LM, da. Silva BGC. Effects of omega-3 supplementation on body weight and body fat mass: a systematic review. *Clin Nutr ESPEN.* (2021) 44:122–9. doi: 10.1016/j.clnesp.2021.04.023

35. Lanza IR, Blachnio-Zabielska A, Johnson ML, Schimke JM, Jakaitis DR, Lebrasseur NK, et al. Influence of fish oil on skeletal muscle mitochondrial energetics and lipid metabolites during high-fat diet. *Am J Physiol Endocrinol Metab.* (2013) 304:E1391–1403. doi: 10.1152/ajpendo.00584.2012

36. Luo X, Jia R, Yao Q, Xu Y, Luo Z, Luo X, et al. Docosahexaenoic acid attenuates adipose tissue angiogenesis and insulin resistance in high fat diet-fed middle-aged mice *via* a sirt1-dependent mechanism. *Mol Nutr Food Res.* (2016) 60:871–85. doi: 10.1002/mnfr.201500714

37. Lalia AZ, Lanza IR. Insulin-sensitizing effects of omega-3 fatty acids: lost in translation? *Nutrients*. (2016) 8:329. doi: 10.3390/nu8060329

38. Yang K, Zeng L, Bao T. Ge J. Effectiveness of Omega-3 fatty acid for polycystic ovary syndrome: a systematic review and meta-analysis. *Reprod Biol Endocrinol.* (2018) 16:27. doi: 10.1186/s12958-018-0346-x

39. Rodríguez-Cruz M, Atilano-Miguel S, Barbosa-Cortés L, Bernabé-García M, Almeida-Becerril T, Cárdenas-Conejo A, et al. Evidence of muscle loss delay and improvement of hyperinsulinemia and insulin resistance in Duchenne muscular dystrophy supplemented with omega-3 fatty acids: a randomized study. *Clin Nutr.* (2019) 38:2087–97. doi: 10.1016/j.clnu.2018.10.017

40. Félix-Soriano E, Martínez-Gayo A, Cobo MJ, Pérez-Chávez A, Ibáñez-Santos J, Palacios Samper N, et al. Effects of DHA-rich n-3 fatty acid supplementation and/or resistance training on body composition and cardiometabolic biomarkers in overweight and obese post-menopausal women. *Nutrients*. (2021) 13:2465. doi: 10.3390/nu13072465

41. Taouis M, Dagou C, Ster C, Durand G, Pinault M. Delarue J. N-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle American journal of physiology. *Endocrinol Metab.* (2002) 282:E664–671. doi: 10.1152/ajpendo.00320.2001

42. Aas V, Rokling-Andersen MH, Kase ET, Thoresen GH. Rustan AC. Eicosapentaenoic acid (20:5 n-3) increases fatty acid and glucose uptake in cultured human skeletal muscle cells. *J Lipids Res.* (2006) 47:366–74. doi: 10.1194/jlr.M500300-JLR200

43. Lipina C. Hundal HS. Lipid modulation of skeletal muscle mass and function. *J Cachexia Sarcopenia Muscle.* (2017) 8:190–201. doi: 10.1002/jcsm.12144

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 03 November 2022
DOI 10.3389/fnut.2022.1019058

OPEN ACCESS

EDITED BY
Li Cai,
Sun Yat-sen University, China

REVIEWED BY
Da-ya Yang,
The First Affiliated Hospital of Sun Yat-sen University, China
Ye Taochun,
Guangzhou University of Chinese Medicine, China

*CORRESPONDENCE
Yuli Huang
hyuli821@smu.edu.cn
Yunzhao Hu
huyunzhao4406@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 02 September 2022
ACCEPTED 14 October 2022
PUBLISHED 03 November 2022

CITATION
Liu X, Sun L, Wen W, Qiu M, Luo J, Li W, Hao S, He M, Wu J, Hu Y and Huang Y (2022) Association between the ratio of serum eicosapentaenoic acid to arachidonic acid and risk of coronary artery disease in young Chinese patients.
Front. Nutr. 9:1019058.
doi: 10.3389/fnut.2022.1019058

Association between the ratio of serum eicosapentaenoic acid to arachidonic acid and risk of coronary artery disease in young Chinese patients

Xiong Liu[1†], Lichang Sun[1†], Weixing Wen[1], Min Qiu[1], Jianjing Luo[1,2], Weiwen Li[1], Shali Hao[1], Mingli He[1], Jiandi Wu[3], Yunzhao Hu[1*] and Yuli Huang[1,4*]

[1]Department of Cardiology, Shunde Hospital, Southern Medical University, Foshan, China, [2]Department of Internal Medicine, Zhaoqing Medical College, Zhaoqing, China, [3]Department of Cardiology, Affiliated Foshan Hospital, Southern Medical University, Foshan, China, [4]Guangdong Provincial Key Laboratory of Cardiac Function and Microcirculation Research, Guangzhou, China

Objective: Long-chain (LC) omega-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may play an anti-inflammatory effect and decrease the risk of coronary artery disease (CAD). In contrast, omega-6 PUFA, mainly arachidonic acid (AA), has pro-inflammatory and pro-aggregatory effects, which may increase the risk of CAD. This study evaluated the associations between EPA, DHA, AA, and their ratios (EPA/AA and DHA/AA) with the risk of CAD in young Chinese patients.

Methods: A total of 182 young patients with CAD and 143 age-matched controls were included. Traditional cardiovascular risk factors were recorded. Serum EPA, DHA and AA were measured by ultra-performance liquid chromatography-mass spectrometry.

Results: The level of AA was significantly higher, while the level of EPA was lower in the CAD group than that in the control group. There was no significant difference in DHA level in the two groups. Both the ratios of EPA/AA and DHA/AA were lower in the CAD group than that in the control. Multivariate logistic regression analysis showed that higher serum AA level was associated with the increased risk of CAD, while EPA was a protective factor for CAD. There was no significant association between DHA level and the risk of CAD. Although both higher ratios of EPA/AA [per tertile increment, adjusted odds ratios (ORs) (OR) 0.356, 95% confidence intervals (CI) 0.247–0.513] and DHA/AA (adjusted OR = 0.465, 95%CI = 0.332–0.653) were associated with a lower risk of CAD in young patients. Receiver operating characteristic (ROC) curve analysis showed that compared with AA, the diagnostic value was increased in EPA/AA, but not in DHA/AA.

Conclusion: EPA, but not DHA may play a protective role in CAD, while AA may be associated with the increased risk of CAD in young Chinese patients. The ratio of EPA/AA can increase the predictive value for diagnosing CAD than EPA or AA alone.

KEYWORDS

eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, risk factors, coronary artery disease, young

Introduction

Cardiovascular disease (CVD) had become a major health burden and cause of mortality worldwide (1). Although generally prevalent in old age individuals, the prevalence of CVD, especially coronary artery disease (CAD) in younger people had been increasing during the past decades (2, 3). The clinical characteristics and risk factors of CAD are quite different between young and older patients. Conventional cardiovascular risk factors [e.g., hypertension, diabetes mellitus (DM), and dyslipidemia] are less prevalent in young CAD patients (4). A previous study showed that only 36% of young patients attacked with myocardial infarction had no or only one conventional cardiovascular risk factor, thus would be mistakenly classified as low risk if only based on the traditional risk scoring system (5). Therefore, detection and proper management of novel risk factors for CAD in young patients are of vital importance to decrease the global burden of CVD.

Polyunsaturated fatty acids (PUFA) play an important role in cardiovascular health. Long-chain (LC) omega-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), most commonly found in seafood or fish oil, may play an anti-inflammatory effect and decrease the risk of CAD. In contrast with omega-3 PUFA, omega-6 PUFA, mainly arachidonic acid (AA), has pro-inflammatory and pro-aggregatory effects in the human body, which may increase the risk of CAD. Therefore, the ratio of omega-3/omega-6 PUFA may reflect the balance of anti-inflammatory and pro-inflammatory fatty acids in circulation, which may significantly relate to cardiovascular risk. Observational studies from Japan, a country with a large amount of seafood intake, showed that a low EPA/AA ratio is associated with an increased risk of CVD, including CAD, stroke, and PAD (6–9), especially in young individuals. However, whether such association was true in the Chinese population was still unclear. Furthermore, EPA and DHA may play different physical effects, and whether they are similar in the risk of CVD was also controversal (7, 10–12).

Therefore, we evaluated the relation between EPA, DHA, and AA with the risk of CAD in young Chinese patients. We also explored whether the combination of these free fatty acids, calculated as the ratio of EPA/AA and DHA/AA, can increase the predictive value for diagnosing CAD.

Materials and methods

Participants

This study was conducted complying with the Declaration of Helsinki and was approved by the Ethics Committee of Shunde Hospital, Southern Medical University, China (NO: KY20191103). Written informed consent was obtained from all participants. All the participants were recruited from the participated hospitals. Young patients with CAD were defined as those presenting with initial CAD symptoms at $\leq$ 55 or $\leq$ 65 years of age in men or women, respectively. CAD was diagnosed as $\geq$ 50% stenosis of the lumen diameter in at least one major coronary artery (including the left main coronary artery, left anterior descending branch, left circumflex branch and right coronary artery. Two independent interventional cardiologists evaluated coronary artery stenosis), which was quantified by coronary angiography (CAG). CAG was performed using the Judkins technique through the radial artery, if failed, the femoral artery access was chosen as an alternative. The results of CAG were assessed by two independent interventional cardiologists, and further evaluated by one radiologist from the participated hospitals. Hospitalized age-matched individuals without a diagnosis of CAD in the same period were screened and included as the controls.

Patients were excluded if suspected acute myocarditis or stress cardiomyopathy; uncontrolled infectious disease, autoimmune disease, end-stage renal disease, acute hepatitis, psychiatric disorders, or malignancy; or received fish oil or polyunsaturated fatty acid supplement during the past 3 months.

Laboratory detection and definition of covariates for coronary artery disease

Levels of hemoglobin (HgB), platelets, fasting blood glucose (FBG), glycated hemoglobin (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), level of low-density lipoprotein cholesterol (LDL-C), and serum creatinine

(Scr) were measured in the laboratory departments of the participated hospitals, and extracted from the medical records.

Conventional risk factors for CAD included as covariates in our study were as follows: (1) Family history of premature CAD was defined as a diagnosis of CAD in a first-degree male relative aged < 55 years or female relative aged < 65 years. (2) Hypertension was defined according to the current Chinese guidelines for the management of hypertension (13), including those with a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or who had received antihypertensive treatment. (3) Type 2 DM was defined as an FBG of ≥ 7.0 mmol/L, or HbA1c of ≥ 6.5%, or treatment with hypoglycemic medication (14). (4) Dyslipidemia was defined as TC of ≥ 5.18 mmol/L, LDL-C of ≥ 3.37 mmol/L, HDL-C of < 1.04 mmol/L, and/or TG of ≥ 1.7 mmol/L or a history of anti-dyslipidemia treatment (15). (5) For cigarette smoking, participants were classified as smokers if they smoked regularly during the past year. Those who had never smoked or stopped smoking for more than 1 year were classified as non-smokers.

Detections of eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid

Venous blood samples were collected after at least 8 h of fasting and stored at −80°C for future measurements of free fatty acid. Detections of DHA, EPA, and AA were performed in a commercial company (BiotechPack ANALYTICAL, Beijing, China) using ultra-performance liquid chromatography-mass spectrometry methods according to previous reports (16, 17). In brief, the stored serum samples were thawed at 4°C, and 50 mg of the samples were homogenized with 100 μL distilled water. Added with 0.5 ml of methanol, samples were extracted by vortexing for 30 min. After centrifuging at 14,000 rpm at 4°C for 5 min, the supernatant was added with 5 μL of the inter-standard solution (FA19:0 25 μg/ml, diluted with methanol), then vortexed for 10 s, stored in a 2 ml injection vial for the test.

After that, the UPLC analysis was performed using a Waters ACQUITY I-class LC system (Waters, Milford, MA, USA). Chromatographic separation was conducted on a Waters ACQUITY UPLC BEH C18 column (1.7 μm particle size, 2.1 mm × 100 mm), maintained at 55°C. The mobile phase consisted of solvent A (Acetonitrile: water, 1:10, 1 mmol/L ammonium acetate) and solvent B (Isopropanol: Acetonitrile, 1:1). Gradient elution was carried out at a flow rate of 0.30 ml/min, with the injection volume of 1 μL. Mass spectrometry was performed using a Xevo TQ-S micro spectrometer (Waters, Milford, MA, USA). The following negative ion ESI parameters were used: turbo spray temperature 150°C, spray voltage −2.5 kV, cone voltage 21 V, desolvation temperature 500°C, and desolvation gas flow 1,000 L/h.

The system was controlled by the Masslynx Analysis software (version 4.1, SCIEX, Boston, MA, USA). The Skyline software (MacCoss, WA, USA) was used to analyze the raw data.

Statistical analysis

Categorical variables were presented as numbers and percentages. Continuous variables were presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Baseline characteristics of CAD patients and controls were compared by the Wilcoxon rank-sum test for non-normally distributed continuous variables, two-tailed *t*-test for normally distributed continuous variables, and the chi-square test with Yates' correction for continuity or Fisher's exact test for categorical variables, as appropriate.

Correlations between covariates and AA, DHA and EPA were evaluated by the Pearson product-moment correlation coefficient (*r*). In this analysis, variables with non-Gaussian distribution were logarithmically transformed. To evaluate the association between the serum free fatty acids and the risk of CAD, EPA, DHA, AA, EPA/AA, and DHA/AA were divided into tertiles according to their levels, respectively. Multivariate logistic regression analysis was used to evaluate the associated factors for CAD, with adjustment of age, sex, smoking, hypertension, diabetes mellitus, TC, TG, HDL-C, and LDL-C using an enter method. The adjusted odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated.

A receiver operating characteristic (ROC) curve was performed, and the area under the curve (AUC) was calculated to evaluate the diagnostic value of EPA, DHA, AA, EPA/AA, and DHA/AA for CAD in young patients. Pairwise comparisons of ROC curves were performed according to the method proposed by Hanley and Hajian-Tilaki (18).

All the statistical analysis was performed using SPSS Statistics for Windows (Version 23.0, IBM Corp., Armonk, NY, USA) and MedCalc (Version 20.0, MedCalc Software Ltd., Belgium). All *P*-values were two-sided, and a $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of the patients

In this case-control study, we included 182 young patients with CAD (83.0% male) and 143 age-matched

TABLE 1 Demographic and clinical characteristics of CAD patients and controls.

	All participants (n = 325)	CAD group (n = 182)	Control group (n = 143)	P-value
Age (years)	49.0 (45.0, 53.0)	49.0 (45.0, 53.0)	50.0 (45.0, 54.0)	0.456
Men [n(%)]	214 (65.8%)	151 (83.0%)	63 (44.1%)	< 0.001
CAD family history [n(%)]	20 (6.2%)	9 (4.9%)	11 (7.7%)	0.429
Current smokers [n(%)]	129 (39.7%)	90 (49.5%)	39 (27.3%)	0.0001
HR (beats/minute)	76.0 (67.0, 86.0)	76.0 (66.0, 86.0)	77.0 (70.0, 86.0)	0.241
Hypertension [n(%)]	169 (52.0%)	97 (53.3%)	72 (50.3%)	0.677
SBP (mm Hg)	127.0 (115.0, 144.0)	126.0 (114.0, 140.0)	131.0 (116.25, 147.0)	0.106
DBP (mm Hg)	82.0 (73.75, 91.0)	82.0 (72.0, 91.0)	81.0 (75.0, 91.0)	0.840
DM [n(%)]	90 (27.7%)	59 (32.4%)	31 (21.7%)	0.043
FBG (mmol/L)	5.80 (5.10, 7.44)	5.93 (5.15, 8.12)	5.69 (5.05, 6.79)	0.024
HbA1c (%)	5.8 (5.4, 6.21)	5.8 (5.5, 6.4)	5.7 (5.4, 6.1)	0.030
HgB (g/L)	138.0 (125.0, 147.0)	139.0 (127.0, 147.0)	133 (121.0, 147.0)	0.043
TC (mmol/L)	4.37 (3.71, 5.27)	4.24 (3.60, 5.23)	4.51 (3.96, 5.28)	0.163
LDL-C (mmol/L)	2.58 (2.06, 3.11)	2.58 (2.04, 3.28)	2.56 (2.09, 2.93)	0.312
HDL-C (mmol/L)	1.05 (0.87, 1.24)	0.98 (0.85, 1.19)	1.13 (0.95, 1.30)	0.001
TG (mmol/L)	1.47 (1.07, 2.08)	1.59 (1.17, 2.34)	1.35 (0.98, 1.88)	< 0.001
PLT (×10^9/L)	237.17 ± 66.0	243.18 ± 62.90	229.53 ± 69.23	0.064
ALT (U/L)	24.0 (18.0, 46.72)	28.0 (20.0, 94.0)	22.0 (16.0, 29.0)	< 0.001
AST (U/L)	28.0 (19.0, 46.0)	31.84 (21.0, 56.37)	23.0 (17.0, 34.95)	< 0.001
Scr (μmol/L)	75.5 (65.80, 89.0)	76.31 (68.0, 88.4)	72.58 (61.25, 90.05)	0.068
AA (μmol/L)	6.38 (4.31, 8.89)	7.26 (4.70, 10.23)	5.91 (3.90, 7.23)	< 0.001
EPA (μmol/L)	0.58 (0.38, 0.77)	0.53 (0.37, 0.74)	0.63 (0.44, 0.80)	0.017
DHA (μmol/L)	2.42 (1.58, 3.47)	2.44 (1.36, 3.60)	2.37 (1.78, 3.34)	0.755
EPA/AA	0.09 (0.07, 0.12)	0.08 (0.06, 0.10)	0.11 (0.08, 0.15)	< 0.001
DHA/AA	0.38 (0.28, 0.51)	0.35 (0.25, 0.44)	0.46 (0.33, 0.58)	< 0.001

Data are presented as percentages, mean and SD, median and interquartile range. AA, arachidonic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAD, coronary artery disease; DBP, diastolic blood pressure; DM, diabetes mellitus; DHA, docosahexaenoic acid; DHA/AA, ratio of DHA and AA; EPA, eicosapentaenoic acid; EPA/AA, ratio of EPA and AA; FBG, fasting blood glucose; HDL-C, high density lipoprotein-cholesterol; HgB, hemoglobin; HR, heart rate; LDL-C, low density lipoprotein-cholesterol; PLT, platelets; SBP, systolic blood pressure; Scr, serum creatinine; TC, total cholesterol; TG, triglyceride.

controls (44.1% male) for analysis, according to the predefined inclusion criteria. The baseline demographic and clinical characteristics of all the participants are shown in Table 1. The median age of all the participants was 49.0 (IQR 45.0, 53.0) years old, similar in the CAD group (median 49.0, IQR 45.0, 53.0) and the control group (median 50.0, IQR 45.0, 54.0) (P = 0.456). There was a higher proportion of male sex, current smokers, and DM in patients with CAD compared with the controls. Furthermore, the levels of FBG, HbA1c, TG, ALT, and AST were higher, while the level of HDL-C was lower in the CAD patients than those in the control group (all $P < 0.05$). There were no significant differences in other traditional risk factors of CVD between the two groups.

Ultra-performance liquid chromatography-mass spectrometry based analysis showed that the level of AA was significantly higher (7.26 vs. 5.94 μmol/L, $P < 0.001$), while the level of EPA was lower (0.53 vs. 0.63 μmol/L, P = 0.017) in the CAD group than that in the control group. There was no significant difference in DHA level in the two groups. Both the ratios of EPA/AA (0.08 vs. 0.11, $P < 0.001$) and DHA/AA (0.25 vs. 0.46, $P < 0.001$) were significantly lower in the CAD group than that in the control (Table 1).

Correlation between serum arachidonic acid level and other baseline variables

The correlations between serum AA level and other baseline clinical variables were presented in Table 2. We only found that the level of AA was negatively correlated with female (r = –0.13, P = 0.019), but not with other covariates, including SBP, DBP, FBG, HbA1C, TC, HDL-C, TG or Scr (all $P > 0.05$). However, there was a significant positive correlation between AA level and EPA (r = 0.534, $P < 0.001$), as well as DHA level (r = 0.746, $P < 0.001$).

TABLE 2 Correlation of arachidonic acid and other baseline variables.

Variables	*R*-value	*P*-value
Age	0.015	0.978
Female	−0.130	0.019
Smoking	0.106	0.056
SBP	−0.046	0.410
DBP	0.021	0.708
FBG	0.072	0.197
HbA1C	0.062	0.266
TC	−0.088	0.115
LDL-C	−0.042	0.450
HDL-C	0.017	0.757
TG	−0.093	0.093
Scr	−0.020	0.717
EPA	0.534	< 0.001
DHA	0.746	< 0.001

Age, AA, SBP, DBP, FBG, HbA1C, TC, HDL-C, LDL-C, TG, Scr, EPA, and DHA were skewed variables and logarithmically transformed. DBP, diastolic blood pressure; DHA, docosahexaenoic acid; DEPA, eicosapentaenoic acid; FBG, fasting blood glucose; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; PLT, platelets; SBP, systolic blood pressure; Scr, serum creatinine; TC, total cholesterol; TG, triglyceride.

Association of serum eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, and their ratios with coronary artery disease in young patients

Multivariate logistic regression analysis showed that after adjustment of age, sex, smoking, hypertension, diabetes mellitus, TC, TG, HDL-C, and LDL-C, higher serum AA level (per tertile increment, adjusted OR = 1.593, 95%CI = 1.149–2.209) was associated with the increased risk of CAD in young patients, while higher level EPA was a protective factor for CAD (adjusted OR = 0.675, 95%CI = 0.486–0.937). There was no significant association between DHA level and risk of CAD (adjusted OR = 0.873, 95%CI = 0.636–1.198). Interestingly, both higher levels of EPA/AA (per tertile increment, adjusted OR = 0.356, 95%CI = 0.247–0.513) and DHA/AA (adjusted OR = 0.465, 95%CI = 0.332–0.653) were associated with a lower risk of CAD in young patients (Table 3).

ROC analysis showed the diagnostic value of serum EPA, DHA, AA and their ratios for CAD in young patients (Figure 1). In decreasing order of AUC, EPA/AA (AUC = 0.700, $P < 0.0001$), DHA/AA (AUC = 0.690, $P < 0.0001$), AA (AUC = 0.630, $P < 0.0001$), and EPA (AUC = 0.577, $P = 0.017$), but not DHA (AUC = 0.510, $P = 0.753$) showed a significant effective value for predicting CAD in young patients (Table 4). Furthermore, pairwise comparisons of ROC curves showed that the EPA/AA ratio was more predictive than EPA [difference between areas (DBA) = 0.123, $P = 0.0001$] or AA (DBA = 0.070, $P = 0.031$] alone, while DHA/AA ratio was similar with the AA (DBA = 0.06, $P = 0.137$) level to predict the risk of CAD in young patients (Table 5).

TABLE 3 Association of serum EPA, DHA, AA and their ratios with CAD in young patients by multivariate logistic regression analysis.

Risk factors	Adjusted OR	95% CI	*P*-value
AA (per tertile increment)	1.593	1.149–2.209	0.005
EPA (per tertile increment)	0.675	0.486–0.937	0.019
DHA (per tertile increment)	0.873	0.636–1.198	0.40
EPA/AA (per tertile increment)	0.356	0.247–0.513	< 0.0001
DHA/AA (per tertile increment)	0.465	0.332–0.653	< 0.0001

Data were adjusted by age, sex, smoking, hypertension, diabetes mellitus, TC, TG, HDL-C, and LDL-C. AA, arachidonic acid; CAD, coronary artery disease; DHA, docosahexaenoic acid; DHA/AA, ratio of DHA and AA; EPA, eicosapentaenoic acid; EPA/AA, ratio of EPA and AA; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride.

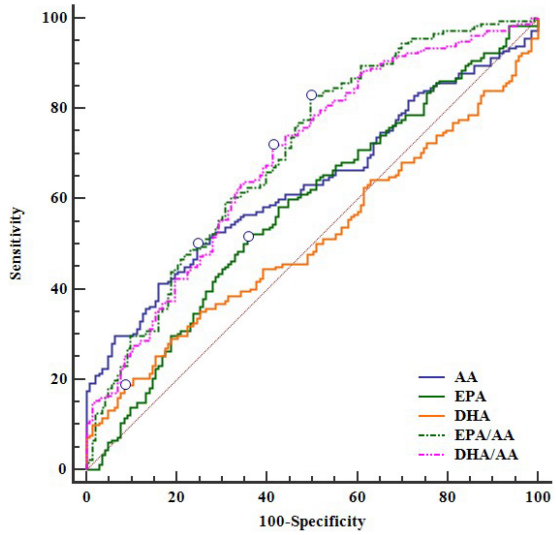


FIGURE 1
The ROC curve of EPA, DHA, AA and their ratios for CAD. AA, arachidonic acid; CAD, coronary artery disease; DHA, docosahexaenoic acid; DHA/AA, ratio of DHA and AA; EPA, eicosapentaenoic acid; EPA/AA, ratio of EPA and AA; ROC, receiver operating characteristic.

