British Journal of Nutrition (2024), 131, 1720–1729 doi:10.1017/S0007114524000266
© The Author(s), 2024. Published by Cambridge University Press on behalf of The Nutrition Society

Association between *n*-3 PUFA and lung function: results from the NHANES 2007–2012 and Mendelian randomisation study

Jingli Li[1], Jian Sun[1], Lingjing Liu[2], Chunyi Zhang[1] and Zixiang Liu[1]*

[1]*Department of Pulmonary and Critical Care Medicine, Shaoxing People's Hospital, Shaoxing 312000, Zhejiang, People's Republic of China*

[2]*Department of Pulmonary and Critical Care Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, People's Republic of China*

(Submitted 19 September 2023 – Final revision received 29 December 2023 – Accepted 23 January 2024 – First published online 26 January 2024)

Abstract

This study aimed to investigate the association between *n*-3 PUFA and lung function. First, a cross-sectional study was conducted based on the National Health and Nutrition Examination Survey (NHANES) 2007–2012 data. *n*-3 PUFA intake was obtained from 24-h dietary recalls. A multivariable linear regression model was used to assess the observational associations of *n*-3 PUFA intake with lung function. Subsequently, a two-sample Mendelian randomisation (MR) was performed to estimate the potential causal effect of *n*-3 PUFA on lung function. Genetic instrumental variables were extracted from published genome-wide association studies. Summary statistics about *n*-3 PUFA was from UK Biobank. Inverse variance weighted was the primary analysis approach. The observational study did not demonstrate a significant association between *n*-3 PUFA intake and most lung function measures; however, a notable exception was observed with significant findings in the highest quartile for forced vital capacity (FVC) and % predicted FVC. The MR results also showed no causal effect of circulating *n*-3 PUFA concentration on lung function (forced expiratory volume in one second (FEV_1), $\beta = 0{\cdot}01301$, SE = 0·01932, $P = 0{\cdot}5006$; FVC, $\beta = -0{\cdot}001894$, SE = 0·01704, $P = 0{\cdot}9115$; FEV_1:FVC, $\beta = 0{\cdot}03118$, SE = 0·01743, $P = 0{\cdot}07359$). These findings indicate the need for further investigation into the impact of higher *n*-3 PUFA consumption on lung health.

Keywords: ***n*-3 PUFA: Lung function: National Health and Nutrition Examination Survey: Mendelian randomisation**

Lung function is a critical predictor of various lung diseases and overall health in the general population[1]. The assessment of lung function is typically performed by utilising measures such as forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the ratio of FEV_1 to FVC (FEV_1:FVC)[2]. Environmental exposures, such as smoking, air pollution and occupational hazards, have been recognised as major contributors to the deterioration of lung function[3–5]. This decline can culminate in the clinical diagnosis of chronic obstructive pulmonary disease, which poses growing social and economic challenges as the third leading cause of morbidity globally[6]. It is thus of great clinical and public health interest to improve lung function.

In recent years, there has been a growing interest in the health benefits of *n*-3 PUFA. EPA and DHA, the two major essential *n*-3 PUFA, have been proven to have anti-inflammatory, antioxidant and immunoregulatory properties[7]. The potential of *n*-3 PUFA for the improvement of lung health is an emerging field of research. Nevertheless, the results from epidemiological studies have not been entirely consistent. While one observational cohort study found a positive correlation between dietary intake of *n*-3 PUFA and lung function[8], a cross-sectional study from a representative sample of Dutch adults showed no improvement in lung function with dietary *n*-3 PUFA intake[9]. Therefore, the association between *n*-3 PUFA and lung function remains equivocal and needs further investigation.

Of note, dietary intake of *n*-3 PUFA is often associated with a variety of clinical and social factors, making it challenging to determine the causal effects of diets on various outcomes. Additionally, the observed association between *n*-3 PUFA intake and lung function may also be influenced by confounders, such as smoking and occupational exposures, which cannot be adequately controlled for using observational study designs[10]. Mendelian randomisation (MR) has emerged as a powerful approach to address the limitations of observational studies by using genetic variants as instrumental variables (IV) to estimate

Abbreviations: FEV1, forced expiratory volume in one second; FVC, forced vital capacity; GWAS, genome-wide association studies; IV, instrumental variables; IVW, inverse variance weighted; MR, Mendelian randomisation; NHANES, National Health and Nutrition Examination Survey.

* **Corresponding author:** Zixiang Liu, email zxshaoxingrmyy@163.com

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

the potential causal effect of exposures (e.g. serum *n*-3 PUFA) on outcomes (e.g. lung function)[11]. Given genetic variants are randomly allocated at conception, MR studies are less susceptible to biases of confounding, residual bias and reverse causality[12]. Hence, MR studies, which are similar in concept to randomised controlled trials, can substantially improve causal inference from observational investigations[13]. Our study adds to the current literature by employing MR to address these potential confounders that have challenged previous observational research. This is especially pertinent in light of previous research that has produced varying results. With this approach, our study provides new insights into the causal relationships between *n*-3 PUFA intake and lung function.

Therefore, in the present study, we performed a cross-sectional study utilising data from the National Health and Nutrition Examination Survey (NHANES), to assess the observational association between *n*-3 PUFA intake and lung function. Subsequently, we conducted the two-sample MR analysis to further estimate the causal relationship between circulating *n*-3 PUFA concentration and lung function.

Methods

Cross-Sectional study

Study population. NHANES is a continuous, stratified, multistage sampling, cross-sectional study designed to evaluate the health and nutritional status of the non-institutionalised civilian population of the USA[14]. This study is performed by the National Center for Health Statistics (NCHS), a branch of the Centers for Disease Control and Prevention (CDC) and is approved by the NCHS Research Ethics Review Board[15]. All participants provided written informed consent. The data collected from the study are publicly available through the CDC website (http://www.cdc.gov/nchs/nhanes.htm). For the present study, publicly accessible data from three NHANES cycles (2007–2008, 2009–2010 and 2011–2012) were analysed, as lung function data were only available during these cycles. A total of 29 353 participants were included in the 2007–2012 cycles, with 10 693 participants aged 20 years or older without pregnancy being screened in the analysis. Participants who had missing information on dietary *n*-3 PUFA intake (*n* 1092), had incomplete lung function test results or did not meet the American Thoracic Society data collection standards (quality grades C–F, *n* 4780) were excluded. Additionally, participants with missing data on BMI, marital status, drinking, smoking, hypertension, diabetes, emphysema, chronic bronchitis and asthma were also excluded (*n* 1653). In examining the sociodemographic characteristics of the excluded sample in comparison with the analytic sample, we observed a consistent distribution across key variables. Both samples exhibited similar profiles in terms of age, sex, race, educational attainment and income levels. After all exclusions, the final analysis sample consisted of 9378 participants (online Supplementary Fig. S1).

Lung function measurement. Spirometry was available in NHANES 2007–2012. Participants who were receiving supplemental oxygen or exhibited symptoms such as chest pain, difficulties with forceful expiration, recent surgical procedures in the eye, chest, or abdomen, recent heart attack, stroke, tuberculosis exposure, or coughing up of blood, or a history of detached retina, collapsed lung, or aneurysm were not eligible for spirometry testing. Eligible participants underwent spirometry testing using an Ohio 822/827 dry-rolling seal volume spirometer, in accordance with the guidelines established by the American Thoracic Society and European Respiratory Society[16]. The best FEV_1, FVC and the FEV_1:FVC ratio were selected for data analysis, which are commonly used clinical indicators of lung function. In addition to the primary lung function indicators, peak expiratory flow (PEF) and forced expiratory volume in 25 %–75 % of FVC (FEV25 %–75 %) were also measured. The percent predicted lung function (FEV_1 and FVC) were calculated using Global Lung Initiative equations that account for age, sex, race and height[17].

Dietary intake assessment. The daily intake of *n*-3 PUFA in the NHANES survey was assessed through two 24-h dietary recall interviews included in the NHANES Individual Foods files. The first interview was conducted in-person by trained interviewers in the Mobile Examination Center (MEC), while the second was a follow-up telephone interview conducted from the home office 3–10 d later, but not on the same day of the week as the first interview[18]. In cases where a second day of dietary recall data was available, only data from the first dietary interview were used. The total and subtypes of *n*-3 PUFA intake were calculated as an average of the 2 d of dietary recalls, which were then adjusted for body weight (mg/kg/d) and divided into quartiles. The *n*-3 PUFA analysed in this study included octadecatrienoic acid (linolenic, ALA, 18:3*n*-3), octadecatetraenoic acid (stearidonic, SDA, 18:4*n*-3), EPA (20:5*n*-3), docosapentaenoic acid (DPA, 22:5*n*-3) and DHA (22:6*n*-3).

Covariates of interest. The following variables were included as potential covariates in this study: age (categorised into 20–39 years, 40–59 years and ≥ 60 years), sex (male and female), ethnicity (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black and other race), educational level (below high school, high school graduate/GED, and some college or above), family poverty:income ratio (< 1·0, 1·0 to < 3·0 and ≥ 3·0), marital status (married/living with a partner, and windowed/divorced/separated/never married), smoking status (never smoker, former smoker and current smoker), drinking status (none, moderate and heavy), BMI (< 25 kg/m^2, 25 to < 30 kg/m^2 and ≥ 30 kg/m^2), emphysema, chronic bronchitis, asthma, hypertension, diabetes, total energy intake, protein intake, dietary fibre intake, fat intake, cholesterol intake, saturated fat, MUFA and PUFA. Smoking status was defined by CDC and NCHS as follows: never (has never smoked or has smoked less than 100 cigarettes in their lifetime), current smoker (has smoked 100 cigarettes in their lifetime and is currently smoking) or former (has smoked more than 100 cigarettes in their lifetime but has quit smoking)[19]. Drinkers were defined as participants who consumed ≥ 12 alcoholic drinks in a year. Moderate drinkers were defined as those who consumed < 1 drink per d (for female) or < 2 drinks per d (for male), while heavy drinkers were defined as those who consumed ≥ 1 drink

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

per d (for female) or ≥ 2 drinks per d (for male)[20]. Participants with physician-diagnosed diabetes, use of oral antidiabetic agents or insulin injections, fasting plasma glucose level of 7·0 mmol/l or higher, or glycosylated Hb (HbA1c) level of 6·5 % or higher were considered to have diabetes mellitus[21]. Hypertension was defined as average systolic blood pressure ≥ 140 mmHg, average diastolic blood pressure ≥ 90 mmHg, a self-reported physician diagnosis of hypertension or use of antihypertensive medications[22]. Emphysema was defined as a positive answer to the question: 'Has a doctor or other health professional ever told you that you have emphysema?'. Current chronic bronchitis was defined as a positive answer to both questions 'Has a doctor or other health professional ever told you that you have chronic bronchitis?' and 'Do you still have chronic bronchitis?'.