TABLE 4 ROC curves of serum EPA, DHA, AA and their ratios for diagnosis of CAD in young patients.

Variable	AUC	95% CI	*P*-value
EPA/AA	0.700	0.647–0.749	< 0.0001
DHA/AA	0.690	0.637–0.740	< 0.0001
AA	0.630	0.575–0.683	< 0.0001
EPA	0.577	0.521–0.631	0.017
DHA	0.510	0.454–0.566	0.753

AA, arachidonic acid; CAD, coronary artery disease; DHA, docosahexaenoic acid; DHA/AA, ratio of DHA and AA; EPA, eicosapentaenoic acid; EPA/AA, ratio of EPA and AA; ROC, receiver operating characteristic.

TABLE 5 Pairwise comparisons of ROC curves.

Comparison	Difference between areas	95% CI	*P*-value
AA vs. EPA	0.053	−0.055–0.161	0.335
AA vs. DHA	0.120	0.005–0.235	0.041
EPA vs. DHA	0.067	0.018–0.115	0.007
EPA/AA vs. AA	0.070	0.006–0.134	0.031
EPA/AA vs. EPA	0.123	0.060–0.187	0.0001
DHA/AA vs. AA	0.060	−0.019–0.139	0.137
DHA/AA vs. DHA	0.180	0.120–0.239	< 0.0001
EPA/AA vs. DHA/AA	0.010	−0.043 to 0.064	0.709
DHA/AA vs. EPA	0.113	0.046 to 0.181	0.001
EPA/AA vs. DHA	0.190	0.105 to 0.275	< 0.0001

AA, arachidonic acid; CAD, coronary artery disease; DHA, docosahexaenoic acid; DHA/AA, ratio of DHA and AA; EPA, eicosapentaenoic acid; EPA/AA, ratio of EPA and AA; ROC, receiver operating characteristic.

Discussion

In the present study, we have several novel findings. First, we found that AA may be associated with the increased risk of CAD in young Chinese patients after adjustment for multiple conventional risk factors. EPA may play a protective role on the risk of CAD, which was not observed for DHA. Second, the ratio of EPA/AA can increase the predictive value for diagnosing CAD than EPA or AA alone. Third, the level of EPA/AA in young Chinese individuals was very low, which may contribute to the high prevalence of premature CAD in China.

EPA can reduce the levels of atherogenic lipoproteins (e.g., triglycerides and remnant lipoprotein cholesterol), oxidative stress, and inflammatory cytokines. Furthermore, EPA can also improve endothelial function, inhibit foam cell formation, plaque progression and rupture, platelet aggregation, and thrombus formation (19). All of these factors contribute to the protective effect of EPA on the risk of CAD. In contrast, AA is a metabolic precursor for many prostaglandins, leukotrienes, thromboxanes, and other oxidized derivatives, and is considered to be a predominantly pro-inflammatory fatty acid (19). Therefore, the ratio of EPA/AA can be regarded as a balance of anti-inflammatory/pro-inflammatory and anti-aggregatory/pro-aggregatory status *in vivo*. Although both EPA and DHA were referred to as LC omega-3 PUFAs and significantly correlate with each other, they may have different biologic effects. The inhibition of cholesterol crystalline domains by EPA but not DHA, may result in a difference in endothelial function (20). Furthermore, EPA is more efficiently incorporated into HDL particles, which can increase its ability to inhibit HDL oxidation than DHA (21). Previous clinical studies also supported these basic research findings. Nishizaki et al. enrolled 1,119 patients from a metropolitan area in Japan, and found that individuals with the lowest tertiles of EPA/AA ($\leq$ 0.33) had a greater probability of acute coronary syndrome (OR 3.14, 95% CI 1.16–8.49), while the similar association was not observed for DHA/AA (7). In contrast, they updated the sample size with 1,733 patients, and reported that a high DHA/AA ratio was significantly associated with a low risk of ACS among men (OR = 0.389; 95%CI 0.211–0.716), however, such association was not significant in women (10). A recently individual-participant data meta-analysis comprising 3,022 incident CHD cases (13,104 controls) showed that although circulating DHA was related to lower CAD risk in the fully adjusted model (OR 0.85; 95% CI, 0.76–0.95, per standard unit increment), there was significant heterogeneity among studies and the effect was modified by study design (22). In the current study, we found that DHA was not associated with the risk of CAD. Although the ratio of DHA/AA showed a reverse association with the odds ratio of CAD, this effect was mainly driven by the level of AA. The AUC for predicting CAD was similar in AA and DHA/AA, which further supports that detection of DHA cannot further provide additional information for determining the risk of CAD based on the level of AA.

Based on our results, we proposed that supplementing with EPA (to increase the ratio of EPA/AA), but not DHA, may play a role in the prevention of CVD. The Japan EPA Lipid Intervention Study (JELIS) showed that treating dyslipidemic patients with highly purified EPA and statins significantly reduced the incidence of major adverse cardiac events, compared with that observed in patients administered statins alone (23). Similarly, the REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) showed that among statin-treated patients with elevated triglycerides and CVD or diabetes, highly purified EPA (icosapent ethyl) can substantially reduce the burden of first, subsequent, and total ischemic events (24). In contrast, those studies used a combination of EPA and DHA did not result in a significant difference in the risk of cardiovascular events (25–27). Although not fully explored, the different effects of EPA and DHA on cardiovascular health may attribute to the inconsistent results. However, limited data had been conducted on the young Chinese population, which is urgently needed.

Another astonishing finding in the current study was that the level of EPA/AA was unexpectedly low in our study. As an Asian country, we previously presumed the ratio of EPA/AA may be similar to that reported in Japan. The Hisayama study from the Japanese general population showed that the median ratio of EPA/AA was 0.41 (interquartile range 0.29–0.59) (28). Another study reported that in White, Japanese, and Japanese American men aged 40–49 years, the ratios of EPA/AA were about 0.09, 0.39, and 0.12, respectively (29). In our study, the median ratios of EPA/DHA were 0.09, 0.08 and 0.11 in all participants, CAD patients and controls, respectively, which was very similar with the Whites, but significantly lower than that in Japanese. The westernization of food customs in China during the past decades may explain this phenomenon. Dietary

habits directly influence the EPA/AA ratio. Meat is abundant in AA. The population-based China Health and Nutrition Survey, followed across 24 years, showed that there was a great transition from the traditional to the Western diet, especially on animal source foods (30). These data call an urgently needed to change the dietary profiles of PUFAs to prevent the epidemic of premature CVD in China.

Several limitations should be noted in the current study. First, the case-control design of the study can only show the association, but not causality among the detected free fatty acids and their ratios with CAD. Further prospective cohort studies should be performed to support the findings in our study. Second, other clinical subtypes of ischemic heart disease like coronary microvascular dysfunction may be neglected and not been excluded in the control group by the traditional criteria of CAD ($\geq$ 50% stenosis of the lumen diameter in at least one major coronary artery). However, we considered that the inclusion of potential CAD with coronary microvascular dysfunction in the case group, may weaken, rather than increase the difference of interested markers between the case and control groups. Therefore, such limitation may not alter the predicting value of AA, EPA and the ratio of EPA/AA for CAD. Third, serum levels of fatty acids were closely related to dietary intake. We only included participants from Guangdong province, Southern China, therefore, the results cannot be extended to people from other regions. Fourth, some medicine may interact with the metabolism of fatty acids, e.g., non-steroidal anti-inflammatory drug, was not recorded in all the participants, which is an underlying confounding factors in the current study. Fifth, dietary intake of the interested fatty acids were not recorded in the current, which make it difficult to access whether the low level of EPA/AA ratio was caused by dietary pattern or genetic factors. However, we think this would not alter the effect of circulating EPA/AA ratio on predicting the risk of CAD in our study.

Conclusion

EPA, but not DHA may play a protective role in CAD, while AA may be associated with the increased risk of CAD in young Chinese patients. The ratio of EPA/AA can increase the predictive value for diagnosing CAD than EPA or AA alone. Further studies are needed to explore the effects of EPA supplements for decreasing the risk of CAD in young Chinese individuals.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Shunde Hospital, Southern Medical University, China. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XL, LS, YunH, and YulH were responsible for the initial plan, study design, conducting the study, and data interpretation. XL, LS, WW, MQ, JL, WL, SH, and MH were responsible for data collection and data extraction. XL and YulH performed the statistical analysis and manuscript drafting. XL, LS, JW, YunH, and YulH were responsible for interpreting the data and critically revised the manuscript. JW, YunH, and YulH were guarantors and had full access to all of the data, including statistical reports and tables, and took full responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Guangdong Basic and Applied Basic Research Fund (Key Project of Guangdong-Foshan Joint Fund) (2019B1515120044), the Science and Technology Innovation Project from Foshan, Guangdong (FS0AA-KJ218-1301-0006), and Outstanding Young Medical Staff in Guangdong Province (600001).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet.* (2017) 390:1211–59.

2. Vedanthan R, Seligman B, Fuster V. Global perspective on acute coronary syndrome: a burden on the young and poor. *Circ Res.* (2014) 114:1959–75. doi: 10.1161/CIRCRESAHA.114.302782

3. Li J, Li X, Wang Q, Hu S, Wang Y, Masoudi FA, et al. ST-segment elevation myocardial infarction in China from 2001 to 2011 (the China PEACE-Retrospective Acute Myocardial Infarction Study): a retrospective analysis of hospital data. *Lancet.* (2015) 385:441–51. doi: 10.1016/S0140-6736(14)60921-1

4. Huang Y, Zhou H, Wu Y, Yang Y, Li W, Lu J, et al. Soluble urokinase plasminogen activator receptor and the risk of coronary artery disease in young chinese patients. *Dis Markers.* (2017) 2017:4719403. doi: 10.1155/2017/4719403

5. Matsis K, Holley A, Al-Sinan A, Matsis P, Larsen PD, Harding SA. Differing clinical characteristics between young and older patients presenting with myocardial infarction. *Heart Lung Circ.* (2017) 26:566–71. doi: 10.1016/j.hlc.2016.09.007

6. Nishibe T, Dardik A, Nishibe M, Kano M, Matsumoto R, Nakano Y, et al. Relationship between serum eicosapentaenoic acid to arachidonic acid ratio and atherosclerotic risk factors in patients with peripheral artery disease. *Vasc Endovascular Surg.* (2021) 55:112–6. doi: 10.1177/1538574420967130

7. Nishizaki Y, Shimada K, Tani S, Ogawa T, Ando J, Takahashi M, et al. Significance of imbalance in the ratio of serum n-3 to n-6 polyunsaturated fatty acids in patients with acute coronary syndrome. *Am J Cardiol.* (2014) 113:441–5. doi: 10.1016/j.amjcard.2013.10.011

8. Serikawa T, Miura S, Okabe M, Hongo H, Tokutome M, Yoshikawa T, et al. Ratio of eicosapentaenoic acid to arachidonic acid is a critical risk factor for acute coronary syndrome in middle-aged older patients as well as younger adult patients. *J Cardiol.* (2014) 63:35–40. doi: 10.1016/j.jjcc.2013.06.016

9. Shojima Y, Ueno Y, Tanaka R, Yamashiro K, Miyamoto N, Hira K, et al. Eicosapentaenoic-to-arachidonic acid ratio predicts mortality and recurrent vascular events in ischemic stroke patients. *J Atheroscler Thromb.* (2020) 27:969–77. doi: 10.5551/jat.52373

10. Nishizaki Y, Shimada K, Tani S, Ogawa T, Ando J, Takahashi M, et al. Association between the docosahexaenoic acid to arachidonic acid ratio and acute coronary syndrome: a multicenter observational study. *BMC Cardiovasc Disord.* (2016) 16:143. doi: 10.1186/s12872-016-0299-y

11. Ueno Y, Tanaka R, Yamashiro K, Miyamoto N, Hira K, Kurita N, et al. Age stratification and impact of eicosapentaenoic acid and docosahexaenoic acid to arachidonic acid ratios in ischemic stroke patients. *J Atheroscler Thromb.* (2018) 25:593–605. doi: 10.5551/jat.40691

12. Iwamatsu K, Abe S, Nishida H, Kageyama M, Nasuno T, Sakuma M, et al. Which has the stronger impact on coronary artery disease, eicosapentaenoic acid or docosahexaenoic acid? *Hypertens Res.* (2016) 39:272–5. doi: 10.1038/hr.2015.143

13. Joint Committee for Guideline Revision. 2018 Chinese guidelines for prevention and treatment of hypertension-a report of the revision committee of chinese guidelines for prevention and treatment of hypertension. *J Geriatr Cardiol.* (2019) 16:182–241.

14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2003) 26(Suppl. 1):S5–20. doi: 10.2337/diacare.26.2007.S5

15. Joint Committee for Developing Chinese guidelines on Prevention and Treatment of Dyslipidemia in Adults. Chinese guidelines on prevention and treatment of dyslipidemia in adults. *Zhonghua Xin Xue Guan Bing Za Zhi.* (2007) 35:390–419.

16. Li H, Xu QY, Xie Y, Luo JJ, Cao HX, Pan Q. Effects of chronic HBV infection on lipid metabolism in non-alcoholic fatty liver disease: a lipidomic analysis. *Ann Hepatol.* (2021) 24:100316. doi: 10.1016/j.aohep.2021.100316

17. Wang D, Sun X, Maziade M, Mao W, Zhang C, Wang J, et al. Characterising phospholipids and free fatty acids in patients with schizophrenia: a case-control study. *World J Biol Psychiatry.* (2021) 22:161–74. doi: 10.1080/15622975.2020.1769188

18. Hanley JA, Hajian-Tilaki KO. Sampling variability of nonparametric estimates of the areas under receiver operating characteristic curves: an update. *Acad Radiol.* (1997) 4:49–58. doi: 10.1016/S1076-6332(97)80161-4

19. Nelson JR, Raskin S. The eicosapentaenoic acid:arachidonic acid ratio and its clinical utility in cardiovascular disease. *Postgrad Med.* (2019) 131:268–77. doi: 10.1080/00325481.2019.1607414

20. Mason RP, Jacob RF, Shrivastava S, Sherratt S, Chattopadhyay A. Eicosapentaenoic acid reduces membrane fluidity, inhibits cholesterol domain formation, and normalizes bilayer width in atherosclerotic-like model membranes. *Biochim Biophys Acta.* (2016) 1858:3131–40. doi: 10.1016/j.bbamem.2016.10.002

21. Sherratt S, Mason RP. Eicosapentaenoic acid inhibits oxidation of high density lipoprotein particles in a manner distinct from docosahexaenoic acid. *Biochem Biophys Res Commun.* (2018) 496:335–8. doi: 10.1016/j.bbrc.2018.01.062

22. Borges MC, Schmidt AF, Jefferis B, Wannamethee SG, Lawlor DA, Kivimaki M, et al. Circulating fatty acids and risk of coronary heart disease and stroke: individual participant data meta-analysis in up to 16 126 participants. *J Am Heart Assoc.* (2020) 9:e13131. doi: 10.1161/JAHA.119.013131

23. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet.* (2007) 369:1090–8. doi: 10.1016/S0140-6736(07)60527-3

24. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Effects of icosapent ethyl on total ischemic events: from REDUCE-IT. *J Am Coll Cardiol.* (2019) 73:2791–802. doi: 10.1016/j.jacc.2019.02.032

25. Nicholls SJ, Lincoff AM, Garcia M, Bash D, Ballantyne CM, Barter PJ, et al. Effect of high-dose omega-3 fatty acids vs corn oil on major adverse cardiovascular events in patients at high cardiovascular risk: the STRENGTH randomized clinical trial. *JAMA.* (2020) 324:2268–80. doi: 10.1001/jama.2020.22258

26. Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, Barton J, et al. Effects of n-3 fatty acid supplements in diabetes mellitus. *N Engl J Med.* (2018) 379:1540–50.

27. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. *N Engl J Med.* (2019) 380:23–32. doi: 10.1056/NEJMoa1811403

28. Ninomiya T, Nagata M, Hata J, Hirakawa Y, Ozawa M, Yoshida D, et al. Association between ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cardiovascular disease: the Hisayama Study. *Atherosclerosis.* (2013) 231:261–7. doi: 10.1016/j.atherosclerosis.2013.09.023

29. Motoyama, KR, Curb JD, Kadowaki T, El-Saed A, Abbott RD, Okamura T, et al. Association of serum n-6 and n-3 polyunsaturated fatty acids with lipids in 3 populations of middle-aged men. *Am J Clin Nutr.* (2009) 90:49–55. doi: 10.3945/ajcn.2008.26761

30. Howard AG, Attard SM, Herring AH, Wang H, Du S, Gordon-Larsen P. Socioeconomic gradients in the westernization of diet in china over 20 years. *SSM Popul Health.* (2021) 16:100943. doi: 10.1016/j.ssmph.2021.100943

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 03 November 2022
DOI 10.3389/fnut.2022.990755

OPEN ACCESS

EDITED BY
Muzi Na,
The Pennsylvania State University (PSU), United States

REVIEWED BY
Matteo Della Porta,
University of Milan, Italy
Manja Zec,
University of Arizona, United States
Bo Yang,
Wenzhou Medical University, China

*CORRESPONDENCE
Caixia Zhang
zhangcx3@mail.sysu.edu.cn

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 10 July 2022
ACCEPTED 10 October 2022
PUBLISHED 03 November 2022

CITATION
Zhang Z, Jiang Y, Li X, Shi D, Ma T, Zhou R and Zhang C (2022) Association of dietary *n* - 3 polyunsaturated fatty acids with breast cancer risk: Serial mediating roles of erythrocyte *n* - 3 polyunsaturated fatty acids. *Front. Nutr.* 9:990755. doi: 10.3389/fnut.2022.990755

Association of dietary *n* - 3 polyunsaturated fatty acids with breast cancer risk: Serial mediating roles of erythrocyte *n* - 3 polyunsaturated fatty acids

Zhuolin Zhang[1,2], Yiling Jiang[1], Xue Li[1], Dandan Shi[1], Ting Ma[1], Ruolin Zhou[1] and Caixia Zhang[1,2]*

[1]Department of Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou, China, [2]Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou, China

Background: Dietary *n* – 3 polyunsaturated fatty acids (PUFAs) were found to be inversely associated with breast cancer risk; however, the underlying pathways between them remain uncertain. We aimed to explore serial mediatory roles of erythrocyte *n* – 3 PUFAs in association between dietary *n* – 3 PUFAs and breast cancer risk.

Materials and methods: Using a case-control study, 850 cases and 861 controls completed structured questionnaires with dietary information. Erythrocyte *n* – 3 PUFAs were measured by gas chromatography. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained using multiple unconditional logistic regression models to examine association between dietary *n* – 3 PUFAs and breast cancer risk. Mediation analyses with bootstrapping were conducted to investigate indirect effects.

Results: Higher intake of dietary ALA, long-chain *n* – 3 PUFAs and total *n* – 3 PUFAs was associated with lower risk of breast cancer. The adjusted $OR_{tertile3v.1}$ (95% CI) was 0.70 (0.55, 0.90) for ALA, 0.76 (0.60, 0.97) for long-chain *n* – 3 PUFAs and 0.74 (0.58, 0.94) for total *n* – 3 PUFAs, respectively. Mediation analysis showed that erythrocyte long-chain *n* – 3 PUFAs served as sequential mediators in the relationship between dietary long-chain or total *n* – 3 PUFAs and breast cancer risk. In particular, erythrocyte long-chain *n* – 3 PUFAs completely mediated the association between dietary long-chain *n* – 3 PUFAs and breast cancer risk [indirect effect (95% CI) = −0.982 (−1.529, −0.508)]. The relationship between dietary total *n* – 3 PUFAs and breast cancer risk was partly mediated by erythrocyte long-chain *n* – 3 PUFAs [indirect effect (95% CI) = −0.107 (−0.216, −0.014)], accounting for 19.31%. However, the serial mediation model in dietary ALA and risk of breast cancer was not statistically significant [indirect effect (95% CI) = −0.042 (−0.144, 0.049)].

Conclusion: This study highlights the complexity and inaccuracy in using a simple analysis of individual dietary *n* – 3 PUFAs to examine their associations

with breast cancer risk without considering the variety of metabolic processes. Interventions aimed at increasing erythrocyte long-chain *n* − 3 PUFAs may represent a promising strategy for breast cancer prevention.

KEYWORDS

***n* − 3 polyunsaturated fatty acids, breast cancer, diet, erythrocyte, mediation analysis**

Introduction

Breast cancer is the most commonly diagnosed cancer and the fifth leading cause of cancer mortality globally in women worldwide (1). It is also the most common cancer among Chinese women, with an estimated 429,105 new cases in 2022 (2). Data on the association of various dietary nutrients, including *n* – 3 polyunsaturated fatty acids (*n* – 3 PUFAs), with the occurrence of breast cancer have long been increasing (3). Women with a higher dietary *n* – 3 PUFA intake are at a decreased risk of breast cancer (4, 5). However, the potential protective mechanisms of dietary *n* – 3 PUFAs on breast cancer risk are not clear.

Emerging evidence has revealed that some types of dietary *n* – 3 PUFAs are positively associated with circulating *n* – 3 PUFAs (6, 7). However, factors such as genetic variations, senility, blood lipids, alcohol consumption might also influence the levels of circulating *n* – 3 PUFAs (8). Moreover, the correlation between dietary α-linolenic acid (ALA) and ALA in circulating blood was relative low (9). Plasma docosapentaenoic acid (DPA) was not found to be significantly related to dietary DPA (10). Although an increased consumption of *n* – 3 PUFAs is generally associated with an increase of *n* – 3 PUFAs composition of blood, this remains unsure for ALA and DPA.

There is a series of conversion process of *n* – 3 PUFAs in the body. ALA is endogenously elongated and desaturated into eicosapentaenoic acid (EPA) by delta-6 and delta-5 desaturase and elongase-5 enzymes (11). Next, EPA is elongated to docosapentaenoic acid (DPA), which is then desaturated and finally produces docosahexaenoic acid (DHA) through the Sprecher pathway. Moreover, the delta-4 desaturation can also produce DHA from EPA in mammalian and human cells (12). It was reported that higher consumption of ALA might increase the rate of ALA oxidation, limit its accumulation in circulation and reduce the rate of conversion to EPA (13). Additionally, studies showed that dietary long-chain *n* – 3 PUFAs could down-regulate the conversion of plasma ALA to long-chain *n* – 3 PUFAs (13, 14). Furthermore, the conversion from ALA to DHA could be increased by the absence of dietary DHA (15). Therefore, the possible mechanism of the relationship between dietary *n* – 3 PUFAs and breast cancer risk might involve the conversion of ALA to EPA, DPA, and/or DHA in the body (13); as well as the influence of dietary *n* – 3 PUFAs on the conversion process of circulating *n* – 3 PUFAs.

Human erythrocytes have a life-span in the circulation of approximately 120 days, which can reflect dietary intake over several months and represent an integrative measure of the interaction concerning dietary, metabolic, and genetic factors (16). Therefore, erythrocyte *n* – 3 PUFAs were usually regarded as biomarkers of dietary *n* – 3 PUFAs. Mediation analysis investigates the mechanisms of the observed relationships between the independent and dependent variables and examines how they relate to the mediating variables (17). In terms of serial mediation, it has been hypothesized that variables affect each other sequentially. Such analysis will enable us to estimate the potential mediating roles of erythrocyte *n* – 3 PUFAs. It will also contribute to examine whether each erythrocyte *n* – 3 PUFA play an independent role in breast cancer risk or play roles after being converted to other types of *n* – 3 PUFAs via metabolic enzymes.

In this context, this study aimed to investigate whether erythrocyte *n* – 3 PUFAs would be sequential mediators in the association between dietary *n* – 3 PUFAs and breast cancer risk. The results of the mediation analysis may help to provide insight into the mechanisms underlying the protective effect of dietary *n* – 3 PUFAs on breast cancer risk and provide research basis for developing targeted cancer prevention strategies. This will cultivate the interest in erythrocyte *n* – 3 PUFAs as comprehensive biomarkers related to breast cancer and provide dietary nutritional modification strategies for breast cancer patients.

Materials and methods

Study population

Detailed description for this hospital-based case-control study have been published elsewhere (18). Briefly, eligible breast cases were recruited from two hospitals between September 2011 and December 2019. The inclusion criteria for cases were as follows: females aged 25–70 years with newly diagnosed and histologically confirmed breast cancer no more than 3 months before the interview, natives in Guangdong or having lived in Guangdong for at least 5 years, and understanding

or speaking Mandarin/ Cantonese. In total, 1,677 of 1,884 eligible cases were successfully interviewed (89.01% response rate). Among them, 869 participants provided blood samples and 853 blood samples were adequate for laboratory analyses. Participants with missing information on other covariates were excluded and leave 850 breast cancer cases were included in the final analysis.

Control subjects were female patients without breast cancers and were simultaneously recruited from the same hospitals as the cases. Patients were excluded if they had a prior history of any cancer or did not understand or speak Mandarin/Cantonese. Totally, 1,762 of 1,965 control subjects were recruited with a response rate of 89.67%. Eight hundred and ninety-two controls provided sufficient blood samples for fatty acid measurement. Finally, 861 control subjects, frequency-matched to cases by 5-year age intervals, were included in the analysis.

Data collection

Information on demographic characteristics, anthropometry factors, lifestyle behaviors, first-degree relatives with cancer, menstrual and reproductive history were collected by trained interviewers through face-to-face interviews. Regular smoking was defined as smoking at least one cigarette/day for more than 6 months. Passive smoking was defined as exposure to the smoke from smokers for at least 15 mins/day in the past 5 years. Regular drinking was defined as drinking alcohol at least once a week over the past year. Menopausal status was defined as permanent absence of menses (at least 12 months since the last menstrual period). The body mass index (BMI) was calculated as the current self-reported body weight (kg) divided by the height squared (m^2). The metabolic equivalent (MET) hours per week was used to estimate physical activity, and the detailed methods of calculating MET have been described previously (19).

Assessment of dietary fatty acid intake

Dietary *n* – 3 PUFA intake during the previous year was collected via a validated 81-item food frequency questionnaire (FFQ) (20). For each food item, a standard portion size was specified and the frequency of consumption was questioned. Energy and fatty acids intakes were calculated based on the China Food Composition Table (21). Our study measured the following *n* – 3 PUFA intake variables: ALA, long-chain *n* – 3 PUFAs (EPA + DPA + DHA) and total *n* – 3 PUFAs (ALA + EPA + DPA + DHA). The major food sources of dietary EPA, DPA, and DHA are fish, seafood and fish oil. Few foods contain only one type of long-chain *n* – 3 PUFA. Therefore, long-chain *n* – 3 PUFAs were not disaggregated.

Measurement of erythrocyte fatty acids

Fasting venous blood samples were obtained on the second day of the participants' admission and before any medication, surgery or examination. Erythrocytes were washed three times with normal saline and separated within 2 h of collection and were stored at −80°C for subsequent analysis. Erythrocyte concentrations of fatty acids were measured by gas chromatography (GC).

The extraction of fatty acids was conducted using the method described by Folch et al. (22) with chloroform/methanol (2:1, v/v). The fatty acids were methylated with a 14% boron-trifluoride ether/methanol (1:3, v/v) solution for 60 mins at 90°C. The fatty acid methyl esters were separated using an Agilent 7890A GC system (Agilent, CA, USA) equipped with a DB-23 capillary column (60 m × 0.25 mm internal diameter × 0.15 μm film; Agilent, CA, USA) and a flame ionization detector. The analytical conditions applied were as follows: (1) nitrogen as carrier gas; (2) split ratio of 5:1 with the injection temperature at 250°C. The oven temperature started at 50°C for 1 min and was programmed from 50 to 175°C at a rate of 25°C/min, and the temperature was continuously increased to 230°C at a rate of 3.5°C/min followed by a 15-min hold period. Comparing the retention time of the samples with commercially available standards to identify individual fatty acids, the amount of each fatty acid was expressed as a percentage of the total erythrocyte membrane fatty acids (relative, %). The intra-assay coefficients of variation (CVs) and inter-assay CVs were <10 and <20% for *n* – 3 PUFAs.

Statistical analysis

We used Mann-Whitney *U*-test for continuous variables and χ^2 test for categorical variables to examine differences in characteristics between breast cancer cases and controls. Dietary *n* – 3 PUFA intake was energy-adjusted using the residual method (23) and then categorized as tertiles (T) on the basis of distribution among the controls. Logistic regression model was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for breast cancer risk in relation to dietary *n* – 3 PUFAs. Pearson correlation coefficients were calculated between dietary *n* – 3 PUFAs and each erythrocyte *n* – 3 PUFA. We tested the mediating effect of erythrocyte *n* – 3 PUFAs using a PROCESS plug-in application for SPSS 25.0 provided by Preacher and Hayes (24), where the mediator should be the continuous variable. The mediation analysis model can be expressed by the following three regression equations (25). First, regressing the independent variable X on the dependent variable Y. The main effect is a precondition for the mediating effect, and the regression effect *c* must be significant (regression Equation 1). Second, regression Equation 2 explains the effect of X on the mediating variable M. When the regression

coefficient *a* is significant, it indicates the existence of an effect of the independent variable on the mediating variable. Third, regressing the X and M on Y simultaneously. It reveals the association between X and Y adjusted for M and the association between M and Y adjusted for X (regression Equation 3).

$$Y = i + cX + e_1 \quad (1)$$

$$M = i + aX + e_2 \quad (2)$$

$$Y = i + c'X + bM + e_3 \quad (3)$$

A bootstrapping method was applied to test the significance of mediating effect, which has high statistical power (26). In the serial mediation model, there was a sequential relationship between the mediating variables (27). Model 6 was chosen with a bootstrapped sample size of 5,000, with dietary *n* – 3 PUFAs as X, breast cancer as Y, and erythrocyte *n* – 3 PUFAs as M at 95% confidence interval. A significant mediation effect was established if zero was not between the lower and upper bound. We calculated the direct, indirect and total effects after adjusting for age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake.

Analyses were performed using SPSS version 25.0 (IBM Corp, Armonk, NY, USA). A two-sided *P* value < 0.05 indicated statistically significant.

Results

Participant characteristics

The characteristics of the cases and the control subjects are shown in Table 1. Compared with controls, cases were more likely to have higher BMI, to drink regularly, and have a family history of cancer in first-degree relatives. Cases were also more likely to have lower levels of education and household and recreational activity. Cases consumed lower ALA, long-chain *n* – 3 PUFAs and total *n* – 3 PUFAs than those of controls. Besides, erythrocyte individual and total *n* – 3 PUFAs proportions in cases were lower than those in controls.

Associations between dietary *n* – 3 polyunsaturated fatty acids and breast cancer risk

As presented in Table 2, after adjusting for potential covariates, higher intake of dietary ALA, long-chain *n* – 3 PUFAs and total *n* – 3 PUFAs was associated with decreased risk of breast cancer. The adjusted $OR_{tertile3\ vs\ tertile1}$ (95% CI) was 0.70 (0.55, 0.90) for ALA, 0.76 (0.60, 0.97) for long-chain *n* – 3 PUFAs and 0.74 (0.58, 0.94) for total *n* – 3 PUFAs, respectively.

Correlation analyses

In control subjects, erythrocyte ALA was not related to any dietary *n* – 3 PUFA intake (see Table 3, all $P > 0.05$). Positive correlations were observed between dietary long-chain *n* – 3 PUFAs and erythrocyte long-chain *n* – 3 PUFAs ($r = 0.029$ for EPA, 0.085 for DPA and 0.147 for DHA, all $P < 0.05$). Meanwhile, higher intake of total *n* – 3 PUFAs was related to higher proportions of erythrocyte EPA and DHA ($r = 0.115$ and 0.078, respectively, all $P < 0.05$), but not DPA ($r = 0.048$, $P > 0.05$). Erythrocyte *n* – 3 PUFAs were significantly and positively correlated with each other (r range from 0.082 to 0.445, all $P < 0.05$).

Mediation analysis on dietary α-linolenic acid and breast cancer risk

The results of the mediation analysis on dietary ALA intake and breast cancer risk are reported in Table 4 and Figure 1. The bootstrap analyses revealed three significant indirect effects between dietary ALA intake and breast cancer risk. The direct effect was significant because zero falls outside the confidence intervals [direct effect (95% CI) = –0.556 (–1.018, –0.093)]. The indirect effect eight and nine indicated that higher intake of dietary ALA was associated with higher levels of erythrocyte EPA, which in turn led to higher levels of DPA or DHA and finally associated with the risk of breast cancer [indirect effect (95% CI) = –0.014 (–0.033, –0.002) and –0.012 (–0.026, –0.003), respectively]. Furthermore, in the multiple chain mediation test of erythrocyte EPA, DPA and DHA, the 95% CI of indirect effect fourteen does not contain 0, and the mediation effect is significant [(indirect effect (95% CI) = –0.001 (–0.004, –0.0002)] However, the total indirect effect was not significant from dietary ALA intake to breast cancer risk through erythrocyte *n* – 3 PUFAs [total indirect effect (95% CI) = –0.042 (–0.144, 0.049)].

Mediation analysis on dietary long-chain *n* – 3 polyunsaturated fatty acids and breast cancer risk

Mediating analysis revealed the mediation effect on relationship between consumption of dietary long-chain *n* – 3 PUFAs and breast cancer risk (see Table 5 and Figure 2). The direct effect was not significant [direct effect (95% CI) = 0.282 (–1.261, 1.825)], whereas the total indirect effects was significant [total indirect effect (95% CI) = –0.982 (–1.529, –0.508)]. Erythrocyte EPA or DHA had significant indirect effect on the correlation between dietary long-chain *n* – 3 PUFAs and breast cancer risk [indirect effect (95% CI) = –0.409 (–0.853, –0.018) and –0.167 (–0.361, –0.025), respectively]. The chain mediation of erythrocyte EPA and

10.3389/fnut.2022.990755

TABLE 1 General characteristics of the study subjects[a].

Variables	Case (n = 850)	Control (n = 861)	P-value
Age (years) mean ± SD	48.33 ± 9.59	48.33 ± 9.53	0.991
BMI (kg/m^2) mean ± SD	23.32 ± 3.70	22.68 ± 3.36	<0.001
Household and recreational activities, MET-h/week (mean ± SD)	36.47 ± 23.73	40.50 ± 24.91	0.001
Occupation [n (%)]			0.647
Administrator/other white-collar workers	176 (20.71)	190 (22.07)	
Blue-collar worker	233 (27.41)	243 (28.22)	
Farmer/other	441 (51.88)	428 (49.71)	
Education [n (%)]			0.001
Primary school or below	209 (24.59)	231 (26.83)	
Secondary school	257 (30.24)	198 (22.99)	
High school	198 (23.29)	190 (22.07)	
College or above	186 (21.88)	242 (28.11)	
Income, Yuan/month [n (%)]			0.192
≤2,000	50 (5.88)	47 (5.46)	
2,001–5,000	257 (30.24)	223 (25.90)	
5,001–8,000	307 (36.12)	325 (37.75)	
≥8,001	236 (27.76)	266 (30.89)	
Regular smoker [n (%)]	12 (1.41)	10 (1.16)	0.646
Regular drinker [n (%)]	76 (8.94)	45 (5.23)	0.003
Passive smoker [n (%)]	319 (37.53)	327 (37.98)	0.848
Menopausal status [n (%)]			0.998
Premenopausal	537 (63.18)	544 (63.18)	
Postmenopausal	313 (36.82)	317 (36.82)	
First-degree relatives with cancer [n (%)]	126 (14.82)	81 (9.41)	0.001
Energy intake (kcal/day) mean ± SD	1511.09 ± 405.93	1544.99 ± 399.33	0.081
Dietary fatty acid intake (mean ± SD)[b]			
ALA (g/day)	0.75 ± 0.22	0.77 ± 0.22	0.007
Long-chain n – 3 PUFAs (mg/day)	57.04 ± 6.65	59.51 ± 6.52	0.024
Total n – 3 PUFAs (g/day)	0.81 ± 0.24	0.83 ± 0.23	0.005
Erythrocyte PUFAs, % of total fatty acids, mean ± SD			
ALA	0.41 ± 0.17	0.45 ± 0.18	<0.001
EPA	0.84 ± 0.42	0.94 ± 0.52	<0.001
DPA	1.09 ± 0.54	1.21 ± 0.66	<0.001
DHA	3.20 ± 0.80	3.40 ± 0.86	<0.001
Total n – 3 PUFAs	5.72 ± 1.52	6.35 ± 1.75	<0.001

ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, Eicosapentaenoic acid; PUFA polyunsaturated fatty acid; SD standard deviation.
[a]Mann-Whitney U-test was used for the comparison of continuous variables between cases and controls. Chi-square test was used to test the differences of categorical variables.
[b]Dietary intakes were adjusted for total energy using the residual method.

DPA or DHA between dietary long-chain n – 3 PUFAs and breast cancer was significant [indirect effect (95% CI) = –0.167 (–0.332, –0.033) and –0.139 (–0.237, –0.063), respectively]. The serial mediating effect of erythrocyte EPA, DPA, and DHA was significant [indirect effect (95% CI) = –0.018 (–0.041, –0.005)]. Combined with Figure 2, erythrocyte EPA or DHA alone had a significant mediating effect. However, erythrocyte DPA can play a chain mediating role and were inversely associated with breast cancer risk only when it combined with EPA and DHA.

Mediation analysis on dietary total n – 3 polyunsaturated fatty acids and breast cancer risk

Table 6 and Figure 3 outlined the mediating effect of mediators in association between dietary total n – 3 PUFAs and breast cancer risk. Significant direct effect and total indirect effect were found [direct effect (95% CI) = –0.447 (–0.873, –0.022); total indirect effect (95% CI) = –0.107 (–0.216, –0.014)]. The total mediating effect of dietary total n – 3 PUFAs on

10.3389/fnut.2022.990755

TABLE 2 Associations between dietary *n* – 3 PUFAs and breast cancer risk[a].

	Cases/Controls	Crude-OR (95% CI)	*P*-trend	Adjusted-OR (95% CI)	*P*-trend
Dietary ALA			0.005		0.005
T1	328/287	1.00 (Ref.)		1.00 (Ref.)	
T2	287/287	0.88 (0.70–1.10)		0.89 (0.70–1.12)	
T3	235/287	0.72 (0.57–0.91)		0.70 (0.55–0.90)	
Dietary long-chain *n* – 3 PUFAs			0.010		0.025
T1	340/287	1.00 (Ref.)		1.00 (Ref.)	
T2	255/287	0.75 (0.59–0.94)		0.77 (0.60–0.97)	
T3	255/287	0.74 (0.59–0.94)		0.76 (0.60–0.97)	
Dietary total *n* – 3 PUFAs			0.014		0.015
T1	319/287	1.00 (Ref.)		1.00 (Ref.)	
T2	294/287	0.92 (0.73–1.16)		0.95 (0.75–1.20)	
T3	237/287	0.74 (0.59–0.94)		0.74 (0.58–0.94)	

ALA, α-linolenic acid; CI, confidence interval; OR, odds ratio; PUFA, polyunsaturated fatty acid; T, tertile.
[a]Adjusted for age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake.

TABLE 3 Correlations between dietary and erythrocyte *n* – 3 PUFAs in control subjects.

	(1) Dietary ALA	(2) Dietary long-chain *n* – 3 PUFAs	(3) Dietary total *n* – 3 PUFAs	(4) Erythrocyte ALA	(5) Erythrocyte EPA	(6) Erythrocyte DPA	(7) Erythrocyte DHA
(1) Dietary ALA	1.000						
(2) Dietary long-chain *n* – 3 PUFAs	–	1.000					
(3) Dietary total *n* – 3 PUFAs	–	–	1.000				
(4) Erythrocyte ALA	−0.030	0.031	−0.019	1.000			
(5) Erythrocyte EPA	0.056	0.029**	0.115**	0.142**	1.000		
(6) Erythrocyte DPA	0.019	0.085*	0.048	0.154**	0.445**	1.000	
(7) Erythrocyte DHA	0.047	0.147**	0.078*	0.082*	0.171**	0.120**	1.000

ALA, a-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.
**P <0.01, *P <0.05.

risk of breast cancer was 19.31%. Erythrocyte EPA and DPA partially mediated the effect of dietary total *n* – 3 PUFAs and breast cancer risk [indirect effect (95% CI) = –0.025 (–0.053, –0.006)]. Erythrocyte EPA and DPA accounted for 4.51% on the correlation between dietary total *n* – 3 PUFAs and breast cancer risk. Similarly, erythrocyte EPA and DHA partially mediated the effect [indirect effect (95% CI) = –0.021 (–0.039, –0.008)], accounting for a mediation ratio of 3.79%. The association between dietary total *n* – 3 PUFA intake and breast cancer risk was partly mediated by erythrocyte EPA, DPA, and DHA sequentially [indirect effect (95% CI) = –0.003 (–0.006, –0.001)], accounting for a mediation ratio of 0.54%.

Discussion

The current study examined the association between dietary intake of *n* – 3 PUFAs and breast cancer risk, and further explored the serial mediating role of erythrocyte *n* – 3 PUFAs within the linkage of dietary *n* – 3 PUFAs and breast cancer risk. The inverse association of dietary long-chain or total *n* – 3 PUFAs with breast cancer risk could be accounted for the potential beneficial actions of dietary long-chain or total *n* – 3 PUFAs on increasing erythrocyte long chain *n* – 3 PUFAs. However, erythrocyte *n* – 3 PUFAs were not mediators in the association between dietary ALA and breast cancer risk.

The present study showed that the total indirect effect of erythrocyte *n* – 3 PUFAs was not significant in the association between dietary ALA intake and breast cancer risk. There are some possible explanations. First, we failed to find the indirect effect of erythrocyte ALA on the relationship between dietary ALA and breast cancer. ALA, an essential fatty acid, cannot be produced endogenously and is closely tied to dietary exposure. However, consistent with previous studies (28, 29), dietary ALA was not found to be associated with erythrocyte ALA in the present study. ALA was oxidized by families of enzymes, including cyclooxygenases, lipoxygenases and cytochrome P450 enzymes (30). ALA converted to oxylipins at a higher rate and in a higher amount, which might be the reason why ALA did not accumulate in tissue as much as long-chain *n* – 3

TABLE 4 Direct and indirect effect of the chain mediation model with mediator of erythrocyte *n* − 3 PUFAs in association between dietary ALA intake and breast cancer risk[a].

	Effect	95% CI	SE
Direct effect			
Dietary ALA → breast cancer	−0.556	-1.018, -0.093	0.236
Indirect effect			
(1) Dietary ALA → RALA → breast cancer	0.014	−0.024, 0.057	0.020
(2) Dietary ALA → REPA → breast cancer	−0.029	−0.082, 0.003	0.022
(3) Dietary ALA → RDPA → breast cancer	0.008	−0.031, 0.047	0.019
(4) Dietary ALA → RDHA → breast cancer	−0.013	−0.062, 0.030	0.023
(5) Dietary ALA → RALA → REPA → breast cancer	0.002	−0.004, 0.011	0.004
(6) Dietary ALA → RALA → RDPA → breast cancer	0.001	−0.002, 0.005	0.002
(7) Dietary ALA → RALA → RDHA → breast cancer	0.0002	−0.001, 0.002	0.001
(8) Dietary ALA → REPA → RDPA → breast cancer	−0.014	−0.033, −0.002	0.008
(9) Dietary ALA → REPA → RDHA → breast cancer	−0.012	−0.026, −0.003	0.006
(10) Dietary ALA → RDPA → RDHA → breast cancer	0.001	−0.002, 0.007	0.002
(11) Dietary ALA → RALA → REPA → RDPA → breast cancer	0.001	−0.002, 0.005	0.002
(12) Dietary ALA → RALA → REPA → RDHA → breast cancer	0.001	−0.002, 0.004	0.001
(13) Dietary ALA → RALA → RDPA → RDHA → breast cancer	0.0001	−0.0002, 0.0005	0.0002
(14) Dietary ALA → REPA → RDPA → RDHA → breast cancer	−0.001	−0.004, −0.0002	0.001
(15) Dietary ALA → RALA → REPA → RDPA → RDHA → breast cancer	0.0001	−0.0002, 0.0005	0.0002
Total indirect effect			
Dietary ALA → breast cancer	−0.042	-0.144, 0.049	0.049

RALA, erythrocyte α-linolenic acid; CI, confidence interval; RDHA, erythrocyte docosahexaenoic acid; RDPA, erythrocyte docosapentaenoic acid; REPA, erythrocyte eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; SE, standard error.

[a]Adjusted for age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake.

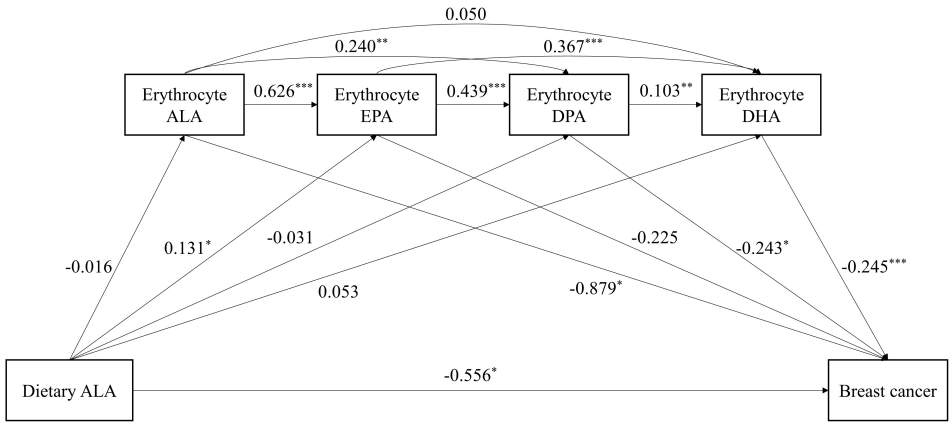


FIGURE 1
The chain mediating effect of erythrocyte *n* − 3 PUFAs on the relationship between dietary ALA intake and breast cancer risk. ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid. Standardized estimating of 5,000 bootstrap sample. Age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake were controlled. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

PUFAs (31). Moreover, a competition between ALA and linoleic acid (LA) may interfere with the correlation between dietary and circulating ALA. Only high supplementation of dietary ALA was reported to result in modest increases in plasma ALA concentrations, because the LA concurrently intake could reduce ALA accumulation (32). Second, we observed a direct effect of dietary ALA on breast cancer risk, but not mediated by erythrocyte ALA. ALA originated from a variety of foods, such as vegetable oils, green leafy vegetables, and common seeds and nuts (5). Consumption of other nutrients from ALA-rich foods might be important confounding factors underlying the inverse relationship between dietary ALA and breast cancer

TABLE 5 Direct and indirect effect of the chain mediation model with mediator of erythrocyte *n* – 3 PUFAs in association between dietary long-chain *n* – 3 PUFA intake and breast cancer risk[a].

	Effect	95% CI	SE
Direct effect			
Dietary long-chain *n* – 3 PUFAs → breast cancer	0.282	−1.261, 1.825	0.787
Indirect effect			
(1) Dietary long-chain *n* – 3 PUFAs → RALA → breast cancer	−0.060	−0.237, 0.070	0.075
(2) Dietary long-chain *n* – 3 PUFAs → REPA → breast cancer	−0.409	−0.853, −0.018	0.212
(3) Dietary long-chain *n* – 3 PUFAs → RDPA → breast cancer	0.002	−0.126, 0.143	0.064
(4) Dietary long-chain *n* – 3 PUFAs → RDHA → breast cancer	−0.167	−0.361, −0.025	0.086
(5) Dietary long-chain *n* – 3 PUFAs → RALA → REPA → breast cancer	−0.011	−0.049, 0.011	0.015
(6) Dietary long-chain *n* – 3 PUFAs → RALA → RDPA → breast cancer	−0.004	−0.018, 0.005	0.006
(7) Dietary long-chain *n* – 3 PUFAs → RALA → RDHA → breast cancer	−0.001	−0.009, 0.005	0.003
(8) Dietary long-chain *n* – 3 PUFAs → REPA → RDPA → breast cancer	−0.167	−0.332, −0.033	0.076
(9) Dietary long-chain *n* – 3 PUFAs → REPA → RDHA → breast cancer	−0.139	−0.237, −0.063	0.044
(10) Dietary long-chain *n* – 3 PUFAs → RDPA → RDHA → breast cancer	−0.0002	−0.015, 0.015	0.007
(11) Dietary long-chain *n* – 3 PUFAs → RALA → REPA → RDPA → breast cancer	−0.004	−0.018, 0.005	0.006
(12) Dietary long-chain *n* – 3 PUFAs → RALA → REPA → RDHA → breast cancer	−0.004	−0.014, 0.004	0.005
(13) Dietary long-chain *n* – 3 PUFAs → RALA → RDPA → RDHA → breast cancer	−0.0004	−0.002, 0.001	0.001
(14) Dietary long-chain *n* – 3 PUFAs → REPA → RDPA → RDHA → breast cancer	−0.018	−0.041, −0.005	0.009
(15) Dietary long-chain *n* – 3 PUFAs → RALA → REPA → RDPA → RDHA → breast cancer	−0.001	−0.002, 0.001	0.001
Total indirect effect			
Dietary long-chain *n* – 3 PUFAs → breast cancer	−0.982	−1.529, −0.508	0.261

RALA, erythrocyte α-linolenic acid; CI, confidence interval; RDHA, erythrocyte docosahexaenoic acid; RDPA, erythrocyte docosapentaenoic acid; REPA, erythrocyte eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; SE, standard error.