Statistical analysis. In this study, the normality of continuous variables was evaluated using the Kolmogorov–Smirnov test and histogram; if a variable was found to be normally distributed, it was described by the mean and standard error, otherwise, it was expressed as the median and interquartile range. Categorical data were presented in terms of counts and percentages. The participants were divided into four groups according to quartiles of *n*-3 PUFA intake, with the lowest quartile (Q1) serving as the reference category. The comparison of continuous variables was performed using either one-way ANOVA test if the variance was homogeneous, or Kruskal–Wallis non-parametric test if not. Meanwhile, χ^2 tests were used to compare the percentages of categorical variables. Population-weighted univariate and multivariable linear regression models were used to evaluate the association between lung function measurements and dietary *n*-3 PUFA intake, with the results presented as β-coefficients along with 95 % CI. The intake of *n*-3 PUFA was divided into quartiles (quartile 1: < 25th percentile, quartile 2: ≥ 25th to 50th percentile, quartile 3: ≥ 50th to 75th percentile and quartile 4: ≥ 75th percentile) according to their distributions. Univariate regression analysis (model 1) examined the association without adjustments. The multivariable regression analyses were performed with adjustments for potential confounders including age, sex, race, educational level, poverty:income ratio, BMI, drinking, smoking, diabetes and hypertension (model 2). Selection of these covariates for model 2 was based on their statistical significance with lung function from univariate analysis. A stepwise regression then refined these covariates, retaining those with significant contributions. To ensure model robustness, we excluded covariates with high multicollinearity. A two-sided *P*-value of less than 0·05 was considered statistically significant. All statistical analyses were performed using Stata 15.0 software (Stata Corporation), taking into account the weighted data and complex design of the NHANES sample.

Mendelian randomisation study

Study design. This study used a two-sample MR study design to estimate the potential causality between exposures and outcomes, using genetic variants as IV. Briefly, we analysed summary statistics from multiple genome-wide association studies (GWAS) to identify SNP that were associated with serum levels of *n*-3 PUFA and lung function. The identified SNP were then combined to evaluate their causal relationship. The validity of MR relies on the following assumptions[23], as demonstrated in online Supplementary Fig. S2: (1) the genetic IV are associated with exposure factor; (2) IV are independent of known and unknown confounders; (3) IV are associated with outcome only via exposure factors. This study's analysis is a secondary examination of publicly available data, not involving new human or animal research. All utilised GWAS datasets are openly accessible, negating the need for ethical approval or informed consent.

Selection of genetic instruments for n-3 PUFA. In the MR analysis, we extracted SNP strongly associated with circulating *n*-3 PUFA concentration as instruments from the most recent and largest available GWAS study, which contained 114 999 participants of European ancestry from the UK Biobank[24]. To select SNP as IV, we screened the genome-wide significant SNP ($P < 5 \times 10^{-8}$) that showed a strong relationship with exposure factors. We performed a clustering process with a pruning threshold of $R^2 < 0{\cdot}001$ and a clumping distance of 10 000 kb to eliminate linkage disequilibrium between genetic variants. Additionally, we excluded palindrome SNP to avoid potential strand ambiguity and set a minimum allele frequency of 0·01 without the use of SNP proxies. The total coefficient of determination (R2) and mean F statistics across selected SNP were estimated to judge the strength of the selected IV, with an F statistic threshold of 10 indicating sufficient strength for MR analyses, as previously described[25,26].

Genetic summary data for lung function. Summary statistics of lung function were extracted from the largest available GWAS meta-analysis of UK Biobank, which consists of 321 047 individuals[27]. Lung function measurements in this study included FEV_1, FVC and FEV_1:FVC. To ensure data quality, the inclusion criteria were limited to participants who had at least two measurements of FEV_1 and FVC, and full details for age, sex, height, ever-smoking status, and spirometry method used were included. In addition, given the important roles of airflow limitation in diagnosing chronic obstructive pulmonary disease, we also utilised summary-level data obtained from a GWAS that analysed the risk of FEV_1:FVC < 0·7 in individuals of European descent (*n* 353 315)[28].

Statistical analysis. We used inverse variance weighted (IVW) as the primary MR analysis approach to evaluate the causal association of genetically predicted circulating *n*-3 PUFA concentration and lung function[29]. We also performed multiple sensitivity analyses, including MR-Egger, weighted median and weighted mode to validate the robustness of the IVW results[30]. In the current study, an association was deemed statistically significant when the IVW achieved a significance level of $P < 0{\cdot}05$, and the results of other analytical methods pointed in the same direction as that of the IVW results. In addition, we used MR-Egger regression to assess the presence of potential pleiotropic effects of IV[12]. The absence of horizontal pleiotropy can be inferred when the MR-Egger intercept term was close to 0 and $P > 0{\cdot}05$. The heterogeneity of SNP was examined using

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

Cochran's Q test in both MR-Egger and IVW methods[31]. A leave-one-out sensitivity analysis was also adopted by removing each SNP one by one to evaluate the influence of each SNP on the overall results obtained using MR-Egger and IVW methods[32]. All statistical analyses were conducted utilising R version 4.0.3 and the TwoSampleMR R package version 0.5.5[33].