[a]Adjusted for age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake.

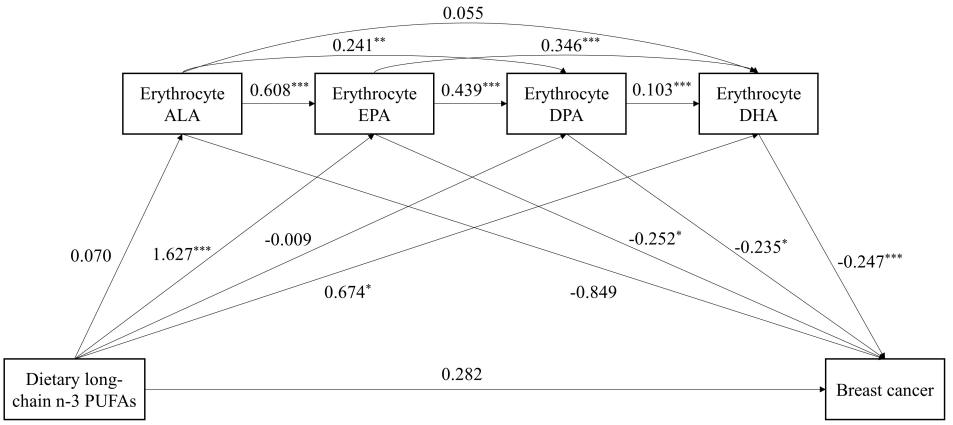


FIGURE 2
The chain mediating effect of erythrocyte *n* – 3 PUFAs on the relationship between dietary long-chain *n* – 3 PUFA intake and breast cancer risk. ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid. Standardized estimating of 5,000 bootstrap sample. Age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake were controlled. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

risk. Third, we observed that the association between dietary ALA and breast cancer risk could be partially explained by erythrocyte long-chain *n* – 3 PUFAs. This might be that long-chain *n* – 3 PUFAs can also be metabolized from ALA in *vivo* by successive desaturation and elongation reactions (33), although the conversion efficiency was poor. The conversion rate of ALA to EPA is 21% in women with a higher activity of delta-6 desaturase expression caused by sex hormones (34), while the conversions rate of ALA to DPA or DHA appear to be lower (6 and 9%, respectively) (35, 36). All of these might explain why the total indirect effect of erythrocyte *n* – 3 PUFAs on breast cancer risk was not significant.

TABLE 6 Direct and indirect effect of the chain mediation model with mediator of erythrocyte *n* – 3 PUFAs in association between dietary total *n* – 3 PUFA intake and breast cancer risk[a].

	Effect	95% CI	SE	Proportion mediated (%)
Direct effect				
Dietary total *n* – 3 PUFAs → breast cancer	−0.447	−0.873, −0.022	0.217	
Indirect effect				
(1) Dietary total *n* – 3 PUFAs → RALA → breast cancer	0.007	−0.032, 0.044	0.018	N/A
(2) Dietary total *n* – 3 PUFAs → REPA → breast cancer	−0.051	−0.122, 0.007	0.032	N/A
(3) Dietary total *n* – 3 PUFAs → RDPA → breast cancer	0.006	−0.029, 0.043	0.018	N/A
(4) Dietary total *n* – 3 PUFAs → RDHA → breast cancer	−0.023	−0.072, 0.014	0.021	N/A
(5) Dietary total *n* – 3 PUFAs → RALA → REPA → breast cancer	0.001	−0.005, 0.008	0.003	N/A
(6) Dietary total *n* – 3 PUFAs → RALA → RDPA → breast cancer	0.0004	−0.002, 0.004	0.001	N/A
(7) Dietary total *n* – 3 PUFAs → RALA → RDHA → breast cancer	0.0001	−0.001, 0.002	0.001	N/A
(8) Dietary total *n* – 3 PUFAs → REPA → RDPA → breast cancer	−0.025	−0.053, −0.006	0.012	4.51
(9) Dietary total *n* – 3 PUFAs → REPA → RDHA → breast cancer	−0.021	−0.039, −0.008	0.008	3.79
(10) Dietary total *n* – 3 PUFAs → RDPA → RDHA → breast cancer	0.001	−0.002, 0.006	0.002	N/A
(11) Dietary total *n* – 3 PUFAs → RALA → REPA → RDPA → breast cancer	0.001	−0.002, 0.004	0.001	N/A
(12) Dietary total *n* – 3 PUFAs → RALA → REPA → RDHA → breast cancer	0.0004	−0.002, 0.003	0.001	N/A
(13) Dietary total *n* – 3 PUFAs → RALA → RDPA → RDHA → breast cancer	0.0000	−0.0002, 0.0004	0.0001	N/A
(14) Dietary total *n* – 3 PUFAs → REPA → RDPA → RDHA → breast cancer	−0.003	−0.006, −0.001	0.001	0.54
(15) Dietary total *n* – 3 PUFAs → RALA → REPA → RDPA → RDHA → breast cancer	0.0001	−0.0003, 0.0004	0.0002	N/A
Total indirect effect				
Dietary total *n* – 3 PUFAs → breast cancer	−0.107	−0.216, −0.014	0.051	19.31

RALA, erythrocyte α-linolenic acid; CI, confidence interval; RDHA, erythrocyte docosahexaenoic acid; RDPA, erythrocyte docosapentaenoic acid; REPA, erythrocyte eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; SE, standard error.

[a]Adjusted for age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake.

[b]N/A is due to no mediating effect.

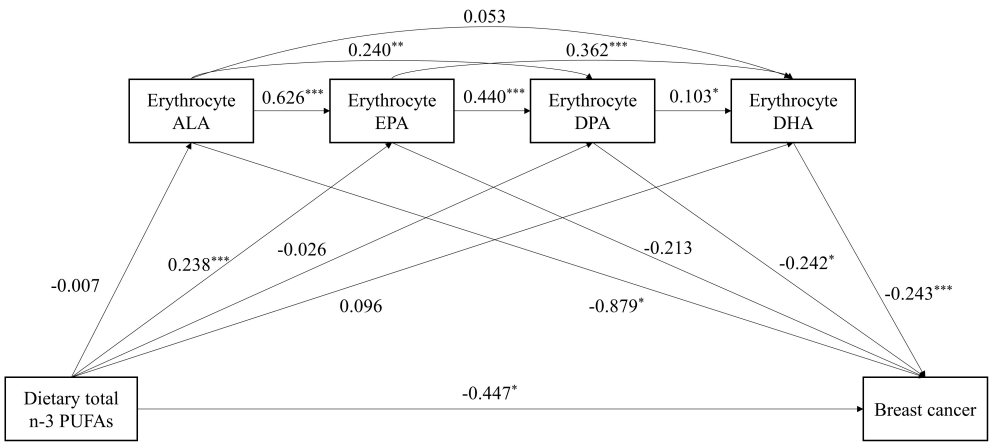


FIGURE 3
The chain mediating effect of erythrocyte *n* – 3 PUFAs on the relationship between dietary total *n* – 3 PUFA intake and breast cancer risk. ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid. Standardized estimating of 5,000 bootstrap sample. Age, BMI, MET-h/week, education, passive smoking, regular drinking, first-degree relatives with cancer and energy intake were controlled. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

The inverse association between dietary long-chain *n* – 3 PUFAs and breast cancer risk was fully mediated by erythrocyte long-chain *n* – 3 PUFAs. Our study showed that the consumption of long-chain *n* – 3 PUFAs was significantly associated with erythrocyte EPA, DPA and DHA. Consistent with our results, other researches also reported that dietary EPA and DHA was positively associated with the levels of erythrocyte EPA and DHA (37, 38). Previous studies observed inverse associations between erythrocyte long-chain *n* – 3 PUFAs and breast cancer risk (39, 40). As a result, the association

between dietary long-chain *n* – 3 PUFAs might be mediated by erythrocyte long-chain *n* – 3 PUFAs. Notably, the omega-3 index is defined as the percentage of erythrocyte EPA plus DHA in total fatty acids and was thought to be associated with lower risk for health events, especially cardiovascular events (41). We also demonstrated the relationship between erythrocyte EPA and DHA and dietary long-chain *n* – 3 PUFAs and their mediating roles in the association between dietary long-chain *n* – 3 PUFAs/total *n* – 3 PUFAs and breast cancer. Therefore, it is worthwhile for future research to explore the relationship between omega-3 index and health events such as cancer. In terms of our observation of the fully mediated effect of erythrocyte long-chain *n* – 3 PUFAs, a few more points needed to be interpreted with care. It should be noted that the full mediation depends on whether the regression coefficient *c*' is significant, and the significance of *c*' is affected by the sample size. When a large enough sample is collected, the previous conclusion of full mediation may become partial mediation. Furthermore, full mediation actually still implies that there may be other mediating variables (42).

The data herein demonstrated that erythrocyte DPA not independently, but sequentially mediated (with EPA and DHA) the relationship between dietary long-chain *n* – 3 PUFAs and breast cancer risk. Dyall et al. (43) reviewed studies on metabolic differences among long-chain *n* – 3 PUFAs and noted the potential role of DPA. It might serve as a reservoir for EPA and DHA. Several reports indicate that interconversion of EPA, DPA, and DHA via retro-conversion and elongation pathways may occur (44, 45). DPA supplementation could increase the plasma concentrations of several arachidonic acid (AA)-derivative, including total PGE_2 levels and other AA- and dihomo-gamma-linolenic acid-derived PG species (44). AA-derived metabolites contribute to angiogenesis (46). The DPA alone therefore could not play a mediating role in the inverse association between dietary long-chain *n* – 3 PUFAs and breast cancer risk, but rather plays a mediating role through interconversion with EPA and DHA. We also observed that the indirect effect of erythrocyte EPA was higher than erythrocyte DHA. It may be due to the difference in metabolism and incorporation of EPA and DHA in different blood components. Brown et al. (47) demonstrated that erythrocyte EPA was a stronger indicator of *n* – 3 PUFA intake than DHA. DHA is incorporated into the inner erythrocyte leaflet and are more influenced by erythrocyte turnover, whereas EPA is incorporated into the outer erythrocyte leaflet and its content largely depends on the equilibrium with plasma (47).

We found that the association between total consumed *n* – 3 PUFAs and breast cancer risk was partly mediated by erythrocyte long-chain *n* – 3 PUFAs. As mentioned above, the source of dietary ALA was complex and diverse. For example, the inverse association of ALA from fruits and vegetables with the risk of breast cancer might be associated with other compounds in fruits and vegetables, such as folate (48) or fiber (49). Given the fact that ALA accounts for the majority of dietary total *n* – 3 PUFAs [the proportion of dietary ALA to total dietary *n* – 3 PUFAs in cases and controls was 93.17 (±6.42)% and 93.32 (±6.41)%, respectively], it was possible that the relationship between total *n* – 3 PUFAs and breast cancer risk was influenced by other nutrients. The partial mediation implied that there might be other mediators' worth exploring in the association between dietary total *n* – 3 PUFAs and breast cancer risk. For example, it was found that mice fed with diets containing increasing amounts of EPA + DHA had decreasing levels of erythrocyte *n* - 6 PUFAs and increasing levels of erythrocyte saturated fatty acids (SFAs) (50). Prisco et al. illustrated that healthy male volunteers supplemented with *n* – 3 PUFAs for 4 months had decreased level of erythrocyte *n*-6 PUFAs and increased level of erythrocyte monounsaturated fatty acids (MUFAs) (51). This indicated that erythrocyte SFAs, MUFAs, and *n*-6 PUFAs may also be mediators of the association dietary *n* – 3 PUFAs and breast cancer risk.

Strengths of this study include the relatively large sample size and a number of potential confounders. We were further able to apply mediation analysis of four mediators on the association between dietary *n* – 3 PUFAs consumed and breast cancer risk for the first time. Several limitations should be taken into account. Firstly, as a case-control study, the causal sequence of variables cannot be curtained. Longitudinal studies were needed to verify the causality of all variables in the future. Secondly, subjective self-report and recall bias might exist. To diminish it, we used photographs with usual portion size of foods to obtain intake as accurately as possible and recruited patients who were diagnosed less than 3 months before the interview. Thirdly, we only had a single measure of erythrocyte *n* – 3 PUFAs, which may not ideally reflect long-term exposure. However, one study showed that no statistically significant increase was observed in the level of *n* – 3 PUFAs over time in the Cardiovascular Health Study across 13 consecutive years of measures (52). Finally, we cannot completely deny that there may still be some residual confounding factors and these results should be extended to the general population in other regions for confirmation.

Conclusion

Our findings suggest that the influence of dietary total *n* – 3 PUFAs on breast cancer risk was at least partially explained by beneficial effects of total *n* – 3 PUFA intake on increasing the erythrocyte long-chain *n* – 3 PUFAs. Importantly, erythrocyte long-chain *n* – 3 PUFAs may fully explain the protective effect of consumed long-chain *n* – 3 PUFAs on breast cancer risk. The links between dietary *n* – 3 PUFAs and breast cancer risk may be mediated by erythrocyte long-chain *n* – 3 PUFAs, whereas evidence of a mediating role of erythrocyte ALA was not supported. This study highlights the complexity in using a simple analysis of dietary individual *n* – 3 PUFA to predict

breast cancer risk without considering the variety of metabolic processes. We demonstrated the possibility of erythrocyte *n* – 3 PUFAs to interpret the role of dietary *n* – 3 PUFAs in breast cancer risk. Interventions aimed at increasing erythrocyte long-chain *n* – 3 PUFAs may represent a promising strategy for breast cancer prevention. Identifying the ratios between long-chain *n* – 3 PUFAs that are best for human health, including those between *n*-6 PUFAs could be investigated in future studies.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of the School of Public Health, Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZZ collected the data, performed the experiments, analyzed the data, and wrote the manuscript. YJ, XL, DS, TM, and RZ participated in data collection and experiments. CZ was responsible for designing and writing grants, supervision of the research, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (Nos: 81973020 and 81102188).

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. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2021) 71:209–49. doi: 10.3322/caac.21660

2. Xia C, Dong X, Li H, Cao M, Sun D, He S, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. *Chin Med J.* (2022) 135:584–90. doi: 10.1097/cm9.0000000000002108

3. Liu J, Ma D. The role of n-3 polyunsaturated fatty acids in the prevention and treatment of breast cancer. *Nutrients.* (2014) 6:5184–223. doi: 10.3390/nu6115184

4. Witte T, Hardman W. The effects of omega-3 polyunsaturated Fatty Acid consumption on mammary carcinogenesis. *Lipids.* (2015) 50:437–46. doi: 10.1007/s11745-015-4011-2

5. Thiébaut A, Chajès V, Gerber M, Boutron-Ruault M, Joulin V, Lenoir G, et al. Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. *Int J Cancer.* (2009) 124:924–31. doi: 10.1002/ijc.23980

6. Smith C, Follis J, Nettleton J, Foy M, Wu J, Ma Y, et al. Dietary fatty acids modulate associations between genetic variants and circulating fatty acids in plasma and erythrocyte membranes: Meta-analysis of nine studies in the CHARGE consortium. *Mol Nutr Food Res.* (2015) 59:1373–83. doi: 10.1002/mnfr.201400734

7. Hodson L, Skeaff C, Fielding B. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res.* (2008) 47:348–80. doi: 10.1016/j.plipres.2008.03.003

8. Arab L. Biomarkers of fat and fatty acid intake. *J Nutr.* (2003) 133:925S–32S. doi: 10.1093/jn/133.3.925S

9. Chen L, Hu Q, Li G, Zhang L, Qin L, Zuo H, et al. Dietary intake and biomarkers of α-linolenic acid and mortality: a meta-analysis of prospective cohort studies. *Front Nutr.* (2021) 8:743852. doi: 10.3389/fnut.2021.743852

10. Richter C, Bisselou K, Nordgren T, Smith L, Appiah A, Hein N, et al. n-3 Docosapentaenoic Acid intake and relationship with plasma long-chain n-3 fatty acid concentrations in the United States: NHANES 2003-2014. *Lipids.* (2019) 54:221–30. doi: 10.1002/lipd.12146

11. Sprecher H, Luthria D, Mohammed B, Baykousheva S. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *J Lipid Res.* (1995) 36:2471–7.

12. Park H, Park W, Kothapalli K, Brenna J. The fatty acid desaturase 2 (FADS2) gene product catalyzes Δ4 desaturation to yield n-3 docosahexaenoic acid and n-6 docosapentaenoic acid in human cells. *FASEB J.* (2015) 29:3911–9. doi: 10.1096/fj.15-271783

13. Vermunt S, Mensink R, Simonis M, Hornstra G. Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid. *Lipids.* (2000) 35:137–42. doi: 10.1007/bf02664762

14. Burdge G, Wootton S. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr.* (2002) 88:411–20. doi: 10.1079/bjn2002689

15. Bradbury J. Docosahexaenoic acid (DHA): an ancient nutrient for the modern human brain. *Nutrients.* (2011) 3:529–54. doi: 10.3390/nu3050529

16. Kohlmeier L. Future of dietary exposure assessment. *Am J Clin Nutr.* (1995) 61:702S–9S. doi: 10.1093/ajcn/61.3.702S

17. Valeri L, Vanderweele T. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods.* (2013) 18:137–50. doi: 10.1037/a0031034

18. Zhang C, Ho S, Chen Y, Fu J, Cheng S, Lin F. Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. *Int J Cancer.* (2009) 125:181–8. doi: 10.1002/ijc.24358

19. Ainsworth B, Haskell W, Herrmann S, Meckes N, Bassett D, Tudor-Locke C, et al. 2011 Compendium of physical activities : a second up date of codes and MET values. *Med Sci Sports Exerc.* (2011) 43:1575–81. doi: 10.1249/MSS.0b013e31821ece12

20. Zhang C, Ho S. Validity and reproducibility of a food frequency Questionnaire among Chinese women in Guangdong province. *Asia Pac J Clin Nutr.* (2009) 18:240–50.

21. Yang Y. *China Food Composition Table*. 6th Edn. Peking: Peking University Medical Press (2019).

22. Lepage G, Roy C. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res.* (1986) 27:114–20.

23. Willett W, Howe G, Kushi L. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65:1220S–8S. doi: 10.1093/ajcn/65.4.1220S

24. Preacher K, Hayes A. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Methods Instrum Comput.* (2004) 36:717–31. doi: 10.3758/bf03206553

25. Baron R, Kenny D. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol.* (1986) 51:1173–82. doi: 10.1037//0022-3514.51.6.1173

26. Taylor A, MacKinnon D, Tein J-Y. Tests of the Three-Path Mediated Effect. *Organ Res Methods.* (2008) 11:241–69. doi: 10.1177/1094428107300344

27. Padmanabhanunni A, Pretorius T. The loneliness-life satisfaction relationship: the parallel and serial mediating role of hopelessness, depression and ego-resilience among young adults in South Africa during COVID-19. *Int J Environ Res Public Health.* (2021) 18:3613. doi: 10.3390/ijerph18073613

28. Khor B, Sahathevan S, Sualeheen A, Ali M, Narayanan S, Chinna K, et al. Dietary fatty acid intake in hemodialysis patients and associations with circulating fatty acid profiles: a cross-sectional study. *Nutrition.* (2019) 63-64:14–21. doi: 10.1016/j.nut.2019.01.005

29. Olsen S, Hansen H, Sandström B, Jensen B. Erythrocyte levels compared with reported dietary intake of marine n-3 fatty acids in pregnant women. *Br J Nutr.* (1995) 73:387–95. doi: 10.1079/bjn19950041

30. Gabbs M, Leng S, Devassy J, Monirujjaman M, Aukema H. Advances in our understanding of oxylipins derived from dietary PUFAs. *Adv Nutr.* (2015) 6:513–40. doi: 10.3945/an.114.007732

31. Caligiuri S, Love K, Winter T, Gauthier J, Taylor C, Blydt-Hansen T, et al. Dietary linoleic acid and α-linolenic acid differentially affect renal oxylipins and phospholipid fatty acids in diet-induced obese rats. *J Nutr.* (2013) 143:1421–31. doi: 10.3945/jn.113.177360

32. Arterburn L, Hall E, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr.* (2006) 83:1467S–76S. doi: 10.1093/ajcn/83.6.1467S

33. Barceló-Coblijn G, Murphy E. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Prog Lipid Res.* (2009) 48:355–74. doi: 10.1016/j.plipres.2009.07.002

34. Lohner S, Fekete K, Marosvölgyi T, Decsi T. Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publications. *Ann Nutr Metab.* (2013) 62:98–112. doi: 10.1159/000345599

35. Williams C, Burdge G. Long-chain n-3 PUFA: plant v. marine sources. *Proc Nutr Soc.* (2006) 65:42–50. doi: 10.1079/pns2005473

36. Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care.* (2004) 7:137–44. doi: 10.1097/00075197-200403000-00006

37. Orchard T, Ing S, Lu B, Belury M, Johnson K, Wactawski-Wende J, et al. The association of red blood cell n-3 and n-6 fatty acids with bone mineral density and hip fracture risk in the women's health initiative. *J Bone Miner Res.* (2013) 28:505–15. doi: 10.1002/jbmr.1772

38. Itomura M, Fujioka S, Hamazaki K, Kobayashi K, Nagasawa T, Sawazaki S, et al. Factors influencing EPA+DHA levels in red blood cells in Japan. *In Vivo.* (2008) 22:131–5.

39. Kuriki K, Hirose K, Wakai K, Matsuo K, Ito H, Suzuki T, et al. Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese. *Int J Cancer.* (2007) 121:377–85. doi: 10.1002/ijc.22682

40. Shannon J, King I, Moshofsky R, Lampe J, Gao D, Ray R, et al. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China. *Am J Clin Nutr.* (2007) 85:1090–7. doi: 10.1093/ajcn/85.4.1090

41. von Schacky C, Harris W. Cardiovascular benefits of omega-3 fatty acids. *Cardiovasc Res.* (2007) 73:310–5. doi: 10.1016/j.cardiores.2006.08.019

42. Preacher K, Hayes A. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods Instrum Comput.* (2008) 40:879–91. doi: 10.3758/brm.40.3.879

43. Dyall S. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Front Aging Neurosci.* (2015) 7:52. doi: 10.3389/fnagi.2015.00052

44. Markworth J, Kaur G, Miller E, Larsen A, Sinclair A, Maddipati K, et al. Divergent shifts in lipid mediator profile following supplementation with n-3 docosapentaenoic acid and eicosapentaenoic acid. *FASEB J.* (2016) 30:3714–25. doi: 10.1096/fj.201600360R

45. Cao J, Schwichtenberg K, Hanson N, Tsai M. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem.* (2006) 52:2265–72. doi: 10.1373/clinchem.2006.072322

46. Sakai M, Kakutani S, Horikawa C, Tokuda H, Kawashima H, Shibata H, et al. Arachidonic acid and cancer risk: a systematic review of observational studies. *BMC Cancer.* (2012) 12:606. doi: 10.1186/1471-2407-12-606

47. Brown A, Pang E, Roberts D. Erythrocyte eicosapentaenoic acid versus docosahexaenoic acid as a marker for fish and fish oil consumption. *Prostaglandins Leukot Essent Fatty Acids.* (1991) 44:103–6. doi: 10.1016/0952-327890191-7

48. Gao C, Tang J, Cao H, Ding J, Wu J, Wang J, et al. MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. *J Hum Genet.* (2009) 54:414–8. doi: 10.1038/jhg.2009.57

49. Deschasaux M, Zelek L, Pouchieu C, His M, Hercberg S, Galan P, et al. Prospective association between dietary fiber intake and breast cancer risk. *PLoS One.* (2013) 8:e79718. doi: 10.1371/journal.pone.0079718

50. Fenton J, Gurzell E, Davidson E, Harris W. Red blood cell PUFAs reflect the phospholipid PUFA composition of major organs. *Prostaglandins Leukot Essent Fatty Acids.* (2016) 112:12–23. doi: 10.1016/j.plefa.2016.06.004

51. Prisco D, Filippini M, Francalanci I, Paniccia R, Gensini G, Abbate K, et al. Effect of n-3 polyunsaturated fatty acid intake on phospholipid fatty acid composition in plasma and erythrocytes. *Am J Clin Nutr.* (1996) 63:925–32. doi: 10.1093/ajcn/63.6.925

52. Lai H, de Oliveira Otto M, Lemaitre R, McKnight B, Song X, King I, et al. Serial circulating omega 3 polyunsaturated fatty acids and healthy ageing among older adults in the Cardiovascular Health Study: prospective cohort study. *BMJ.* (2018) 363:k4067. doi: 10.1136/bmj.k4067

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 04 November 2022
DOI 10.3389/fnut.2022.1005857

OPEN ACCESS

EDITED BY
Muzi Na,
The Pennsylvania State University (PSU), United States

REVIEWED BY
Vittorio Calabrese,
University of Catania, Italy
Meilin Zhang,
Tianjin Medical University, China

*CORRESPONDENCE
Yuandi Xi
xiaoer711@163.com

SPECIALTY SECTION
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

RECEIVED 28 July 2022
ACCEPTED 13 October 2022
PUBLISHED 04 November 2022

CITATION
Wang X, Li T, Ding H, Liu Y, Liu X, Yu K, Xiao R and Xi Y (2022) The role of dietary patterns and erythrocyte membrane fatty acid patterns on mild cognitive impairment. *Front. Nutr.* 9:1005857. doi: 10.3389/fnut.2022.1005857

The role of dietary patterns and erythrocyte membrane fatty acid patterns on mild cognitive impairment

Xuan Wang[1], Tiantian Li[1], Huini Ding[1], Yuru Liu[2], Xiaoqiang Liu[3], Kang Yu[4], Rong Xiao[1] and Yuandi Xi[1]*

[1]Beijing Key Laboratory of Environmental Toxicology, School of Public Health, Capital Medical University, Beijing, China, [2]Fangshan District Center for Disease Control and Prevention, Beijing, China, [3]Shijiaying Health Service Center, Beijing, China, [4]Peking Union Medical College Hospital, Beijing, China

Background: Dietary fatty acids have been shown to be associated with the development of cognition. However, research on the role of fatty acid intake in dietary patterns and fatty acid patterns (FAPs) in the development of cognitive function is limited. The aim of this study was to explore the correlation between dietary patterns and FAPs and to provide available evidence for preventing mild cognitive impairment (MCI) through these patterns.