Results

In Table 1, we present the characteristics of the study participants from the US NHANES sample. Overall, 49·5 % of the eligible participants were men and 50·5 % female, with a mean age of 46·0 years and a mean BMI of 29·08 kg/m^2. Of the participants, 55·6 % reported never having smoked, 23·8 % were former smokers and 21·4 % were current smokers. The mean levels of FEV_1, FVC, FEV_1:FVC, PEF and FEV25 %–75 % were 3952·0 ml, 3096·1 ml, 78·3 %, 8140·6 ml/s and 2947·7 ml/s, respectively. Participants with higher *n*-3 PUFA intake were found to be predominantly male, younger, non-Hispanic White, with higher family income and education level, as well as lower BMI and CRP level ($P < 0·001$). Conversely, individuals with lower *n*-3 PUFA intake tended to be smokers, drinkers, and patients with hypertension and diabetes. In addition, FEV_1, FVC, PEF and FEV25 %–75 % were significantly higher in participants with higher *n*-3 PUFA intake compared with those with lower *n*-3 PUFA intake.

Table 2 displays the findings of a linear regression analysis investigating the relationship between dietary *n*-3 PUFA intake and lung function measurements. In the univariate analysis of the entire study population (model 1), a positive association was found between *n*-3 PUFA intakes and lung function. However, after adjusting for relevant confounders (model 2), participants in the highest quartile intake of *n*-3 PUFA showed only higher FVC and percent predicted FVC compared with the lowest quartile of intake (43·67 ml and 0·86 % for FVC and percent predicted FVC, respectively, $P = 0·009$ and 0·04). There was no statistically significant association identified between the intake of *n*-3 PUFA and FEV_1 or percent predicted FEV_1. In the stratified analysis by smoking status, including current, former, and never smokers, there was no statistically significant association identified between the intake of *n*-3 PUFA and lung function (online Supplementary Table S1). Upon excluding ALA from the *n*-3 PUFA intake, the association between *n*-3 PUFA intake and lung function measurements remained consistent with the non-significant associations observed in the initial models, suggesting that ALA does not drive the observed lack of association within our study population (online Supplementary Table S2).

After harmonising the SNP effects, thirty-nine SNP associated with circulating *n*-3 PUFA concentration were used as IV in our two-sample MR analysis, as shown in online Supplementary Table S3. Detailed summary information of these instruments is presented in Table 3. The mean F-statistic for *n*-3 PUFA was above 200, considerable weak instrument bias would not be expected. There was no genetic evidence of a causal relationship between circulating *n*-3 PUFA concentration and lung function using IVW method; MR-Egger regression, weighted median, weighted mode and simple mode methods presented similar results (Table 4). The scatter plots and forest plots of these MR results are presented in Fig. 1 and online Supplementary Fig. S3, respectively. There was evidence for heterogeneity existed, measured by Cochran's *Q* test ($P < 0·001$) based on both IVW and MR-Egger methods (Table 4). Hence, a multiplicative random effects model (inverse variance-weighted regression) was employed to reassess the causal effects, and the findings were consistent (FEV_1, $P = 0·5006$; FVC, $P = 0·9115$; FEV1:FVC, $P = 0·07359$; the risk of FEV_1:FVC < 0·7, $P = 0·2932$). The MR-Egger intercept did not deviate significantly from 0 ($P > 0·05$), indicating no evidence of horizontal pleiotropy (Table 4). In addition, the results of the leave-one-out sensitivity analysis showed that the observed causal association was relatively credible by removing any SNP (online Supplementary Fig. S4).

Discussion

In this study, we sought to investigate the association between *n*-3 PUFA intake and lung function by conducting observational analyses based on the NHANES data and then estimate the potential causal relationship between circulating *n*-3 PUFA concentration and lung function through a two-sample MR analysis. Our findings revealed that *n*-3 PUFA intake was not associated with lung function, and that elevated circulating *n*-3 PUFA concentration, as genetically predicted, did not result in improvement in lung function. These results suggest that increasing *n*-3 PUFA intake to elevate circulating *n*-3 PUFA concentration is unlikely to provide a clinical benefit for enhancing lung function. Our study stands out as the first to utilise both cross-sectional and MR analysis to explore the relationship between *n*-3 PUFA and lung function, providing a unique contribution to the field. The incorporation of NHANES data and MR adds a novel dimension to the current understanding, particularly in a US context where dietary intake is often suboptimal.

n-3 PUFA are considered as essential nutrients and primarily obtained from exogenous sources, such as seafood, due to the low efficiency of endogenous synthesis from precursors[34]. The potent anti-inflammatory and antioxidant properties of *n*-3 PUFA have been demonstrated to have beneficial effects in several chronic inflammatory diseases, such as CVD, diabetes, rheumatoid arthritis and even cancer[35]. There is increasing evidence indicating that *n*-3 PUFA may have a protective effect on the lungs against the detrimental impacts of chronic inflammation and oxidative stress[36]. Some studies have reported that supplementation with *n*-3 PUFA can decrease inflammatory markers and improve asthma symptoms[37,38]. A study conducted in the USA with a sample size of 8960 found that intake of *n*-3 PUFA through fish consumption protect smokers from chronic obstructive pulmonary disease and deterioration of lung function[39]. The most plausible explanation for these observations is that *n*-3 PUFA could modulate inflammatory processes, which may have a therapeutic effect in the pathophysiology of chronic inflammatory lung diseases[40]. Despite these promising findings, there remains a paucity of epidemiological data on the direct relationship between *n*-3 PUFA and lung function.