Materials and methods: The 973 participants aged between 65 and 85 were recruited from 2020 to 2021 for this multicenter research in Beijing. Neuropsychological tests were used for cognitive evaluation, and data of dietary intake in the past 12 months were collected with semi-quantitative food frequency questionnaire. The erythrocyte membrane fatty acid profile was tested by chromatography and mass spectrometry lipid profiling. Factor analysis was used to derive the main dietary patterns and FAPs. Pearson's correlation or Spearman's correlation was used to explore the association between dietary patterns and FAPs. Binary logistic regression was applied to examine the relationship between patterns and cognitive function.

Results: Six dietary patterns and six FAPs were identified, explaining 53.4 and 80.9% of the total variance separately. After adjusting all potential confounders, T3 of the pattern 1 and FAP2 were the independent protect factors for MCI, respectively (OR 0.601, 95% CI [0.395, 0.914]; OR 0.108, 95% CI [0.019, 0.623]). Rich of SM (26:0), SM (24:1), and SM (26:1) is the characteristic of FAP2. A positive correlation was found between component scores of dietary pattern1 and FAP2 (r = 0.441, p = 0.001). People who adhered to a reasonable intake of animal flesh consumed more various long-chain fatty acids as well.

Conclusion: The erythrocyte membrane metabolites, SM (26:0), SM (24:1), and SM (26:1), might function as early biomarkers for predicting or monitoring of cognitive aging in the elderly. The dietary pattern with recommended animal flesh consumption was significantly associated with FAP characterized

by very long-chain SMs. This dietary pattern affected FAP, which might achieve the ultimate goal of neuroprotection through the very long-chain SMs. A rational intake of dietary fatty acids might be an effective way on preventing MCI in the elderly.

KEYWORDS

fatty acid, cognitive impairment, dietary pattern, erythrocyte membrane fatty acid profile, sphingomyelin

Introduction

Mild cognitive impairment (MCI) is a situation in which individuals show cognitive impairment with minimal damage of instrumental activities of daily living (IADL) (1–3). This refers to an intermediate stage from normal aging to dementia (1). Older adults with MCI have the highest risk of progression to dementia (4). By 2050, it is estimated that there will be 2 billion people aged 60 years and over, and 131 million of whom are expected to be influenced by dementia (5).

Dietary nutrition is an important way to promote healthy aging and prevent age-related diseases (6). In recent years, dietary factors, especially fat intake (7), have been shown to be involved in the development of hippocampal neurogenesis and cognition (8–10). Fatty acids in tissues, the important composition of fat, can reflect both the quantity and quality of dietary fat intake and have been recognized as reliable biomarkers in epidemiologic studies (11). Previous studies have shown associations between intake of dietary nutrients or dietary patterns and cognition function (10, 11). Long-chain polyunsaturated fatty acids (LCPUFAs) have been reported to be potential mediators that might protect nervous system. They also get involved in the mechanisms leading to cognitive impairment or inflammation in elderly subjects (12). However, evidence indicates that saturated fatty acids (SFAs) with different carbon chain lengths have various effects on the process of Aβ generation, and fatty acids with longer chain (C20:0 and C26:0) are more likely to promote Aβ production (13). In addition, dietary fatty acid saturation is reported to be harmful to cognitive function in human studies (14). Studies about the circulating fatty acid patterns (FAPs) further find that neuroprotective potential fatty acids binding to specific phospholipids are more valuable to improve neuro-function (15). For example, the major components of polyunsaturated fatty acids (PUFAs, C20:3, C20:4, C22:5, and C22:6) in sphingomyelin (SM) and ceramide (Cer) are believed to decline more preferentially in the brain of aged mouse. However, compared with the plasma fatty acid profiles, the erythrocyte membrane fatty acid profile could reflect a long-term intake of fatty acids (16–18). Therefore, the aim of this study was to explore dietary patterns with suitable fatty acid intake and FAPs by principal components analysis (PCA), find the correlation of both two patterns, and provide available evidence for preventing MCI.

Materials and methods

Study design and participants

Participants aged 65–85 in this study were collected in several centers of our research in Beijing from 2020 to 2021 (ChiCTR2100054969). The workflow and standards were referenced from our prior study (19, 20). Finally, 973 participants were collected in this study, and 50 of them were selected for lipid analyses. This study was carried out in accordance with the Declaration of Helsinki and ethically approved by the Ethics Committee of Capital Medical University (Z2019SY052). All informed consents were signed by participants before they were included.

Cognitive assessment

Cognitive impairment was assessed by the Montreal Cognitive Assessment (MoCA), while mini-mental state examination (MMSE) score was applied to exclude any AD (21). Two-step procedure was used to diagnose MCI individuals according to our previous study (20). Briefly, neurologists would perform a secondary examination of participants to determine the clinical diagnosis, if they were suspected of having MCI based on their MoCA presentation.

Dietary assessment

The information of dietary intake was collected by the food frequency questionnaire (FFQ) of 2002 China National Nutrition and Health Survey (CNHS 2002) (22) that asked about habitual intake of food over the past year. The energy and nutrient intake were calculated by using the China Food Composition Database (Version 6) (23). The energy-adjusted

amounts of all dietary nutrients were calculated by the residual method (24).

Lipid analysis

Erythrocytes were prepared for lipidomic detection. About 250 μl of water was added into each 50 μl of erythrocyte lysates. After 30s vortex, the samples were frozen and thawed with liquid nitrogen for three times. The samples were then sonicated for 10 min in the ice-water bath. Then, 50 μl of normalized protein concentration of the sample was mixed with 150 μl water and 480 μl extraction liquid (VMTBE: Vmethanol = 5:1) containing internal standard. After 60s vortex, the samples were sonicated for 10 min in the ice-water bath. Then, the samples were centrifuged at 3,000 rpm for 15 min at 4°C. About 250 μl of the supernatant was transferred to a fresh tube. The rest of the sample was added with 250 μl of MTBE, followed by vortex, sonication, and centrifugation, and another 250 μl of the supernatant was taken out. This step was repeated twice. The final supernatants were combined and dried in a vacuum concentrator at 37°C. Then, the dried samples were reconstituted in 100 μl of resuspension buffer (Vdichloromethane: Vmethanol: Vwater = 60:30:4.5) by 30s vortex and sonication on ice for 10 min. The constitution was then centrifuged at 12,000 rpm for 15 min at 4°C, and 30 μl of the supernatant was transferred to a fresh glass vial for LC-MS analysis. The quality control (QC) sample was prepared by mixing 15 μl of the supernatants from all samples.

The UHPLC separation was carried out using a SCIEX ExionLC series UHPLC System. Lipid profiling was performed by a UHPLC system (1290 series, Agilent Technologies, USA) equipped with a Kinetex C18 column (2.1 × 100 mm, 1.7 μm, Phenomen) coupled to Q Exactive (QE)-MS/MS (Thermo Fisher Scientific, Bremen, Germany). Therefore, a binary solvent system consisting of 40% water, and 60% acetonitrile (solvent A) and 10% acetonitrile, and 90% isopropanol (solvent B), both acidified with ammonium acetate (10 mM), was used to establish a gradient elution program following the set of solvent B: 0–12.0 min, 40–100%; 12.0–13.5 min, 100%; 13.5–13.7 min, 100–40%; 13.7–18.0 min, 40%. The injection volume was 4 μl for positive ion mode and 6 μl for negative ion mode, respectively. The column temperature was 40°C. The auto-sampler temperature was 6°C, and the injection volume was 2 μl. Typical ion source parameters were as follows: Ionspray voltage: +5,500/−4,500 V, curtain gas: 40 psi, temperature: 350°C, ion source gas 1:50 psi, ion source gas 2:50 psi, DP: ± 80 V.

In this study, the UHPLC separation was carried out by using a SCIEX ExionLC series UHPLC System. AB Sciex QTrap 6,500 + mass spectrometer was applied for analytical development. Multiple reaction monitoring (MRM) mode was used in mass spectrometry analysis. These analyses resulted in 350 lipids, including 12 SM species and 15 Cer species.

Statistical analysis

Data of continuous variables were presented as means ± standard deviation (SD) or medians (interquartile ranges, IQR). Discrete variables were expressed as percentages (%). Analysis of variance (ANOVA) or the Kruskal–Wallis rank test was applied for continuous variables, while the chi-square test and Wilcoxon rank-sum test were used for descriptive analysis. Component scores were obtained by dietary pattern of each subject.

PCA was used to identify major dietary patterns and FAPs. The factors were rotated by an orthogonal rotation (varimax) for increasing the explanation and simplifying the structure (25). In the final analysis, factor scores of each participant were produced by multiple regression for each component, and factor loadings were based on the dietary intake or levels of fatty acids.

Factor analysis revealed six major dietary patterns which explained 53.3% of the total variance together in dietary intake. An eigenvalue cutoff > 1, scree plot, and component interpretability were used to decide the number of components to retain. A significant chi-square ($p < 0.001$) for the Bartlett's test of sphericity and the Kaiser–Meyer–Olkin test > 0.6 could indicate the strong correlation among the variables to allow for factor analysis. Dimension reduction was performed on the original 12 SMs and 15 Cers by PCA. Factor analysis revealed six major FAPs which together explained 80.9% of the total variance.

Tertiles were classified based on the distribution of scores for each pattern across the whole population. They were used to characterize each pattern, build regression models, and so on (26). The effect of each factor on MCI was analyzed by logistic regression model.

For all analyses, the lowest tertile of dietary pattern score or lipid pattern score was considered as reference. Pearson's correlation and Spearman's correlation were used to analyze the association between dietary pattern parameters and lipid pattern parameters. Statistical significance was set at a two-sided $p < 0.05$. All statistical analyses were performed through the IBM SPSS Statistics 26. Graphs were drawn using the software program GraphPad Prism 8 and R studio.

Results

Demographic characteristics of participants

The demographic characteristics of all subjects are described in **Table 1**. About 59.8% of 973 participants were female. No

TABLE 1 Demographic characteristics of subjects.

	Total	Control	MCI	*p*
N	973	442	531	
Age	69 (67, 73)	70 (67, 73)	69 (67, 73)	0.857
Female, *n* (%)	627 (59.8%)	313 (65.1%)	314 (55.4%)	< 0.003**
MoCA score	21 (17, 23)	22 (20, 25)	19 (15, 22)	< 0.001**
BMR, kcal	1,259 (1,156, 1,385)	1,265 (1,157, 1,376)	1,254 (1,155, 1,388)	0.844
Education, *n* (%)				< 0.001**
Illiterate	218 (22.4%)	153 (34.6%)	65 (12.2%)	
Primary school	323 (33.2%)	175 (39.6%)	148 (27.9%)	
Junior high school	350 (36.0%)	81 (18.3%)	269 (50.7%)	
High school and above	82 (8.4%)	33 (7.5%)	49 (9.2%)	

BMR, basal metabolic rate; MCI, mild cognitive impairment. **$p < 0.01$.

TABLE 2 Factor-loading matrix for the dietary patterns and food groups in sample.

	Pattern 1	Pattern 2	Pattern 3	Pattern 4	Pattern 5	Pattern 6
Fish	0.634*	−0.115	0.175	0.206	−0.086	0.002
Liquor	0.614*	−0.013	−0.201	−0.320	0.165	−0.131
Poultry	0.606*	0.096	−0.067	0.120	0.001	0.003
Red meat	0.600*	−0.013	0.205	0.079	0.139	0.157
Fats and oil	−0.047	0.825*	−0.048	−0.114	0.017	0.046
Condiment	0.049	0.815*	0.09	0.087	0.052	−0.007
Legumes and nuts	0.364	0.001	0.582*	−0.055	−0.098	0.061
Vegetables	0.184	−0.092	0.56*	0.275	0.241	−0.166
Coarse grains	−0.109	0.079	0.546*	0.006	0.071	0.063
Dairy	0.112	−0.03	−0.026	0.677*	−0.213	0.040
Fruits	−0.028	−0.033	0.338	0.590*	0.076	0.027
Eggs	0.287	0.085	−0.358	0.544*	0.230	0.018
Tubers	0.042	0.053	−0.082	−0.078	0.739*	0.122
Wheats and rice	0.056	0.018	0.235	0.021	0.732*	−0.001
Beverages	0.077	−0.050	0.078	−0.025	−0.031	0.790*
Cakes	−0.019	0.087	−0.041	0.088	0.146	0.712*
Percentage of variance explained	11.2%	8.8%	8.7%	8.6%	8.4%	7.7%

*Means factor loading with absolute value ≥0.5.

difference was found between MCI and control individuals in age and basal metabolic rate (BMR). Compared to control individuals, MCI participants were more likely to be male ($p = 0.001$) and had higher levels of education ($p < 0.001$).

Dietary pattern

The characteristics of six dietary patterns are shown in Table 2. These were strongly correlated within the pattern, if food groups with absolute factor loading coefficients are greater than or equal to 0.5. Pattern 1, which explained 11.2% of the total variance, was characterized by the consumption of alcohol and animal flesh which included fish, liquor, poultry, and red meat (pork, beef, and mutton). People in pattern 2, which explained 8.8%, were more likely to consume oil, salt, and soy sauce. Pattern 3, which explained 8.7%, had higher consumption of soybean, nuts, vegetables, and coarse grains. Pattern 4, which explained 8.6%, included milk, fruits, and eggs. The characteristic of pattern 5 (explained 8.4%) was that tubers were the main source of potatoes and cereals. Pattern 6, which explained 7.7%, included sugary beverages and desserts.

The effect of dietary pattern on mild cognitive impairment

The results of the logistic regression analysis are manifested in Table 3. Subjects were divided into three subgroups based on tertiles of factor scores of each dietary pattern. First, six dietary patterns were tested separately after adjusting

TABLE 3 Effect of dietary pattern on MCI.

	T1		T2		T3	
	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
Pattern 1	1[Ref.]	NA	0.957 (0.678, 1.352)	0.804	0.636 (0.443, 0.913)	0.014*
Pattern 2	1[Ref.]	NA	0.93 (0.663, 1.305)	0.675	0.884 (0.63, 1.242)	0.478
Pattern 3	1[Ref.]	NA	1.119 (0.794, 1.578)	0.520	0.678 (0.481, 0.956)	0.026*
Pattern 4	1[Ref.]	NA	0.74 (0.526, 1.042)	0.085	0.658 (0.467, 0.928)	0.017*
Pattern 5	1[Ref.]	NA	1.332 (0.948, 1.872)	0.098	1.11 (0.787, 1.565)	0.552
Pattern 6	1[Ref.]	NA	1.082 (0.769, 1.522)	0.652	1.182 (0.839, 1.664)	0.339

Data were all adjusted by age, gender, education, and BMR. OR, odds ratio. *$p < 0.05$.

TABLE 4 Dietary intake across tertiles of dietary pattern 1.

Fatty acids, g/d	Pattern 1		*p*
	T1	T3	
Total fatty acid	40.3124 (24.1172, 55.1897)	45.4194 (22.0369, 74.287)	0.014*
SFA	15.1984 (10.4227, 18.0028)	18.1353 (12.7871, 24.2102)	< 0.001**
C14:0	1.1675 (0.7280, 2.2506)	1.3452 (0.6115, 2.4480)	0.001**
C16:0	8.0887 (5.9447, 9.8052)	10.3661 (7.3520, 13.6752)	< 0.001**
C17:0	0.2396 (0.1326, 0.3214)	0.3375 (0.2713, 0.4003)	< 0.001**
C18:0	3.0782 (2.1903, 3.7259)	4.1063 (2.8245, 5.3274)	< 0.001**
C19:0	0.0214 (0.0153, 0.0259)	0.0281 (0.0204, 0.0384)	< 0.001**
MUFA	14.3328 (8.0274, 19.936)	16.1768 (7.6206, 27.999)	0.003**
C14:1	0.0073 (0.0042, 0.0143)	0.0100 (0.0046, 0.0169)	< 0.001**
C15:1	0.0003 (0.0002, 0.0005)	0.0004 (0.0002, 0.0006)	0.005**
C16:1	0.2297 (0.1698, 0.2908)	0.3682 (0.2914, 0.4638)	< 0.001**
C17:1	0.0194 (0.0112, 0.0263)	0.0267 (0.0209, 0.0309)	< 0.001**
C18:1	5.2796 (2.9140, 7.4868)	6.2249 (2.9700, 11.8298)	0.001**
C20:1	0.0694 (0.0000, 0.1534)	0.0071 (0.0000, 0.1800)	0.004**
C24:1	0.0034 (0.0000, 0.0083)	0.0000 (0.0000, 0.0097)	0.003**
PUFA	10.8729 (4.1025, 16.7700)	9.3347 (0.7928, 24.5094)	0.996
C16:2	0.0034 (0.0021, 0.0054)	0.0054 (0.0034, 0.0074)	< 0.001**
C20:2	0.0027 (0.0018, 0.0035)	0.0033 (0.0021, 0.0049)	< 0.001**
C20:3	0.0052 (0.0000, 0.0114)	0.0007 (0.0000, 0.0132)	0.003**
C20:4	0.0052 (0.0031, 0.0068)	0.0065 (0.0041, 0.0100)	< 0.001**
C22:3	0.0000 (0.0000, 0.0000)	0.0000 (0.0000, 0.0001)	< 0.001**
C22:4	0.0003 (0.0002, 0.0004)	0.0005 (0.0003, 0.0008)	< 0.001**
C22:5	0.0000 (0.0000, 0.0001)	0.0001 (0.0000, 0.0003)	< 0.001**
C22:6	0.0023 (0.0022, 0.0024)	0.0026 (0.0023, 0.0031)	< 0.001**

T1, the lowest tertile of dietary patterns; T3, the highest tertile of dietary patterns. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. *$p < 0.05$, **$p < 0.01$.

TABLE 5 Dietary intake across tertiles of dietary pattern 1.

Dietary intake	Pattern 1		*p*
	T1	T3	
Fish, g/d	21.00 (12.25, 35.75)	73.13 (47.06, 109.25)	< 0.001**
Liquor, g/d	0.00 (0.00, 3.50)	10.50 (4.88, 21.00)	< 0.001**
Poultry, g/d	1.50 (0.00, 4.50)	14.00 (7, 24.50)	< 0.001**
Red meat, g/d	0.00 (0.00, 0.00)	1.26 (0.00, 32.53)	< 0.001**
SM, mg/d	2.78 (1.57,3.50)	5.66 (4.59,7.27)	< 0.001**

T1, the lowest tertile of dietary patterns; T3, the highest tertile of dietary patterns. SM, sphingomyelin. **$p < 0.01$.

for age, gender, education, and BMR. Compared with the reference group, T3 of the pattern 1 was an independent protective factor for MCI (OR 0.636, 95% CI [0.443, 0.913]). Besides, T3 of the pattern 3 and T3 of pattern 4 were independent protective factors for MCI (OR 0.678, 95% CI [0.481, 0.956]; OR 0.658, 95% CI [0.467, 0.928]). No difference could be found between MCI and control individuals in other dietary patterns.

Dietary intake across tertiles of pattern 1, pattern 3, and pattern 4

Compared with T1 participants of pattern 1, the uptake of C14:0, C16:0, C17:0, C18:0, C19:0, C14:1, C15:1, C16:1 (palmitoleic acid, POA), C17:1, and C18:1 (oleic acid, OA) was significantly elevated in T3 of pattern 1 ($p = 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.005$, $p < 0.001$, $p < 0.001$, and $p = 0.001$). They were all long-chain saturated fatty acids (LCSFAs) or long-chain monounsaturated fatty acids (LCMUFAs). However, the consumption of C20:1 and C24:1, which are very long-chain monounsaturated fatty acids (VLCMUFAs), was significantly higher in T1 than T3 of pattern 1 ($p = 0.004$, $p = 0.003$). In addition, most of the LCPUFA and very long-chain polyunsaturated fatty acids (VLCPUFAs), such as C16:2, C20:2, C20:4 (arachidonic acid, AA), C22:3, C22:4, C22:5 (docosapentaenoic acid, DPA), and C22:6 (docosahexenoic acid, DHA), were significantly elevated in T3 of pattern 1 ($p < 0.001$) (Table 4).

Besides, food group and SM intake across tertiles of pattern 1 are shown in **Table 5**. Compared with T1, the consumption of fish, poultry, red meat, liquor, and SM was significantly higher in participants in the top tertile of pattern 1 ($p < 0.001$).

We also explored the fatty acid profiles in pattern 3 and pattern 4. In T3 of pattern 3, people tended to consume fewer fatty acids (**Supplementary Table 1**). Compared with T1 participants of pattern 4, the consumption of total fatty acids, MUFA, and PUFA (**Supplementary Table 2**) was significantly lower in T3 of pattern 4 ($p < 0.001$). However, the results of the consumption of C14:1, C15:1, C16:1 (POA), C17:1, C16:2, C20:4 (AA), and C22:4 were reversed in pattern 4 ($p < 0.001$).

Between-group differences in SM and Cer FAPs

Dimension reduction with PCA resulted in six FAPs with eigenvalues > 1 (**Supplementary Table 3**). The main contributors to each rotational FAP were defined as those with a factor loading > 0.7. All other contributors to each rotational FAP (with a factor loading < 0.7) were excluded from further consideration. Finally, five of six components, FAP1, FAP2, FAP3, FAP4, and FAP6, remained to be further analyzed. **Figure 1** illustrates the profile of group difference between control and MCI for FAP2 ($p < 0.05$). FAP2 was characterized by SM (26:0), SM (24:1), and SM (26:1), which were three very long-chain saturated fatty acids (VLCSFAs) and VLCMUFA (**Supplementary Table 3**).

Association between dietary patterns or SM intake with FAP2

Associations between dietary pattern1 or SM intake and FAP2 are shown in **Figures 2A–C**. The significantly positive correlations were found between component scores of dietary pattern 1 and FAP2 either in total subjects or in each groups, respectively ($r = 0.441$, $p = 0.001$; $r = 0.635$, $p = 0.003$; $r = 0.475$, $p = 0.008$). Furthermore, there was no linear correlation between FAP2 and patterns 3 or 4 ($r = 0.021$, $p = 0.884$; $r = -0.134$, $p = 0.355$). Moreover, the intake of SM was positively correlated with FAP2 overall score ($r = 0.293$ $p = 0.039$) and was also shown in **Figure 2**, and the same relationship could be found in the consumption of SM with FAP2 scores in MCI ($r = 0.398$, $p = 0.029$).

The effect of FAP2 on mild cognitive impairment

There were no statistically differences in age, gender, and education among between control and MCI participants who take the quantitative lipidomic analysis (**Supplementary Table 4**). The results of the logistic regression analysis are manifested in **Table 6**. Subjects were divided into three subgroups according to the tertiles of FAP2 factor score. Compared with T1 of FAP2, T3 was an independent protective factor for MCI (OR 0.152, 95% CI [0.032, 0.713]). They also showed that the model was improved in predicting MCI when adjusted for BMR. The *p*-value was lower than before, and the odds ratio of T3 on MCI was decreased (adjusted OR 0.108, adjusted 95% CI [0.019,0.623]) as well.

Discussion

The relationship between dietary patterns and cognition has received increasing attention due to the complex interactions between various nutrients and food (27–29). The association between circulation or tissue FAPs with cognitive decline has been identified (30, 31). However, few studies have combined dietary patterns with FAPs to explore the association between nutrition and MCI. The main findings of the present study indicated that MCI was cross-sectionally associated with dietary patterns that differ in fatty acid intake. Dietary pattern was closely correlated with erythrocyte membrane FAP. The elderly with higher levels of long-chain SM (LCSMs) had a lower risk of developing MCI.

First, the results showed that males and highly educated people were at higher risk of MCI. This is similar to other studies (32–34). It is indicated that higher education is related with faster cognitive decline on global cognition (35). In this cross-sectional study, six different dietary patterns were identified. Pattern 1 was characterized by high intakes of animal flesh which included fish, liquor, poultry, and red meat (pork, beef, and mutton). It was indicated that people who adhered to a reasonable intake of animal flesh (especially fish) had a lower risk of MCI after considering all potential confounding variables. These foods play a potentially beneficial role in cognition, which have showed in previous studies (36–38). Specifically, we found that participants in the T3 of pattern 1 had much lower ORs on MCI than T1. Moreover, a large longitudinal study demonstrates that lower animal flesh intake is significantly associated with an increased risk of MCI compared with regular intake (39). However, there is another opinion that higher animal flesh consumption leads to high intake of SFA, which can increase inflammation to make a negative impact on cognition (14). In the present study, the intake of poultry plus red meat in the top tertile of pattern 1 score was just up to the recommended amount (300–500 g/week) of the Chinese Dietary Guidelines, and so did fish. It was implied that adequate intake of animal flesh is crucial for neurological health. In addition, less than 40 g/d alcohol consumption has anti-inflammatory effects (40, 41). In general, people in the top tertile of pattern 1 consumed less PUFA, but more total fatty

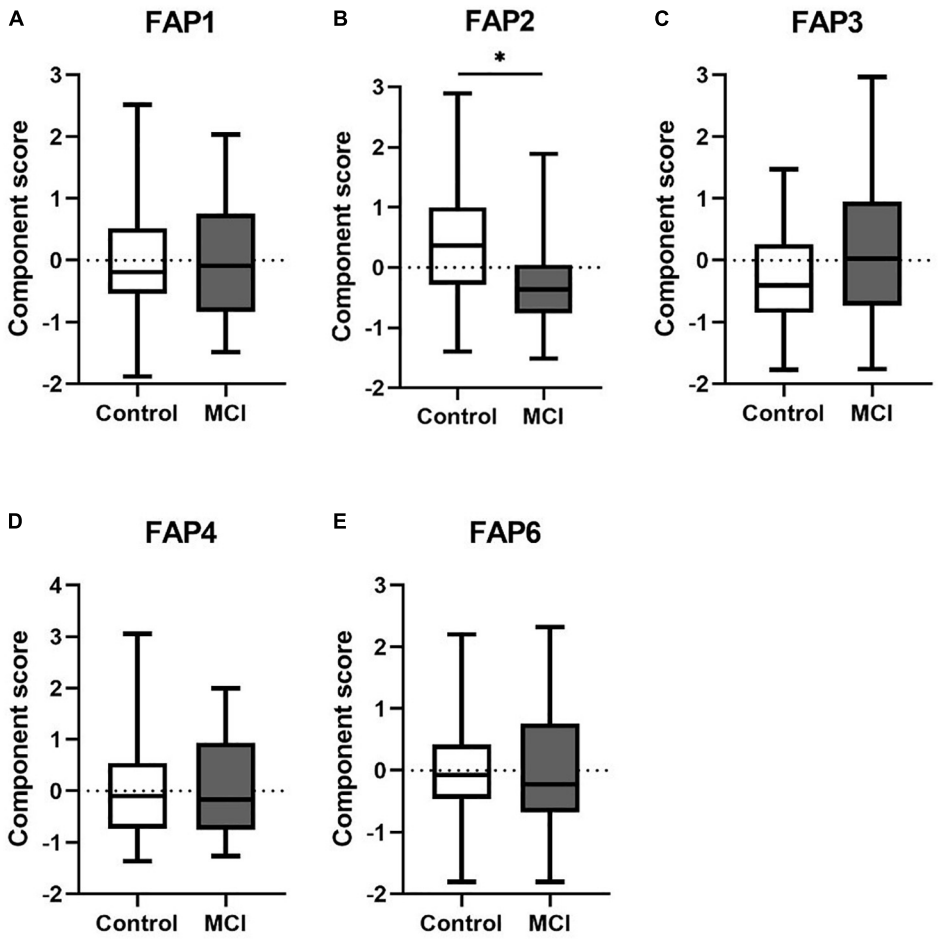


FIGURE 1
(A–E) The differences of FAPs of Cer and SM between control and MCI. Cer, ceramide; SM, sphingomyelin; FAP, fatty acid pattern. *$p < 0.05$.

acids, SFA, and MUFA. However, in terms of carbon chain length, those people consumed more LCSFAs, LCMUFAs, and LCPUFAs, but less VLCMUFAs. It showed that VLCMUFAs had no effect on preventing cognitive decline. The percentage of VLCMUFAs and VLCPUFAs in VLCFAs might be related to MCI. Furthermore, an *in vitro* study reports that SFAs with longer chain (C20:0 and C26:0) were more likely to promote Aβ production (42). Therefore, the reasonable ratio of fatty acid intake and its effect on cognitive function for elderly people remains needs more evidence.

The pattern 3 was also positively associated with cognitive functions. The brain-friendly food groups in pattern 3 are legumes, nuts, vegetables, and grains. They are rich in antioxidants such as fiber, beta-carotene, vitamins, folate, and magnesium (38, 43). It has been established that oxidative stress and inflammation contribute to cognitive decline (44). It has been proved that these antioxidants have anti-inflammatory effect and significant association with the prevention of cognitive impairment (45). Our findings also showed that eggs, milk, and dairy products, which are included in pattern 4, are nutritious foods that contain a variety of nutrients associated with improving cognitive function, such as folate, vitamin B12, choline, and protein (38). A systematic review in 2019 indicates that dairy products may help prevent cognitive decline (46). This is consistent with our results. However, fatty acid patterns were no linear correlated with dietary patterns 3 and 4, which might affect cognitive function through the aforementioned non-fatty acid pathways, but it is worth noting that some fatty acids believed having neuroprotection were also found in mode 4 and that might be another way of neuroprotection.

We further focused on LCFAs in our lipidomic results. SM and Cer, as major components of myelin sheath, are related to synaptic dysfunction, neuroinflammation, and neuronal apoptosis in AD (47). Membrane-associated oxidative stress is closely related to activating sphingomyelinases, which cleave SM to generate Cer (48). Excessively, high amounts of ceramide can trigger a form of programmed cell death called apoptosis (48). Cer generated in response to membrane-associated oxidative

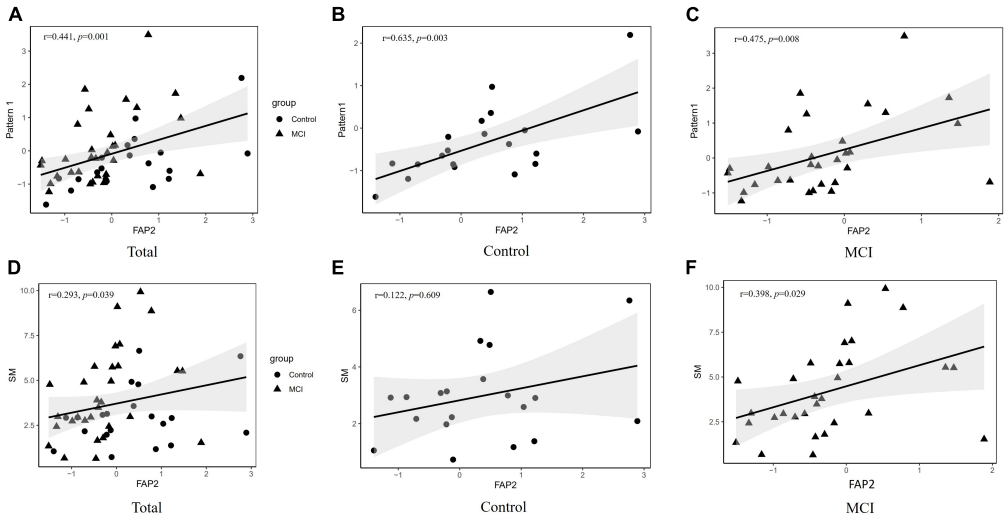


FIGURE 2

Association between dietary pattern 3 and FAP2. **(A)** The association between component scores of dietary pattern 1 and FAP2 in all subjects. **(B)** The association between component scores of dietary pattern 1 and FAP2 in control. **(C)** The association between component scores of dietary pattern 1 and FAP2 in MCI. **(D)** The association between SM intakes and component scores of FAP2 in all subjects. **(E)** The association between SM intakes and component scores of FAP2 in control. **(F)** The association between SM intakes and component scores of FAP2 in MCI. FAP, fatty acid pattern; SM, sphingomyelin.

TABLE 6 Logistic regression analyses of FAPs for predicting MCI.

	Unadjusted			Adjusted[a]		
	β	OR (95%CI)	*p*	β	OR (95%CI)	*p*
FAP1			0.403			0.297
T1	1[Ref.]	1[Ref.]	NA	1[Ref.]	1[Ref.]	NA
T2	−0.724	0.485 (0.122, 1.922)	0.303	−1.100	0.333 (0.072, 1.529)	0.157
T3	0.182	1.200 (0.281, 5.124)	0.806	−0.132	0.876 (0.184, 4.173)	0.868
FAP2			0.024*			0.018*
T1	1[Ref.]	1[Ref.]	NA	1[Ref.]	1[Ref.]	NA
T2	−0.143	0.867 (0.187, 4.007)	0.855	−0.048	0.953 (0.185, 4.907)	0.954
T3	−1.887	0.152 (0.032, 0.713)	0.017*	−2.223	0.108 (0.019, 0.623)	0.013*
FAP3			0.172			0.226
T1	1[Ref.]	1[Ref.]	NA	1[Ref.]	1[Ref.]	NA
T2	−0.608	0.544 (0.137, 2.167)	0.388	−0.465	0.628 (0.147, 2.690)	0.531
T3	0.822	2.275 (0.518, 9.989)	0.276	0.897	2.453 (0.521, 11.558)	0.257
FAP4			0.649			0.571
T1	1[Ref.]	1[Ref.]	NA	1[Ref.]	1[Ref.]	NA
T2	0.671	0.867 (0.187, 4.007)	0.356	0.790	2.204 (0.487, 9.975)	0.305
T3	0.239	0.152 (0.032, 0.713)	0.730	0.180	1.197 (0.285, 5.033)	0.806
FAP6			0.268			0.393
T1	1[Ref.]	1[Ref.]	NA	1[Ref.]	1[Ref.]	NA
T2	−0.857	0.424 (0.104, 1.724)	0.231	−0.819	0.441 (0.102, 1.910)	0.274
T3	0.269	1.309 (0.310, 5.533)	0.714	0.135	1.145 (0.254, 5.169)	0.860

OR, odds ratio; FAP, fatty acid patterns. [a]Data were adjusted by BMR. *$p < 0.05$.

stress is implicated in the dysfunction and death of cells in a range of disorders, including Alzheimer's disease and amyotrophic lateral sclerosis (48, 49). Therefore, SMs and Cers were included in PCA in this study. It is certain that reduced plasma concentrations of SM are associated with AD (50, 51). The component score of FAP2 was found significantly different between MCI and control group, and SM (26:0), SM (24:1), and SM (26:1) were the main characteristics of this FAP. Dietary intake of SM was significantly correlated with the levels of SM in FAP2. This result suggested that the levels of SM in the erythrocyte membrane fatty acid profile might be affected by dietary SM intake. A systematic review showed that the results of C26:0 levels are not consistent. One study suggests that aMCI and AD patients have lower levels of C26:0, and another research shows upregulation of C26:0 in erythrocytes in AD subjects (52). One more study demonstrated that the levels of SM (OH) C24:1 and several serum metabolites have significant differences between patients of MCI and early-stage AD and that might be potential biomarkers to distinguish MCI patients who will develop to early-stage AD from stable MCI patients (53). In our linear correlation analysis results, the significantly positive correlations were found between component scores of dietary pattern1 and FAP2 no matter in all subjects or in MCI/control groups, respectively. These results not only demonstrated that erythrocyte membrane fatty acids were affected by long-term dietary habits, but also suggested that dietary fatty acids might exert neuroprotective effects by affecting erythrocyte lipid profiles. This result validated that the dietary patterns with different consumption characteristics of fatty acid might affect FAPs. Our results supported that erythrocyte membrane metabolites, SM (26:0), SM (24:1), and SM (26:1), might function as early biomarkers for predicting or monitoring cognitive aging in elderly (13). Dietary pattern 1 affected FAP2, and this pattern might achieve the ultimate goal of neuroprotection through the very long-chain SMs.

There were some limitations in this study. First, it is the cross-sectional design so that evidence of any causal conclusions between dietary patterns, FAPs, and MCI could not be provided. Therefore, validation in clinical trials was required. Moreover, FFQ is a semi-quantitative questionnaire, and the information of dietary intake depends on subject's memory, which may lead to recall bias. Finally, it should be carried out with caution to generalize the results we obtained. However, this study combined dietary patterns and FAPs to explore the association between nutrition and MCI. From the perspective of rational intake of dietary fatty acids, the results provided more available data and precise dietary advice to prevent cognitive decline. It provides novel scientific evidence and strategies for nutritional intervention, and it has significant theoretical support and social significance for encouraging healthy aging.

Conclusion

In conclusion, this study found the suitable dietary patterns and FAPs which have the potential effect on preventing cognitive decline and examined the relationship between dietary patterns and FAPs. It was supported that erythrocyte membrane metabolites, SM (26:0), SM (24:1), and SM (26:1), might function as early biomarkers for predicting or monitoring cognitive aging in the elderly. The dietary pattern with recommended animal flesh intake was significantly associated with FAP characterized by very long-chain SMs. This dietary pattern affected FAP, which might achieve the ultimate goal of neuroprotection through the very long-chain SMs. A rational intake of dietary fatty acids might be an effective way on preventing MCI in the elderly.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XW, TL, and YX contributed to the concept and design of the manuscript. XW and TL performed the statistical analysis. XW was responsible for the drafting of the manuscript. KY, RX, and YX contributed to revision of the manuscript. XW, TL, HD, and YX contributed to the investigation. YL, XL, and YX contributed to project administration. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (82273620 and 81973018); the Key Laboratory of Trace Element and Nutrition, National Health Commission of China (WLKFZ202201); the Nutrition Research Fund of the Chinese Nutrition Society: Feihe Special Fund for Physical Nutrition and Health (CNS-Feihe2021-110); DSM Special Research Foundation (CNS-DSM2018A31); and Danone Institutes Research Prizes of China for Alimentation (DIC2019-02).

Acknowledgments

We thank all of the participants who took part in this study. We gratefully acknowledge Yao Zhao, Baoying Li, and Yuhua Li for their considerable assistance with many aspects. We also sincerely grateful to the Shijiaying Health Service Center, Zhoukoudian Health Service Center, and Center for Disease Control and Prevention, Fangshan District, Beijing, China.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1005857/full#supplementary-material

References

1. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med.* (2004) 256:183–94. doi: 10.1111/j.1365-2796.2004.01388.x

2. Bondi MW, Smith GE. Mild cognitive impairment: A concept and diagnostic entity in need of input from neuropsychology. *J Int Neuropsychol Soc.* (2014) 20:129–34. doi: 10.1017/S1355617714000010

3. Petersen RC, Lopez O, Armstrong MJ, Getchius T, Ganguli M, Gloss D, et al. Practice guideline update summary: Mild cognitive impairment: Report of the guideline development, dissemination, and implementation subcommittee of the american academy of neurology. *Neurology.* (2018) 90:126–35. doi: 10.1212/WNL.0000000000004826

4. Salzman T, Sarquis-Adamson Y, Son S, Montero-Odasso M, Fraser S. Associations of multidomain interventions with improvements in cognition in mild cognitive impairment: A systematic review and meta-analysis. *JAMA Netw Open.* (2022) 5:e226744. doi: 10.1001/jamanetworkopen.2022.6744

5. Moore K, Hughes CF, Ward M, Hoey L, McNulty H. Diet, nutrition and the ageing brain: Current evidence and new directions. *Proc Nutr Soc.* (2018) 77:152–63. doi: 10.1017/S0029665117004177

6. Coelho-Junior HJ, Trichopoulou A, Panza F. Cross-sectional and longitudinal associations between adherence to Mediterranean diet with physical performance and cognitive function in older adults: A systematic review and meta-analysis. *Ageing Res Rev.* (2021) 70:101395. doi: 10.1016/j.arr.2021.101395

7. Ding B, Xiao R, Ma W, Zhao L, Bi Y, Zhang Y. The association between macronutrient intake and cognition in individuals aged under 65 in China: A cross-sectional study. *BMJ Open.* (2018) 8:e18573. doi: 10.1136/bmjopen-2017-018573

8. Murphy T, Thuret S. The systemic milieu as a mediator of dietary influence on stem cell function during ageing. *Ageing Res Rev.* (2015) 19:53–64. doi: 10.1016/j.arr.2014.11.004

9. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: Systematic review and meta-analysis of observational studies. *BMJ.* (2015) 351:h3978. doi: 10.1136/bmj.h3978

10. Brown TJ, Brainard J, Song F, Wang X, Abdelhamid A, Hooper L. Omega-3, omega-6, and total dietary polyunsaturated fat for prevention and treatment of type 2 diabetes mellitus: Systematic review and meta-analysis of randomised controlled trials. *BMJ.* (2019) 366:l4697. doi: 10.1136/bmj.l4697

11. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res.* (2008) 47:348–80. doi: 10.1016/j.plipres.2008.03.003

12. Janssen CI, Kiliaan AJ. Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration. *Prog Lipid Res.* (2014) 53:1–17. doi: 10.1016/j.plipres.2013.10.002

13. Huo Z, Rana BK, Elman JA, Dong R, Engelman CD, Johnson SC, et al. Metabolic profiling of cognitive aging in midlife. *Front Aging Neurosci.* (2020) 12:555850. doi: 10.3389/fnagi.2020.555850

14. Tan BL, Norhaizan ME. Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. *Nutrients.* (2019) 11:2579. doi: 10.3390/nu11112579

15. Tu J, Yin Y, Xu M, Wang R, Zhu ZJ. Absolute quantitative lipidomics reveals lipidome-wide alterations in aging brain. *Metabolomics.* (2017) 14:5. doi: 10.1007/s11306-017-1304-x

16. Djousse L, Biggs ML, Lemaitre RN, King IB, Song X, Ix JH, et al. Plasma omega-3 fatty acids and incident diabetes in older adults. *Am J Clin Nutr.* (2011) 94:527–33. doi: 10.3945/ajcn.111.013334

17. Huang NJ, Pishesha N, Mukherjee J, Zhang S, Deshycka R, Sudaryo V, et al. Genetically engineered red cells expressing single domain camelid antibodies confer long-term protection against botulinum neurotoxin. *Nat Commun.* (2017) 8:423. doi: 10.1038/s41467-017-00448-0

18. Fote G, Wu J, Mapstone M, Macciardi F, Fiandaca MS, Federoff HJ. Plasma sphingomyelins in Late-Onset Alzheimer's disease. *J Alzheimers Dis.* (2021) 83:1161–71. doi: 10.3233/JAD-200871

19. An Y, Zhang X, Wang Y, Wang Y, Liu W, Wang T, et al. Longitudinal and nonlinear relations of dietary and Serum cholesterol in midlife with cognitive decline: Results from EMCOA study. *Mol Neurodegener.* (2019) 14:51. doi: 10.1186/s13024-019-0353-1

20. Zhang X, Wang Y, Liu W, Wang T, Wang L, Hao L, et al. Diet quality, gut microbiota, and microRNAs associated with mild cognitive impairment in middle-aged and elderly Chinese population. *Am J Clin Nutr.* (2021) 114:429–40. doi: 10.1093/ajcn/nqab078

21. Qin H, Zhu B, Hu C, Zhao X. Later-Onset hypertension is associated with higher risk of dementia in mild cognitive impairment. *Front Neurol.* (2020) 11:557977. doi: 10.3389/fneur.2020.557977

22. He Y, Ma G, Zhai F, Li Y, Hu Y, Feskens EJ, et al. Dietary patterns and glucose tolerance abnormalities in Chinese adults. *Diabetes Care.* (2009) 32:1972–6. doi: 10.2337/dc09-0714

23. Ma YH, Shen XN, Xu W, Huang YY, Li HQ, Tan L, et al. A panel of blood lipids associated with cognitive performance, brain atrophy, and Alzheimer's diagnosis: A longitudinal study of elders without dementia. *Alzheimers Dement (Amst).* (2020) 12:e12041. doi: 10.1002/dad2.12041

24. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65(4 Suppl):1220S–8S. doi: 10.1093/ajcn/65.4.1220S

25. Willey J, Wakefield M, Silver HJ. Exploring the diets of adults with obesity and type II diabetes from nine diverse countries: Dietary intakes, patterns, and quality. *Nutrients.* (2020) 12:2027. doi: 10.3390/nu12072027

26. Liu D, Zhao LY, Yu DM, Ju LH, Zhang J, Wang JZ, et al. Dietary patterns and association with obesity of children aged 6(-)17 years in medium and small cities in china: findings from the CNHS 2010(-)2012. *Nutrients.* (2018) 11:3. doi: 10.3390/nu11010003

27. Mantzorou M, Vadikolias K, Pavlidou E, Tryfonos C, Vasios G, Serdari A, et al. Mediterranean diet adherence is associated with better cognitive status and less depressive symptoms in a Greek elderly population. *Aging Clin Exp Res.* (2021) 33:1033–40. doi: 10.1007/s40520-020-01608-x

28. Trichopoulou A, Kyrozis A, Rossi M, Katsoulis M, Trichopoulos D, La Vecchia C, et al. Mediterranean diet and cognitive decline over time in an elderly Mediterranean population. *Eur J Nutr.* (2015) 54:1311–21. doi: 10.1007/s00394-014-0811-z

29. Okubo H, Inagaki H, Gondo Y, Kamide K, Ikebe K, Masui Y, et al. Association between dietary patterns and cognitive function among 70-year-old Japanese elderly: A cross-sectional analysis of the SONIC study. *Nutr J.* (2017) 16:56. doi: 10.1186/s12937-017-0273-2

30. Shen J, Li J, Hua Y, Ding B, Zhou C, Yu H, et al. Association between the erythrocyte membrane fatty acid profile and cognitive function in the overweight and obese population aged from 45 to 75 years old. *Nutrients.* (2022) 14:914. doi: 10.3390/nu14040914

31. Bernath MM, Bhattacharyya S, Nho K, Barupal DK, Fiehn O, Baillie R, et al. Serum triglycerides in Alzheimer disease: Relation to neuroimaging and CSF biomarkers. *Neurology.* (2020) 94:e2088–98. doi: 10.1212/WNL.0000000000009436

32. Fu J, Liu Q, Du Y, Zhu Y, Sun C, Lin H, et al. Age- and Sex-Specific prevalence and modifiable risk factors of mild cognitive impairment among older adults in china: A Population-Based observational study. *Front Aging Neurosci.* (2020) 12:578742. doi: 10.3389/fnagi.2020.578742

33. Qin HY, Zhao XD, Zhu BG, Hu CP. Demographic factors and cognitive function assessments associated with mild cognitive impairment progression for the elderly. *Biomed Res Int.* (2020) 2020:3054373. doi: 10.1155/2020/3054373

34. Yuan Y, Li J, Zhang N, Fu P, Jing Z, Yu C, et al. Body mass index and mild cognitive impairment among rural older adults in China: The moderating roles of gender and age. *BMC Psychiatry.* (2021) 21:54. doi: 10.1186/s12888-021-03059-8

35. van Loenhoud AC, Groot C, Bocancea DI, Barkhof F, Teunissen C, Scheltens P, et al. Association of education and intracranial volume with cognitive trajectories and mortality rates across the Alzheimer disease continuum. *Neurology.* (2022) 98:e1679–91. doi: 10.1212/WNL.0000000000200116

36. Huang Q, Jia X, Zhang J, Huang F, Wang H, Zhang B, et al. Diet-Cognition associations differ in mild cognitive impairment subtypes. *Nutrients.* (2021) 13:1341. doi: 10.3390/nu13041341

37. Zhu J, Xiang YB, Cai H, Li H, Gao YT, Zheng W, et al. A prospective investigation of dietary intake and functional impairments among the elderly. *Am J Epidemiol.* (2018) 187:2372–86. doi: 10.1093/aje/kwy156

38. Huang Q, Jiang H, Zhang J, Jia X, Huang F, Wang H, et al. Dietary patterns are associated with Multi-Dimensional cognitive functions among adults aged 55 and older in china. *Front Nutr.* (2022) 9:806871. doi: 10.3389/fnut.2022.806871

39. Ngabirano L, Samieri C, Feart C, Gabelle A, Artero S, Duflos C, et al. Intake of meat, fish, fruits, and vegetables and Long-Term risk of dementia and alzheimer's disease. *J Alzheimers Dis.* (2019) 68:711–22. doi: 10.3233/JAD-180919