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

Table 1. Baseline characteristics of study participants according to quartiles of dietary *n*-3 PUFA intake in the NHANES 2007–2012

Characteristic	Total participants (*n* 9378)		Q1 (*n* 2345) ≤ 12·50		Q2 (*n* 2344) 12·51–18·78		Q3 (*n* 2344) 18·79–27·77		Q4 (*n* 2345) ≥ 27·78		*P*
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Age (year)											
Mean	46		47·12		46·65		45·79		44·42		< 0·001
SE	0·17		0·34		0·33		0·33		0·33		
Age groups (years)											< 0·001
20–39	3666	39·1	875	37·3	880	37·5	911	38·9	1000	42·6	
40–59	3347	35·7	808	34·5	828	35·3	869	37·1	842	35·9	
≥ 60	2365	25·2	662	28·2	636	27·1	564	24·1	503	21·5	
Sex											< 0·001
Male	4641	49·5	1029	43·9	1116	47·6	1232	52·6	1264	53·9	
Female	4737	50·5	1316	56·1	1228	52·4	1112	47·4	1081	46·1	
Race/ethnicity											< 0·001
Mexican American	1459	15·6	400	17·1	391	16·7	364	15·5	304	13·0	
Other Hispanic	992	10·6	234	10·0	235	10·0	289	12·3	234	10·0	
Non-Hispanic White	4291	45·8	1033	44·1	1113	47·5	1055	45·0	1090	46·5	
Non-Hispanic Black	1899	20·2	572	24·4	455	19·4	430	18·3	442	18·8	
Other race	737	7·9	106	4·5	150	6·4	206	8·8	275	11·7	
Education level											< 0·001
Below high school	2162	23·1	682	29·1	561	23·9	485	20·7	434	18·5	
High school graduate/GED	2094	22·3	567	24·2	542	23·1	475	20·3	510	21·7	
Some college or above	5115	54·6	1096	46·7	1239	52·9	1381	58·9	1400	59·7	
Poverty:income ratio											< 0·001
< 1	1770	19·3	531	22·6	430	18·3	403	17·2	406	17·3	
1–2·99	3511	38·2	975	41·6	899	38·4	851	36·3	786	33·5	
≥ 3	3641	39·7	736	31·4	900	38·4	983	41·9	1022	43·6	
Marital status											0·001
Married/living with a partner	5675	60·5	1341	57·2	1460	62·3	1462	62·4	1412	60·2	
Windowed/divorced/separated/never married	3701	39·5	1004	42·8	883	37·7	882	37·6	932	39·7	
Smoking status											0·062
Never	5214	55·6	1279	54·5	1301	55·5	1347	57·5	1287	54·9	
Former	2086	22·2	507	21·6	546	23·3	518	22·1	515	22·0	
Current	2078	22·2	559	23·8	497	21·2	479	20·4	543	23·2	
Alcohol intake											< 0·001
None	1985	21·2	608	25·9	519	22·1	481	20·5	377	16·1	
Moderate	6426	68·5	1506	64·2	1595	68·0	1640	70·0	1685	71·9	
Heavy	967	10·3	231	9·9	230	9·8	223	9·5	283	12·1	
BMI (kg/m^2)											
Mean	29·08		32·39		29·91		27·99		26·04		< 0·001
SE	0·70		0·16		0·13		0·12		0·10		
< 25	2728	29·1	340	14·5	535	22·8	769	32·8	1084	46·2	< 0·001
25–29·9	3146	33·5	669	28·5	803	34·3	857	36·6	817	34·8	
≥ 30	3504	37·4	1336	57·0	1006	42·9	718	30·6	444	18·9	
Hypertension											< 0·001
No	5849	62·4	1269	54·1	1429	61·0	1513	64·5	1638	69·9	
Yes	3497	37·3	1071	45·7	908	38·7	821	35·0	697	29·7	
Diabetes											< 0·001
No	7818	83·4	1840	78·5	1955	83·4	1948	83·1	2075	88·5	
Yes	1560	16·6	505	21·5	389	16·6	396	16·9	270	11·5	
Emphysema											0·233
No	9274	98·9	2313	98·6	2315	98·8	2326	99·2	2320	98·9	
Yes	104	1·1	32	1·4	29	1·2	18	0·8	25	1·1	
Chronic bronchitis											0·001
No	8931	95·2	2209	94·2	2225	94·9	2243	95·7	2254	96·1	
Former	253	2·7	62	2·6	69	2·9	62	2·6	60	2·6	
Current	194	2·1	74	3·2	50	2·1	39	1·7	31	1·3	
Asthma											0·274
No	8062	86·0	1991	84·9	2011	85·8	2025	86·4	2035	86·8	
Yes	1316	14·0	354	15·1	333	14·2	319	13·6	310	13·2	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
C-reactive protein (mg/dl)	0·18	0·35	0·27	0·47	0·20	0·33	0·16	0·32	0·12	0·24	< 0·001
Total energy intake (kcal/d)	2111·48	9·09	1520·37	11·74	1934·47	12·78	2243·41	14·73	2747·64	21·49	< 0·001
Total protein intake (g/d)	82·60	0·38	60·76	0·53	76·61	0·58	87·53	0·65	105·51	0·89	< 0·001
Total dietary fibre intake (g/d)	16·93	0·94	12·25	0·14	15·72	0·16	18·06	0·17	21·68	0·22	< 0·001
Total fat intake (g/d)	78·52	0·42	49·70	0·48	69·63	0·54	83·92	0·66	110·83	1·00	< 0·001
Total cholesterol intake (mg/d)	289·70	2·02	200·85	2·81	268·96	3·43	307·73	3·84	381·25	4·84	< 0·001
Total SFA (g/d)	25·34	0·15	16·98	0·18	23·07	0·21	27·06	0·26	34·26	0·38	< 0·001