40. Wang X, Li T, Li H, Li D, Wang X, Zhao A, et al. Association of dietary inflammatory potential with blood inflammation: The prospective markers on mild cognitive impairment. *Nutrients.* (2022) 14:2417. doi: 10.3390/nu14122417

41. Imhof A, Woodward M, Doering A, Helbecque N, Loewel H, Amouyel P, et al. Overall alcohol intake, beer, wine, and systemic markers of inflammation in western Europe: Results from three MONICA samples (Augsburg, Glasgow, Lille). *Eur Heart J.* (2004) 25:2092–100. doi: 10.1016/j.ehj.2004.09.032

42. Liu JJ, Zhang W, Wang SS, Jia ZQ, Shi YH, Yang L, et al. Effects of chain length of saturated fatty acids on Abeta generation in SH-SY5Y cells. *Neurosci Lett.* (2019) 698:169–72. doi: 10.1016/j.neulet.2019.01.024

43. Chan R, Chan D, Woo J. A cross sectional study to examine the association between dietary patterns and cognitive impairment in older Chinese people in Hong Kong. *J Nutr Health Aging.* (2013) 17:757–65. doi: 10.1007/s12603-013-0348-5

44. Den H, Dong X, Chen M, Zou Z. Efficacy of probiotics on cognition, and biomarkers of inflammation and oxidative stress in adults with Alzheimer's disease or mild cognitive impairment – a meta-analysis of randomized controlled trials. *Aging (Albany NY).* (2020) 12:4010–39. doi: 10.18632/aging.102810

45. Jiang X, Huang J, Song D, Deng R, Wei J, Zhang Z. Increased consumption of fruit and vegetables is related to a reduced risk of cognitive impairment and dementia: Meta-Analysis. *Front Aging Neurosci.* (2017) 9:18. doi: 10.3389/fnagi.2017.00018

46. Cuesta-Triana F, Verdejo-Bravo C, Fernandez-Perez C, Martin-Sanchez 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

47. Su H, Rustam YH, Masters CL, Makalic E, McLean CA, Hill AF, et al. Characterization of brain-derived extracellular vesicle lipids in Alzheimer's disease. *J Extracell Vesicles.* (2021) 10:e12089. doi: 10.1002/jev2.12089

48. Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ, Mattson MP. Cellular stress responses, the hormesis paradigm, and vitagenes: Novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxid Redox Signal.* (2010) 13:1763–811. doi: 10.1089/ars.2009.3074

49. Perluigi M, Di Domenico F, Giorgi A, Schinina ME, Coccia R, Cini C, et al. Redox proteomics in aging rat brain: Involvement of mitochondrial reduced glutathione status and mitochondrial protein oxidation in the aging process. *J Neurosci Res.* (2010) 88:3498–507. doi: 10.1002/jnr.22500

50. Bergland AK, Proitsi P, Kirsebom BE, Soennesyn H, Hye A, Larsen AI, et al. Exploration of plasma lipids in mild cognitive impairment due to Alzheimer's disease. *J Alzheimers Dis.* (2020) 77:1117–27. doi: 10.3233/JAD-200441

51. Oresic M, Hyotylainen T, Herukka SK, Sysi-Aho M, Mattila I, Seppanan-Laakso T, et al. Metabolome in progression to Alzheimer's disease. *Transl Psychiatry.* (2011) 1:e57. doi: 10.1038/tp.2011.55

52. Cisbani G, Bazinet RP. The role of peripheral fatty acids as biomarkers for Alzheimer's disease and brain inflammation. *Prostaglandins Leukot Essent Fatty Acids.* (2021) 164:102205. doi: 10.1016/j.plefa.2020.102205

53. Weng WC, Huang WY, Tang HY, Cheng ML, Chen KH. The differences of serum metabolites between patients with Early-Stage Alzheimer's disease and mild cognitive impairment. *Front Neurol.* (2019) 10:1223. doi: 10.3389/fneur.2019.01223

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 16 November 2022
DOI 10.3389/fpubh.2022.939827

OPEN ACCESS

EDITED BY
Zheqing Zhang,
Southern Medical University, China

REVIEWED BY
Dennis E. Jewell,
Kansas State University, United States
Li-Ting Kao,
National Defense Medical Center, Taiwan

*CORRESPONDENCE
Zhenchao Jia
zhenchao-1@163.com
Xinghua Tang
tangxinghua@med.uestc.edu.cn

SPECIALTY SECTION
This article was submitted to Public Health and Nutrition, a section of the journal Frontiers in Public Health

RECEIVED 09 May 2022
ACCEPTED 24 October 2022
PUBLISHED 16 November 2022

CITATION
Long L, Jia Z and Tang X (2022) Serum polyunsaturated fatty acids and hearing threshold shifts in adults in the United States: A cross-sectional study. *Front. Public Health* 10:939827. doi: 10.3389/fpubh.2022.939827

Serum polyunsaturated fatty acids and hearing threshold shifts in adults in the United States: A cross-sectional study

Lili Long[1], Zhenchao Jia[2]* and Xinghua Tang[3]*

[1]Department of Otorhinolaryngology, Sichuan University Hospital of Sichuan University, Chengdu, China, [2]Department of Prevention and Health Care, Sichuan University Hospital of Sichuan University, Chengdu, China, [3]Department of Otorhinolaryngology Head and Neck Surgery, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China

Background: Few studies have evaluated the association between polyunsaturated fatty acids (PUFAs) and hearing levels. This study aimed to investigate the association between serum PUFAs and hearing threshold shifts in US adults.

Methods: We investigated 913 adults from the National Health and Nutrition Examination Survey (NHANES) 2011–2012. Multivariate linear regression analyses were conducted to evaluate associations between PUFA and hearing threshold shifts.

Results: Overall, 11 serum PUFAs were inversely associated with low-frequency thresholds, especially in men, and were positively related to high-frequency thresholds, particularly in the 40–59 years old cohort. Furthermore, some serum PUFAs were positively associated with both hearing threshold subgroups in women.

Conclusion: Some PUFAs tend to be beneficial for low-frequency hearing status and detrimental to the high-frequency hearing threshold. The male sex may play a protective role in this association, while the female sex and middle age may be detrimental in the effect of PUFAs on hearing function.

KEYWORDS

polyunsaturated fatty acids, hearing threshold shift, National Health and Nutrition Examination Survey, cross-sectional study, adults

Introduction

Hearing loss (HL) is the most common sensory deficit in humans. More than 30 million adults in the United States, nearly 15% of the total population, have some degree of HL (1). Hearing impairment adversely affects social engagement and is associated with impaired quality of life, dementia, depression, and increased mortality (2–4). The estimated direct and indirect medical costs resulting from hearing impairment have increased from $3.3 million to 12.8 million annually in the United States (5). This health

burden is escalating; hence, studying risk factors to develop preventive and therapeutic strategies is essential to reduce the effect and burden of hearing impairment.

The cochlea in the inner ear is highly vascularized and is supplied by a single feed artery (6). It is assumed that impaired inner ear perfusion and ischemic vascular damage of the cochlea can cause hearing impairment (7). Cardiovascular disease events (e.g., myocardial infarction, ischemic heart disease, and stroke) showed a moderate association with hearing impairment in a cohort study (8). Previous studies have further reported on the relationship between polyunsaturated fatty acids (PUFAs) and many diseases (9). n-3 PUFAs have been shown to exert protective effects against cardiovascular diseases, such as heart failure and stroke (9, 10). Hence, it is plausible that PUFAs may also play an important cochlear protective role for the auditory system.

To date, there have been only a few population-based studies investigating the association between PUFAs and the risk of hearing impairment (11–15), three of which studied the effect of total dietary PUFA intake, particularly n-3 PUFAs on low-frequency or speech-frequency hearing impairment (11, 12, 14). Two studies examined the relationship between plasma PUFAs and hearing status but only in old or young people (13, 15). Therefore, we performed this study using data from the National Health and Nutrition Examination Survey (NHANES) database to investigate whether cross-sectional associations exist between individual serum PUFAs and both low-, and high-frequency hearing threshold shifts in adults aged 20–69 years in the United States.

Methods

Ethics statement

This study utilized publicly accessible data from the NHANES website (https://www.cdc.gov/nchs/nhanes/Index.htm). The NHANES data were approved by the National Center for Health Statistics Institutional Review Board in accordance with the Declaration of Helsinki. Informed consent was obtained from all the eligible subjects.

Study population

The National Health and Nutrition Examination Survey is a national survey conducted every year by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. The survey is combined with a series of physical examinations, interviews, and laboratory tests and uses a complex, multistage, probability sample design to be representative of the civilian, noninstitutionalized US population. Cross-sectional data examined in this study were collected from participants enrolled in the 2011–2012 cycle of the NHANES, as this is the only cycle containing results of serum fatty acid tests. The complete selection procedure for the study is shown in Figure 1. Audiometry examinations were conducted in adults aged 20–69 years. Participants lacking complete data on the otoscopic test, tympanogram test, audiometry test, and PUFAs measurement or with missing covariate data were excluded, as were participants with abnormal otoscopic results, poor-quality tympanogram results, or tympanogram with compliance $\leq$ 0.3 ml. Participants with the subsample weight value assigned as "0" in their records were excluded, as they did not provide blood specimens. Four participants with outlier values of PUFAs were also excluded. Finally, 913 adults were included in the study.

Blood PUFAs measurement

Serum samples were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA for testing. Fasting serum fatty acid concentrations were measured by electron capture negative-ion mass spectrometry based on a modification of the method outlined by Lagerstedt et al. (16). More details regarding the PUFA quantification procedure and analytical methods are available on the NHANES website (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/FAS_G.htm#LBXED1).

Audiometric measurement

Standardized pure-tone air conduction audiometric measurements were conducted in a dedicated sound-isolated room by a trained examiner. Hearing thresholds were tested on both ears of the participants at frequencies between 500 and 8,000 Hz. Pure-tone average (PTA) hearing thresholds were calculated at low (0.5, 1, and 2 kHz) and high (4, 6, and 8 kHz) frequencies. More details about the audiometry procedure and analytical methods are available on the NHANES website (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/AUX_G.htm).

Covariates

Potential covariates considered in the analyses included age, sex, race/ethnicity, education level, body mass index (BMI), diabetes, hypertension, serum cotinine, firearm noise exposure, occupational noise exposure, and recreational noise exposure. Information on age, sex, race/ethnicity, education level, diabetes, hypertension, and noise exposure was obtained from the in-home self-reported questionnaire. BMI data were calculated

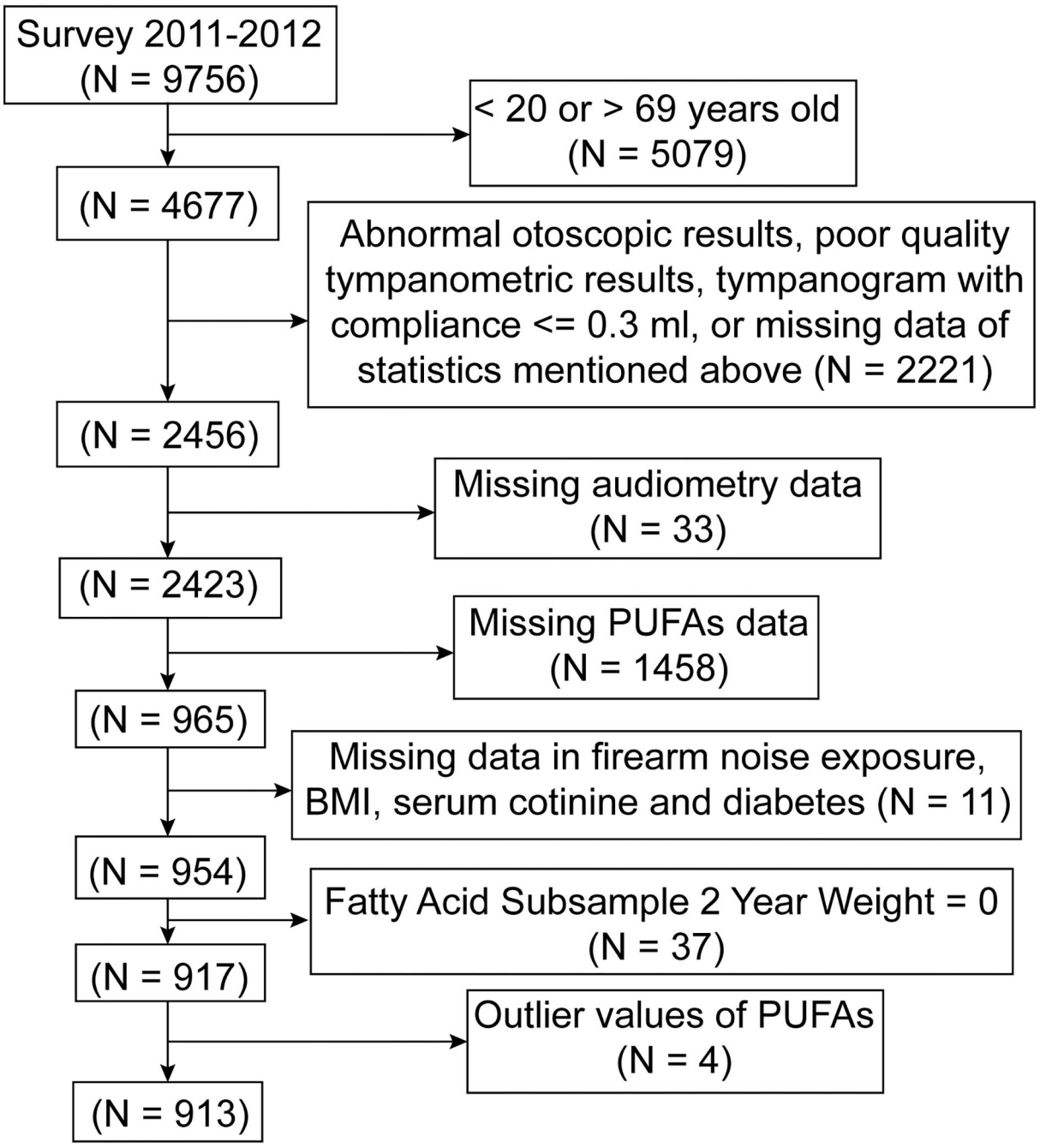


FIGURE 1
Flow chart of the patient selection process. NHANES, National Health and Nutrition Examination Survey.

from the weight and height data recorded during the physical examination. Diabetes was defined as "other than during pregnancy, ever been told by a doctor or health professional had diabetes or sugar diabetes." The answer of "borderline" was also considered as diabetes (17). Hypertension was defined as "ever been told by a doctor or other health professional had hypertension, also called high blood pressure" (17). Firearm noise exposure was defined as "ever used firearms for any reason," occupational noise exposure was defined as "ever had a job or combination of jobs exposed to loud sounds or noise for 4 or more hours a day, several days a week," and recreational noise exposure was defined as "ever been exposed to very loud noise or music for 10 or more hours a week" (17).

Statistical analysis

The study used Fatty Acid Subsample 2 Year Weight of the 2011–2012 NHANES cycle to estimate representative measures for the United States population, following the guidelines of the NCHS (18, 19). Serum concentrations of saturated, monounsaturated, and polyunsaturated fatty acids were measured in the 2011–2012 NHANES survey that included a total of 11 serum n-3 and n-6 PUFAs, which were used for analyses in our study. These PUFAs were linoleic acid (LA, 18:2n-6), γ-linolenic acid (GLA, 18:3n-6), eicosadienoic acid (EDA, 20:2n-6), homo-γ-linolenic acid (HGLA, 20:3n-6), arachidonic acid (AA, 20:4n-6), docosatetraenoic acid (DTA, 22:4n-6), docosapentaenoic acid (DPAn-6, 22:5n-6), α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). Weighted statistical differences in demographic and potential hearing-related covariables between samples grouped by sex were evaluated (Table 1). Categorical data were shown as percentages, and continuous data were presented as mean ± standard deviation (SD). The total number of participants (N = 913) was divided into tertiles for each PUFA, from the lowest concentration of each PUFA to the highest level, with almost the same number of subjects in each tertile (33%). The range of PUFA values for each tertile is shown in Supplementary Table S1. Multivariate linear regression analysis was performed to determine regression coefficients (β) and 95% confidence intervals (CIs) between PUFAs and hearing threshold shifts, adjusting for potential confounders, including age, sex, race/ethnicity, education level, BMI (categorical), diabetes, hypertension, serum cotinine, firearm noise exposure, occupational noise exposure, and recreational noise exposure. Tests for a linear trend across tertiles of serum PUFAs were conducted using the median serum PUFAs in each tertile as a continuous variable. The interactions of PUFAs with age and sex in influencing hearing thresholds were evaluated. Multivariate linear regression analysis stratified by age and sex was performed. All statistical analyses were conducted using the R programming language (version 3.6.1). A p-value of less than 0.05 was considered statistically significant.

TABLE 1 Weighted demographic characteristics of the study participants.

Variables	Male (N = 474)	Female (N = 439)	P-value[a]
Continuous variables, mean ± SD			
Age (years)	41.68 ± 13.75	42.35 ± 13.95	0.4692
BMI (kg/m^2)	28.89 ± 5.80	29.28 ± 7.39	0.3792
Low-frequency PTA (dB)[b]	7.37 ± 7.65	7.50 ± 7.75	0.7876
High-frequency PTA (dB)[b]	21.82 ± 18.70	16.20 ± 12.83	**<0.0001**
Categorical variables, %			
Race/Ethnicity			0.3074
Mexican American	7.08	8.93	
Non-Hispanic White	69.13	64.20	
Non-Hispanic Black	10.02	13.02	
Other races	13.77	13.84	
Education level			**0.0024**
Below high school	13.86	12.85	
High school	21.39	13.08	
Above high school	64.75	74.06	
BMI (categorical)			**0.0208**
Underweight (<18.5 kg/m^2)	0.53	2.15	
Normal (≥18.5 kg/m^2, <25 kg/m^2)	25.45	30.00	
Overweight (≥25 kg/m^2, <30 kg/m^2)	37.04	29.97	
Obesity (≥30 kg/m^2)	36.97	37.87	
Diabetes	7.19	7.72	0.7636
Hypertension	27.27	25.39	0.5195
Serum cotinine (≥10 ng/ml)	31.92	18.52	**<0.0001**
Firearm noise exposure	60.76	29.76	**<0.0001**
Occupational noise exposure	44.11	23.32	**<0.0001**
Recreational noise exposure	17.80	7.71	**<0.0001**

BMI, body mass index; PTA, pure-tone average. [a]p-values of continuous variables and categorical variables were calculated by the weighted linear regression model and weighted chi-square test, respectively. [b]Low-frequency and high-frequency PTA values in the better ear were computed from the average hearing thresholds of 0.5, 1, and 2 kHz and 4, 6, and 8 kHz, respectively.
The bold values indicate the significant values.

Results

Characteristics of the study participants

The study sample included 913 participants that included 439 women (weighted mean, 42.35 ± 13.95 years) and 474 men (weighted mean, 41.68 ± 13.75 years) aged between 20 and 69 years, sampled from the US population. The means ± SD of low-frequency and high-frequency PTA hearing thresholds were 7.37 ± 7.65 and 21.82 ± 18.70 dB, in male participants, respectively, and 7.50 ± 7.75 and 16.20 ± 12.83 dB in female subjects, respectively. The average high-frequency hearing status of men was worse than that of women. The education levels of men were lower than those of women. The BMI of men was higher than that of women, and men were more likely to be overweight than women. The level of serum cotinine, the biomarker of passive and positive smoking exposure, was higher in men than that in women. Men were exposed to more firearm noise, occupational noise, and recreational noise than women (all $p < 0.05$).

Multivariate regression analysis: Association between PUFAs and hearing thresholds

Table 2 shows the associations between 11 individual PUFAs with low-frequency and high-frequency hearing thresholds using a multivariate linear regression model. All PUFAs were converted to a categorical variable (tertiles) and were used as a continuous variable to calculate the linear trend. In the unadjusted model (crude model), the *p*-value for trend indicates that almost all 11 PUFAs were positively associated with low-frequency and high-frequency PTA hearing threshold shifts. Only the associations of LA, AA, DPAn-6, ALA, and DHA with low-frequency PTA were not significant. In the fully adjusted model (model 2), AA and DHA were inversely related to low-frequency PTA, while EDA, HGLA, and DPAn-6 showed positive associations with high-frequency PTA. However, significant *p* for trend was not observed among the tertiles of 6 other PUFAs and hearing threshold shifts (all *p* for trend ≥ 0.05).

Multivariate regression analysis stratified by age: Association between PUFAs and hearing thresholds

Table 3 and Supplementary Table S2 show the results for 11 PUFAs in analyses stratified by age. In general, 5 PUFAs, HGLA, DTA, DPAn-6, EPA, and DHA were associated with hearing threshold shifts that differed by age (Table 3). People aged 40–59 years in the highest tertile of HGLA, DTA, DPAn-6, EPA, and DHA and people aged 20–39 years in the highest tertile of HGLA had higher high-frequency PTA as compared to those in the lowest tertile of these PUFAs after adjusting for age, sex, race/ethnicity, education level, BMI, diabetes, hypertension, serum cotinine level, firearm noise exposure, occupational noise exposure, and recreational noise exposure (β = 2.03, 4.38, 5.90, 6.09, 6.27, and 2.03, *p* for trend = 0.0457, 0.0216, 0.0020, 0.0017, 0.0014, and 0.0457, respectively, all *p* for interaction $<$ 0.05; Table 3). However, DTA demonstrated an inverse trend with high-frequency PTA in subjects aged 60–69 years (β = −10.76, *p* for trend = 0.0020, *p* for interaction = 0.0371; Table 3). LA, EDA, and ALA showed no statistically significant interactions with age on the prediction of hearing threshold shifts (Supplementary Table S2).

Multivariate regression analysis stratified by sex: Association between PUFAs and hearing thresholds

Table 4 and Supplementary Table S3 show the associations of the tertiles of 11 PUFAs with different groups of hearing threshold shifts stratified by sex. Men in the highest tertile of EDA, AA, and DHA had better low-frequency hearing levels as compared to those in the lowest tertile after adjusting for age, race/ethnicity, education level, BMI, diabetes, hypertension, serum cotinine level, firearm noise exposure, occupational noise exposure, and recreational noise exposure (β = −1.75, −2.69, and −2.31, *p* for trend = 0.0386, 0.0008, and 0.0057, *p* for interaction = 0.0004, 0.0230, and 0.0313, respectively; Table 4). In contrast, women in the highest tertile of EDA and DPAn-6 had worse low-frequency hearing levels as compared to those in the lowest tertile after adjusting for confounders (β = 1.94 and 2.55, *p* for trend = 0.0168 and 0.0307, *p* for interaction = 0.0004 and 0.0027, respectively), so as ALA with high-frequency PTA (β = 4.13, *p* for trend = 0.0028, *p* for interaction = 0.0016) in women (Table 4). Although 9 out of 11 PUFAs showed statistically significant interactions with sex for the prediction of hearing threshold shifts, most showed no statistically significant relationship with hearing threshold shifts, except the 4 PUFAs mentioned above (Table 4 and Supplementary Table S3).

Discussion

In this nationwide cross-sectional study, we identified a relationship between n-6, n-3 PUFAs and hearing threshold shifts of adults in the United States. This research indicated that some serum PUFAs were inversely associated with low-frequency PTA, especially in men, and were positively related to high-frequency PTA, particularly in the 40–59 years old cohort. Furthermore, some serum PUFAs were found to be positively associated with both hearing threshold subgroups in women after adjusting for confounders (Tables 2–4, Supplementary Tables S1, S2). To the best of our knowledge, this is the first cross-sectional study to investigate the relationship between individual serum PUFAs and hearing threshold shifts of adults in the United States. The findings of this study suggest that PUFAs may exert both beneficial and detrimental effects on human hearing status.

Three previous population-based studies found that higher increases in n-3 PUFAs were associated with reduced HL. HL was estimated using pure-tone audiometry at speech frequency (500, 1,000, 2,000, and 4,000 Hz) or by self-report (11, 12, 14). The study conducted by Dullemeijer et al. (13) testing plasma n-3 PUFAs showed an inverse association between n-3 PUFAs and low-frequency hearing levels, which were consistent with the results of a prior study (13). However, in a recent longitudinal observational cohort study, measuring plasma concentrations of n-3 and n-6 PUFAs, no clear link was found between PUFAs and hearing function (15).

The results of our study showed that AA and DHA were inversely associated with low-frequency PTA after adjusting for related cofounders and that EDA and DHA were inversely related to low-frequency PTA in men. The benefit of PUFAs on low-frequency hearing levels was almost consistent with

TABLE 2 Multivariable linear regression model of outcomes of hearing thresholds.

Variables (umol/L)			Low-frequency PTA (dB)			High-frequency PTA (dB)		
			Crude model	Model 1	Model 2	Crude model	Model 1	Model 2
n-6	LA	β (95% CI)	0.52 (−0.10, 1.15)	−0.24 (−0.81, 0.32)	−0.18 (−0.76, 0.40)	2.91 (1.58, 4.24)	0.89 (−0.16, 1.94)	0.92 (−0.15, 1.98)
		P_{trend}	0.1026	0.3987	0.5472	**<0.0001**	0.0978	0.0928
	GLA	β (95% CI)	1.36 (0.75, 1.97)	0.06 (−0.52, 0.63)	−0.01 (−0.60, 0.58)	4.62 (3.35, 5.89)	0.67 (−0.40, 1.73)	0.51 (−0.57, 1.59)
		P_{trend}	**<0.0001**	0.8487	0.9697	**<0.0001**	0.2192	0.3584
	EDA	β (95% CI)	0.84 (0.24, 1.45)	0.01 (−0.54, 0.56)	−0.07 (−0.64, 0.50)	4.37 (3.11, 5.64)	2.18 (1.17, 3.19)	2.22 (1.19, 3.26)
		P_{trend}	**0.0064**	0.9813	0.8216	**<0.0001**	**<0.0001**	**<0.0001**
	HGLA	β (95% CI)	0.90 (0.28, 1.53)	0.14 (−0.43, 0.71)	0.03 (−0.57, 0.62)	3.49 (2.17, 4.81)	1.57 (0.52, 2.61)	1.35 (0.27, 2.44)
		P_{trend}	**0.0047**	0.6290	0.9318	**<0.0001**	**0.0033**	**0.0148**
	AA	β (95% CI)	0.46 (−0.15, 1.08)	−0.82 (−1.39, −0.26)	−0.80 (−1.37, −0.22)	3.32 (2.03, 4.61)	−0.21 (−1.26, 0.85)	−0.18 (−1.24, 0.89)
		P_{trend}	0.1378	**0.0045**	**0.0070**	**<0.0001**	0.6989	0.7459
	DTA	β (95% CI)	0.74 (0.12, 1.36)	0.02 (−0.54, 0.58)	−0.16 (−0.75, 0.43)	2.96 (1.65, 4.28)	0.64 (−0.40, 1.68)	0.30 (−0.78, 1.39)
		P_{trend}	**0.0192**	0.9485	0.5895	**<0.0001**	0.2293	0.5808
	DPAn−6	β (95% CI)	0.42 (−0.20, 1.03)	0.12 (−0.43, 0.67)	−0.04 (−0.60, 0.53)	2.07 (0.76, 3.38)	1.42 (0.40, 2.44)	1.22 (0.19, 2.26)
		P_{trend}	0.1868	0.6741	0.8938	**0.0021**	**0.0064**	**0.0206**
n-3	ALA	β (95% CI)	0.53 (−0.10, 1.15)	0.16 (−0.40, 0.72)	0.17 (−0.41, 0.74)	2.17 (0.84, 3.49)	1.00 (−0.03, 2.03)	1.00 (−0.06, 2.05)
		P_{trend}	0.0977	0.5711	0.5694	**0.0014**	0.0580	0.0637
	EPA	β (95% CI)	0.94 (0.33, 1.55)	−0.52 (−1.10, 0.05)	−0.37 (−0.96, 0.22)	4.57 (3.30, 5.85)	0.61 (−0.46, 1.67)	0.95 (−0.13, 2.03)
		P_{trend}	**0.0025**	0.0756	0.2137	**<0.0001**	0.2639	0.0863
	DPA	β (95% CI)	1.45 (0.83, 2.07)	−0.23 (−0.83, 0.37)	−0.25 (−0.86, 0.37)	4.54 (3.24, 5.84)	−0.32 (−1.44, 0.79)	−0.41 (−1.53, 0.71)
		P_{trend}	**<0.0001**	0.4538	0.4317	**<0.0001**	0.5693	0.4748
	DHA	β (95% CI)	0.01 (−0.60, 0.62)	−0.91 (−1.46, −0.36)	−0.79 (−1.36, −0.23)	2.26 (0.97, 3.55)	0.28 (−0.75, 1.31)	0.87 (−0.17, 1.92)
		P_{trend}	0.9831	**0.0013**	**0.0061**	**0.0006**	0.5979	0.1008

Crude Model = unadjusted. Model 1 = Crude Model + sex, age. Model 2 = Model 1 + race/ethnicity, education level, firearm noise exposure, occupational noise exposure, recreational noise exposure, serum cotinine, BMI, diabetes, and hypertension. The bold values indicate the significant values.

10.3389/fpubh.2022.939827

TABLE 3 Adjusted[a] associations between PUFAs and hearing threshold shifts stratified by age ($N = 913$).

					P_{trend}	$P_{interaction}$
		HGLA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	20 ≤ y < 40	Ref	0.11 (−1.06, 1.27)	0.77 (−0.52, 2.05)	0.2502	**0.0041**
	40 ≤ y <60	Ref	−0.58 (−2.56, 1.40)	0.13 (−1.77, 2.02)	0.7199	
	60 ≤ y <69	Ref	−1.83 (−7.60, 3.94)	−3.63 (−9.03, 1.78)	0.1846	
High-frequency PTA	20 ≤ y < 40	Ref	−0.01 (−1.76, 1.73)	2.03 (0.10, 3.95)	**0.0457**	**0.0182**
	40 ≤ y <60	Ref	5.49 (1.21, 9.78)	6.38 (2.28, 10.48)	**0.0056**	
	60 ≤ y <69	Ref	−3.72 (−12.10, 4.65)	−3.83 (−11.67, 4.02)	0.3737	
		DTA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	20 ≤ y < 40	Ref	−0.31 (−1.46, 0.84)	0.58 (−0.73, 1.89)	0.4546	0.2038
	40 ≤ y <60	Ref	1.09 (−0.84, 3.02)	0.71 (−1.19, 2.60)	0.5854	
	60 ≤ y <69	Ref	−6.55 (−11.71, −1.39)	−4.46 (−9.03, 0.10)	0.1000	
High-frequency PTA	20 ≤ y < 40	Ref	0.04 (−1.70, 1.78)	1.19 (−0.79, 3.16)	0.2698	**0.0371**
	40 ≤ y <60	Ref	0.80 (−3.40, 4.99)	4.38 (0.26, 8.50)	**0.0216**	
	60 ≤ y <69	Ref	−8.22 (−15.56, −0.87)	−10.76 (−17.25, 4.26)	**0.0020**	
		DPAn−6 (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	20 ≤ y < 40	Ref	1.03 (−0.72, 2.78)	0.42 (−1.33, 2.18)	0.9167	0.8037
	40 ≤ y <60	Ref	1.03 (−0.72, 2.78)	0.42 (−1.33, 2.18)	0.6942	
	60 ≤ y <69	Ref	−0.96 (−6.03, 4.11)	−0.77 (−5.27, 3.72)	0.7439	
High-frequency PTA	20 ≤ y < 40	Ref	1.73 (−2.05, 5.52)	5.90 (2.11, 9.69)	0.0676	**0.0010**
	40 ≤ y <60	Ref	1.73 (−2.05, 5.52)	5.90 (2.11, 9.69)	**0.0020**	
	60 ≤ y <69	Ref	−0.24 (−7.45, 6.97)	−5.93 (−12.31, 0.46)	0.0648	
		EPA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	20 ≤ y < 40	Ref	−0.92 (−2.05, 0.22)	−1.14 (−2.42, 0.14)	0.0628	0.4376
	40 ≤ y <60	Ref	0.48 (−1.50, 2.46)	−0.64 (−2.58, 1.30)	0.3733	
	60 ≤ y <69	Ref	−2.66 (−8.24, 2.92)	0.63 (−4.54, 5.81)	0.6878	
High-frequency PTA	20 ≤ y < 40	Ref	0.99 (−0.72, 2.71)	−0.07 (−2.00, 1.87)	0.9011	**0.0334**
	40 ≤ y <60	Ref	1.22 (−3.06, 5.50)	6.09 (1.90, 10.28)	**0.0017**	
	60 ≤ y <69	Ref	−10.50 (−18.31, −2.69)	−0.37 (−7.61, 6.88)	0.7716	
		DHA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	20 ≤ y < 40	Ref	−1.26 (−2.39, −0.13)	−1.78 (−3.07, −0.48)	**0.0048**	0.3053
	40 ≤ y <60	Ref	−0.99 (−2.73, 0.76)	−0.94 (−2.72, 0.84)	0.3134	
	60 ≤ y <69	Ref	−0.15 (−5.63, 5.33)	−1.92 (−6.33, 2.49)	0.3878	
High-frequency PTA	20 ≤ y < 40	Ref	−0.83 (−2.56, 0.89)	−0.55 (−2.52, 1.43)	0.5064	**0.0028**
	40 ≤ y <60	Ref	2.01 (−1.77, 5.78)	6.27 (2.42, 10.12)	**0.0014**	
	60 ≤ y <69	Ref	−8.00 (−15.82, 0.18)	−5.93 (−12.31, 0.46)	0.4079	

[a]Adjusted for age, sex, race/ethnicity, education level, BMI, diabetes, hypertension, serum cotinine, firearm noise exposure, occupational noise exposure, and recreational noise exposure. The bold values indicate the significant values.

TABLE 4 Adjusted[a] associations between PUFAs and hearing threshold shifts stratified by sex ($N = 913$).

					P_{trend}	$P_{interaction}$
		EDA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	Male	Ref	0.42 (−1.13, 1.98)	−1.75 (−3.37, −0.13)	**0.0386**	**0.0004**
	Female	Ref	0.77 (−0.81, 2.36)	1.94 (0.34, 3.54)	**0.0168**	
High-frequency PTA	Male	Ref	1.42 (−1.78, 4.62)	3.34 (0.01, 6.68)	0.0501	0.1601
	Female	Ref	1.36 (−1.00, 3.71)	5.41 (3.03, 7.78)	<0.0001	
		AA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	Male	Ref	−2.05 (−3.58, −0.51)	−2.69 (−4.24, −1.14)	**0.0008**	**0.0230**
	Female	Ref	0.48 (−1.10, 2.06)	−0.06 (−1.78, 1.67)	0.9547	
High-frequency PTA	Male	Ref	−1.89 (−5.07, 1.30)	−0.56 (−3.78, 2.66)	0.7411	0.2258
	Female	Ref	1.21 (−1.18, 3.59)	0.74 (−1.87, 3.35)	0.5723	
		DPAn-6 (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	Male	Ref	0.20 (−1.55, 1.96)	−1.26 (−3.37, 0.84)	0.2321	**0.0027**
	Female	Ref	1.40 (−0.39, 3.18)	2.55 (0.24, 4.85)	**0.0307**	
High-frequency PTA	Male	Ref	2.13 (−1.46, 5.73)	4.16 (−0.16, 8.48)	0.0594	0.8201
	Female	Ref	2.22 (−0.46, 4.90)	3.54 (0.08, 7.01)	**0.0450**	
		ALA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	Male	Ref	0.96 (−0.67, 2.58)	−0.36 (−2.05, 1.33)	0.6071	**0.0002**
	Female	Ref	0.86 (−0.70, 2.42)	1.51 (−0.28, 3.30)	0.0959	
High-frequency PTA	Male	Ref	−2.07 (−5.40, 1.27)	0.66 (−2.80, 4.13)	0.6347	**0.0016**
	Female	Ref	1.12 (−1.20, 3.45)	4.13 (1.47, 6.79)	**0.0028**	
		DHA (umol/L) β (95% CI)				
		Tertile 1	**Tertile 2**	**Tertile 3**		
Low-frequency PTA	Male	Ref	−0.59 (−2.07, 0.89)	−2.31 (−3.94, −0.67)	**0.0057**	**0.0313**
	Female	Ref	−1.23 (−2.85, 0.39)	−1.54 (−3.22, 0.15)	0.5176	
High-frequency PTA	Male	Ref	−1.61 (−4.67, 1.44)	1.81 (−1.56, 5.18)	0.1018	0.3300
	Female	Ref	1.16 (−1.28, 3.60)	0.16 (−2.37, 2.70)	0.4506	

[a]Adjusted for age, race/ethnicity, education level, BMI, diabetes, hypertension, serum cotinine level, firearm noise exposure, occupational noise exposure, and recreational noise exposure. The bold values indicate the significant values.

findings of previous population-based and animal studies (11–14, 20). Cochlear blood flow must be well regulated to meet the metabolic demand of the inner ear. Impaired cochlear blood flow may lead to damage to hair cells, resulting in the development of hearing impairment. The n-3 PUFAs may benefit hearing by the maintenance of adequate cochlear vascular supply through multiple mechanisms, including triglyceride lowering, hypolipidemic properties, and anti-inflammatory and anti-atherothrombotic properties (21, 22). Evidence has also shown that dietary n-6 PUFA may help to improve endothelial function and chronic inflammation (23).

Though in general, some PUFAs were found to be beneficial for the low-frequency hearing threshold, and EDA, HGLA, and DPAn-6 showed a positive association with high-frequency PTA. DTA, DPAn-6, EPA, and DHA in participants aged 40–59 years and HGLA in participants aged 20–59 years were positively associated with high-frequency PTA. EDA and DPAn-6 were positively associated with low-frequency PTA in women, and

ALA was associated with high-frequency PTA in women. There are some pieces of evidence to show the detrimental effect of PUFAs on the hearing status and auditory development, which support our findings (20, 24–28). The association of PUFAs with hearing level is more obvious in middle-aged participants younger than 60 years, indicating that the onset of PUFAs' effect on age-related hearing impairment is much earlier than that previously reported (11, 13). PUFAs showed a protective role for hearing in men and a detrimental role in women, which is in contrast with previous findings (12, 14). More studies are needed to better understand the differences between men and women to reach a consensus.

Our study has several strengths, including the large and nationally representative sample cohort extracted from the NHANES. The selection was standardized to achieve minimized selection bias. Furthermore, standardized, audiometric testing was used to measure the pure-tone hearing threshold. Participants with abnormal otoscopic examination results, tympanogram compliance $\leq$ 0.3 ml, or poor-quality results in tympanogram were excluded to avoid analyzing data for conductive or mixed hearing loss. Our analyses were further adjusted for confounding factors that included age, sex, race, education level, BMI, diabetes, hypertension, serum cotinine level, and noise exposure that could result in a misinterpretation. The effects of PUFAs on both low-frequency and high-frequency hearing levels were estimated, with the result of broader frequency estimates than those in previous research studies (11, 12, 14). In addition, individual serum PUFAs were used as a valid estimate of dietary intake of fatty acids (29).

Despite these strengths, this study also has some limitations, which should be mentioned. The results of this study did not permit a temporal relation to be examined because of the cross-sectional design of the NHANES (17). Although the status of serum PUFAs may vary widely depending on dietary intake, this study looked at their concentrations at the one-time point. Furthermore, some potential confounders were not calculated in the models; only the main confounders, which have been reported in previous studies, were included. The results would be more accurate if we consider all other confounders.

Conclusion

According to the results of the NHANES data analyses, some serum PUFAs were inversely associated with low-frequency PTA, especially in men, while others were positively related to high-frequency PTA, particularly in the 40–59 years old cohort. Furthermore, some of the serum PUFAs were positively associated with both hearing threshold subgroups in women. In general, serum PUFAs tended to be beneficial for low-frequency hearing status and detrimental to the high-frequency hearing threshold. The male sex may play a protective role in this association, while the female sex and middle age may be detrimental in the effect of PUFAs on hearing function.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2011.

Author contributions

LL, ZJ, and XT completed the conceptualization. LL made a formal analysis of the data, wrote the original draft, and completed the methodology. ZJ and XT completed the review and editing, revising, and final approval and are accountable for all aspects. All authors have approved the final manuscript as submitted.

Acknowledgments

All authors would like to thank NHANES for providing the publicly available data.

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/fpubh.2022.939827/full#supplementary-material

References

1. Mahboubi H, Lin HW, Bhattacharyya N. Prevalence, characteristics, and treatment patterns of hearing difficulty in the United States. *JAMA Otolaryngol. Head Neck Surg.* (2018) 144:65–70. doi: 10.1001/jamaoto.2017.2223

2. Li CM, Zhang X, Hoffman HJ, Cotch MF, Themann CL, Wilson MR. Hearing impairment associated with depression in US adults, national health and nutrition examination survey 2005–2010. *JAMA Otolaryngol. Head Neck Surg.* (2014) 140:293–302. doi: 10.1001/jamaoto.2014.42

3. Zheng Y, Fan S, Liao W, Fang W, Xiao S, Liu J. Hearing impairment and risk of Alzheimer's disease: a meta-analysis of prospective cohort studies. *Neurol Sci.* (2017) 38:233–9. doi: 10.1007/s10072-016-2779-3

4. Kim SY, Min C, Kim HJ, Lee CH, Sim S, Park B, et al. Mortality and cause of death in hearing loss participants: a longitudinal follow-up study using a national sample cohort. *Otol Neuroto.* (2020) 41:25–32. doi: 10.1097/MAO.0000000000002429

5. Huddle MG, Goman AM, Kernizan FC, Foley DM, Price C, Frick KD, et al. The economic impact of adult hearing loss: a systematic review. *JAMA Otolaryngol. Head Neck Surg.* (2017) 143:1040–8. doi: 10.1001/jamaoto.2017.1243

6. Schuknecht HF, Gacek MR. Cochlear pathology in presbycusis. *Ann Otol Rrhinol Laryngol.* (1993) 102:1–16. doi: 10.1177/00034894931020S101

7. Nakashima T, Naganawa S, Sone M, Tominaga M, Hayashi H, Yamamoto H, et al. Disorders of cochlear blood flow. *Brain Res Brain Res Rev.* (2003) 43:17–28. doi: 10.1016/S0165-0173(03)00189-9

8. Gates GA, Cobb JL, D'Agostino RB, Wolf PA. The relation of hearing in the elderly to the presence of cardiovascular disease and cardiovascular risk factors. *Arch Otolaryngol Head Neck Surg.* (1993) 119:156–61. doi: 10.1001/archotol.1993.01880140038006

9. Oppedisano F, Mollace R, Tavernese A, Gliozzi M, Musolino V, Macrì R, et al. PUFA supplementation and heart failure: effects on fibrosis and cardiac remodeling. *Nutrients.* (2021) 13:2965. doi: 10.3390/nu13092965

10. Yamagata K. Dietary docosahexaenoic acid inhibits neurodegeneration and prevents stroke. *J Neurosci Res.* (2021) 99:561–72. doi: 10.1002/jnr.24728

11. Gopinath B, Flood VM, Rochtchina E, McMahon CM, Mitchell P. Consumption of omega-3 fatty acids and fish and risk of age-related hearing loss. *Am J Clin Nutr.* (2010) 92:416–21. doi: 10.3945/ajcn.2010.29370

12. Curhan SG, Eavey RD, Wang M, Rimm EB, Curhan GC. Fish and fatty acid consumption and the risk of hearing loss in women. *Am J Clin Nutr.* (2014) 100:1371–7. doi: 10.3945/ajcn.114.091819

13. Dullemeijer C, Verhoef P, Brouwer IA, Kok FJ, Brummer RJ, Durga J. Plasma very long-chain n-3 polyunsaturated fatty acids and age-related hearing loss in older adults. *J Nutr Health Aging.* (2010) 14:347–51. doi: 10.1007/s12603-010-0078-x

14. Yévenes-Briones H, Caballero FF, Struijk EA, Lana A, Rodríguez-Artalejo F, Lopez-Garcia E. Dietary fat intake and risk of disabling hearing impairment: a prospective population-based cohort study. *Eur J Nutr.* (2022) 61:231–42. doi: 10.1007/s00394-021-02644-7

15. Orlando MS, Dziorny AC, Love T, Harrington D, Shamlaye CF, Watson G, et al. Association of audiometric measures with plasma long chain polyunsaturated fatty acids in a high-fish eating population: the seychelles child development study. *Neurotoxicology.* (2020) 77:137–44. doi: 10.1016/j.neuro.2020.01.005

16. Lagerstedt SA, Hinrichs DR, Batt SM, Magera MJ, Rinaldo P, McConnell JP. Quantitative determination of plasma c8-c26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. *Mol Genet Metab.* (2001) 73:38–45. doi: 10.1006/mgme.2001.3170

17. Long L, Tang Y. Urine caffeine metabolites and hearing threshold shifts in US adults: a cross-sectional study. *Sci Rep.* (2021) 11:21631. doi: 10.1038/s41598-021-01094-9

18. Chen TC, Parker JD, Clark J, Shin HC, Rammon JR, Burt VL. National health and nutrition examination survey: estimation procedures, 2011-2014. *Vital Health Stat 2.* (2018) 177:1–26. Available online at: https://www.cdc.gov/nchs/data/series/sr_02/sr02_177.pdf

19. Lim ZW, Chen WL. Exploring the association of Bone Alkaline Phosphatases And Hearing Loss. *Sci Rep.* (2020) 10:4006. doi: 10.1038/s41598-020-60979-3

20. Honkura Y, Suzuki J, Sakayori N, Inada H, Kawase T, Katori Y, et al. Effects of enriched endogenous omega-3 fatty acids on age-related hearing loss in mice. *BMC Res Notes.* (2019) 12:768. doi: 10.1186/s13104-019-4809-8

21. Connor WE. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr.* (2000) 71:171S—5S. doi: 10.1093/ajcn/71.1.171S

22. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol.* (2011) 58:2047–67. doi: 10.1016/j.jacc.2011.06.063

23. Seah JY, Gay GM, Su J, Tai ES, Yuan JM, Koh WP, et al. Consumption of red meat, but not cooking oils high in polyunsaturated fat, is associated with higher arachidonic acid status in Singapore Chinese adults. *Nutrients.* (2017) 9:101. doi: 10.3390/nu9020101

24. Church MW, Jen KL, Anumba JI, Jackson DA, Adams BR, Hotra JW. Excess omega-3 fatty acid consumption by mothers during pregnancy and lactation caused shorter life span and abnormal ABRs in old adult offspring. *Neurotoxicol Teratol.* (2010) 32:171–81. doi: 10.1016/j.ntt.2009.09.006

25. Church MW, Jen KL, Jackson DA, Adams BR, Hotra JW. Abnormal neurological responses in young adult offspring caused by excess omega-3 fatty acid (fish oil) consumption by the mother during pregnancy and lactation. *Neurotoxicol Teratol.* (2009) 31:26–33. doi: 10.1016/j.ntt.2008.09.001

26. Church MW, Jen KL, Stafferton T, Hotra JW, Adams BR. Reduced auditory acuity in rat pups from excess and deficient omega-3 fatty acid consumption by the mother. *Neurotoxicol Teratol.* (2007) 29:203–10. doi: 10.1016/j.ntt.2006.10.009

27. Haubner LY, Stockard JE, Saste MD, Benford VJ, Phelps CP, Chen LT, et al. Maternal dietary docosahexanoic acid content affects the rat pup auditory system. *Brain Res Bull.* (2002) 58:1–5. doi: 10.1016/S0361-9230(01)00764-X

28. Stockard JE, Saste MD, Benford VJ, Barness L, Auestad N, Carver JD. Effect of docosahexaenoic acid content of maternal diet on auditory brainstem conduction times in rat pups. *Dev Neurosci.* (2000) 22:494–9. doi: 10.1159/000017481

29. Lelli D, Antonelli Incalzi R, Ferrucci L, Bandinelli S, Pedone C. Association between PUFA intake and serum concentration and mortality in older adults: a cohort study. *Clin Nutr.* (2020) 39:510–5. doi: 10.1016/j.clnu.2019.02.030

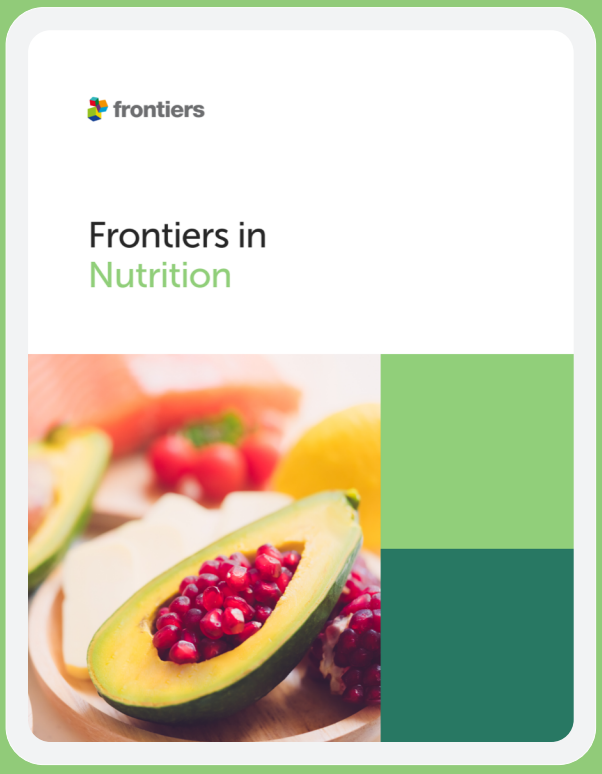
Frontiers in Nutrition

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