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

Table 1. (*Continued*)

Characteristic	Total participants (*n* 9378)		Q1 (*n* 2345) ≤ 12·50		Q2 (*n* 2344) 12·51–18·78		Q3 (*n* 2344) 18·79–27·77		Q4 (*n* 2345) ≥ 27·78		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*P*
Total MFA (g/d)	28·61	0·16	18·44	0·20	25·67	0·22	30·64	0·27	39·68	0·39	< 0·001
Total PUFA (g/d)	17·71	0·10	9·62	0·10	14·64	0·11	18·90	0·14	27·68	0·24	< 0·001
n-3 PUFA (mg/d)	1720·75	11·04	784·50	6·22	1303·63	7·04	1803·46	9·56	2990·88	25·11	< 0·001
n-3 PUFA (excluding ALA) (mg/d)	139·29	2·92	51·49	1·31	83·70	2·36	136·81	4·04	285·16	9·94	< 0·001
EPA + DHA (mg/d)	39·00	80·50	21·50	39·00	33·75	58·00	48·00	96·50	75·00	225·25	< 0·001
Lung function parameters											
FEV_1 (ml)	3096·10	9·27	2969·87	18·75	3061·21	18·34	3149·53	18·48	3203·78	18·20	< 0·001
FVC (ml)	3951·95	11·16	3787·50	22·54	3905·20	21·85	4022·13	22·37	4092·99	22·00	< 0·001
FEV_1:FVC (%)	78·31	0·82	78·30	0·16	78·32	0·16	78·32	0·17	78·28	0·17	0·998
PEF (ml/s)	8140·60	22·76	7815·73	46·15	8098·95	44·98	8274·05	45·98	8373·68	44·10	< 0·001
FEV25 %–75 % (ml/s)	2947·68	13·35	2861·62	26·76	2937·53	27·05	2990·70	26·56	3000·89	26·32	0·001
% predicted FEV_1	95·81	0·16	94·72	0·33	95·78	0·31	96·11	0·31	96·61	0·30	0·001
% predicted FVC	98·16	0·14	96·86	0·29	97·92	0·28	98·48	0·29	99·37	0·28	< 0·001
% predicted FEV_1:FVC	96·99	0·18	97·41	0·18	97·48	0·18	97·38	0·18	96·99	0·18	0·023

NHANES, National Health and Nutrition Examination Survey; IQR, interquartile range; ALA, α-linolenic acid; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow; %, sample-weighted percentages.
Values were presented as mean ± SE or median ± IQR for continuous variables and *n* (%) for categorical variables. All *P*-values are statistically significant ($P < 0·05$).

Table 2. Association between *n*-3 PUFA intake and lung function among participants in NHANES 2007–2012

n-3 PUFA intake quartile (mg/kg/d)	FEV_1 (ml)			FVC (ml)			% predicted FEV_1			% predicted FVC		
	β	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*
Model 1												
Q1		1·00 (Ref.)			1·00 (Ref.)			1·00 (Ref.)			1·00 (Ref.)	
Q2	91·34	40·21, 142·47	< 0·001	117·70	56·18, 179·21	< 0·001	1·06	0·19, 1·92	0·017	1·07	0·28, 1·86	0·008
Q3	179·67	128·54, 230·79	< 0·001	234·63	173·11, 296·15	< 0·001	1·39	0·52, 2·25	0·002	1·62	0·83, 2·41	< 0·001
Q4	233·92	182·79, 285·04	< 0·001	305·49	243·98, 367·00	< 0·001	1·89	1·02, 2·76	< 0·001	2·51	1·73, 3·30	< 0·001
Model 2												
Q1		1·00 (Ref.)			1·00 (Ref.)			1·00 (Ref.)			1·00 (Ref.)	
Q2	10·65	–15·91, 37·22	0·432	13·78	–17·09, 44·66	0·381	0·40	–0·44, 1·25	0·352	0·35	–0·42, 1·12	0·372
Q3	10·22	–17·02, 37·45	0·462	23·04	–8·61, 54·69	0·154	0·31	–0·56, 1·17	0·487	0·45	–0·34, 1·24	0·261
Q4	22·51	–5·59, 50·62	0·116	43·66	10·99, 76·32	**0·009**	0·64	–0·26, 1·53	0·162	0·86	0·04, 1·67	**0·04**

NHANES, National Health and Nutrition Examination Survey; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; *β*, partial regression coefficient. Model 1 is the univariate model that assesses the association without adjustments. Model 2 adjusts for potential confounders including age, sex, race, educational level, poverty:income ratio, BMI, drinking, smoking, diabetes and hypertension. Values in bold indicate statistical significance ($P < 0.05$).

Table 3. Detailed information and datasets of exposure or outcome used in the present study

Exposure or outcome	PMID	First author	GWAS ID	Sample sizes	nSNP	Consortium	Source of population
Circulating *n*-3 PUFA concentration	35692035	Borges MC	met-d-*n*-3	114 999	12321875	UK Biobank	European
FEV_1	30804560	Shrine N	ebi-a-GCST007432	321 047	19674931	UK Biobank	European
FVC	30804560	Shrine N	ebi-a-GCST007429	321 047	19676344	UK Biobank	European
FEV_1:FVC	30804560	Shrine N	ebi-a-GCST007431	321 047	19671887	UK Biobank	European
The risk of FEV_1:FVC < 0·7	34294062	Higbee D	ieu-b-106	353 315	12321875	UK Biobank	European

PMID, PubMed Unique Identifier; GWAS, Genome-Wide Association Study; nSNP, number of SNP; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.

A large cross-sectional cohort study from Honolulu Heart Program showed evidence of a protective effect of high *n*-3 PUFA intake on lung function among smokers[41]. This was one of the earliest studies conducted on a population of Japanese-American men in 1994. Higher *n*-3 PUFA intake was associated with improved lung function in current or former smokers according to the Atherosclerosis Risk in Communities (ARIC) study[42]. Similar results were observed in a nutritional epidemiological study conducted in the Lovelace Smokers cohort, which found that DPA was positively correlated with better average FEV_1 volumes and reduced age-related FEV_1 decline[43]. The potential benefits of *n*-3 PUFA on lung function are biologically plausible, but not all observational studies have demonstrated that *n*-3 PUFA intake has a positive effect on lung function. The available information regarding the association between *n*-3 PUFA and lung function is limited and

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press
British Journal of Nutrition

Table 4. Summary on MR results of circulating *n*-3 PUFA concentration on lung function

				MR			Heterogeneity		Pleiotropy		
Exposures	Outcomes	Methods	nSNP	*β*	SE	*P*	Q	*P*	Intercept	SE	*P*
n-3 PUFA	FEV_1	MR-Egger	39	–0·003346	0·02662	0·9006	448·2	4·55E-72	0·002	0·0023	0·376
		IVW	39	0·01301	0·01932	0·5006	458	1·814E-73			
		Weighted median	39	–0·002627	0·007413	0·7231					
		Weighted mode	39	0·003888	0·006964	0·5799					
n-3 PUFA	FVC	MR-Egger	39	–0·01266	0·02355	0·5939	348·4	2·738E-52	0·0013	0·002	0·002
		IVW	39	–0·001894	0·01704	0·9115	352·6	1·285E-52			
		Weighted median	39	–0·01447	0·007071	0·04068					
		Weighted mode	39	–0·007477	0·00715	0·3023					
n-3 PUFA	FEV_1:FVC	MR-Egger	39	0·01764	0·02397	0·4665	358·1	3·401E-54	0·0017	0·0021	0·413
		IVW	39	0·03118	0·01743	0·07359	364·7	5·398E-55			
		Weighted median	39	0·02307	0·007268	0·001504					
		Weighted mode	39	0·02401	0·006371	0·0005577					
n-3 PUFA	The risk of FEV_1: FVC < 0·7	MR-Egger	40	0·0002181	0·006052	0·9714	177·2	7·307e-20	–0·00061	5e-04	0·237
		IVW	40	–0·004707	0·004478	0·2932	183·9	1·089e-20			
		Weighted median	40	–0·001963	0·00247	0·4268					
		Weighted mode	40	–0·001853	0·002438	0·4518					

MR, Mendelian randomization; nSNP, number of SNP; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; IVW, Inverse variance weighted.

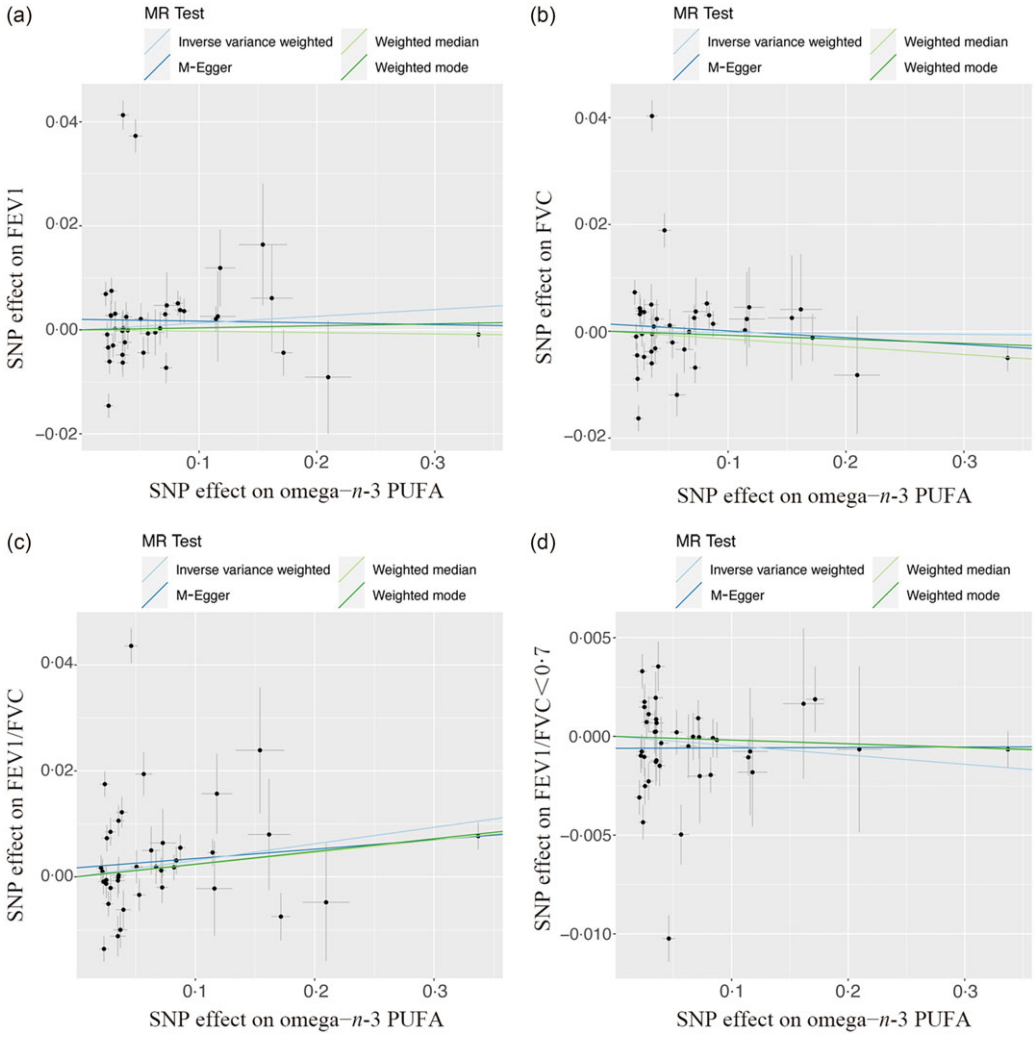

Fig. 1. Scatter plot for MR analyses of causal associations between each circulating *n*-3 PUFA concentration SNP and lung function. The slope of each line represents the causal association and each approach has a different line. FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; MR, Mendelian randomisation.

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

controversial. For instance, a cross-sectional study showed no association between dietary intake of *n*-3 PUFA and FEV_1[9]. A 25-year longitudinal study conducted in the Netherlands also failed to detect a protective role of *n*-3 PUFA on lung function[44]. These findings were generally consistent with our results from the nationally representative NHANES, suggesting *n*-3 PUFA may have no impact on lung function. This aligns with the understanding that the standard American diet is relatively deficient in *n*-3 PUFA, particularly EPA and DHA, which are essential for mitigating inflammation and oxidative stress. This lack of association may highlight the possibility that the intake levels in the USA fall below a threshold necessary for a detectable impact on lung function. The absence of a significant association in our findings challenges the prevailing notion that *n*-3 PUFA intake is universally beneficial for lung function, suggesting a need to reassess dietary guidelines and the potential for individualised nutritional recommendations based on genetic makeup and metabolic capacity. Notwithstanding this overall trend, significant results from Table 2, specifically in model 2 comparisons of the highest and lowest quartiles for FVC and % predicted FVC, warrant attention. Therefore, while our results do not support a broad role for *n*-3 PUFA in lung health at customary intake levels, they do suggest that substantially increased consumption, well above current averages, may be necessary to investigate potential benefits on lung function.

Hence, studies investigating the impact of *n*-3 PUFA intake on lung function have reported conflicting results. Unmeasured or uncontrolled confounding factors (such as environment and selection biases) and reverse causation might partially explain the discrepancy between these observational studies[45]. Studies to date have mostly focused on the dietary intake of *n*-3 PUFA from different food sources, which may lead to conclusions that may be confounded by contamination or food preparation methods in different geographical regions[46]. Moreover, the disadvantages of these studies include variations in study design, limited sample size and short follow-up periods, which may weaken the strength of the observed associations[47]. It is worth mentioning that the effects of dietary intake of *n*-3 PUFA on lung health may be mediated through *n*-3 PUFA in the circulation and tissues[48]. A previous study found that estimated dietary intake of *n*-3 PUFA was associated with plasma and erythrocyte membrane levels of these fatty acids[49]. For example, evidence from a randomised trial demonstrated that the beneficial effect of fish oil supplementation on blood pressure was associated with increased plasma phospholipid levels of *n*-3 PUFA[50]. In addition to diet, there are other determinants of *n*-3 PUFA levels, such as genetic variation in the metabolism of these fatty acid[51]. Glaser et al. indicated that *n*-3 PUFA levels in plasma, breast milk and tissues are affected by the concentration of fatty acid desaturase enzymes in the liver[52]. Therefore, lung function may be impacted by the genetic and metabolic variations that cause variability in *n*-3 PUFA levels. For these reasons, in addition to using the nationally representative observational study in NHANES, our study used MR approach, which is less prone to bias and provides a higher level of evidence[53]. This study selected the GWAS dataset with the most prominent circulating *n*-3 PUFA concentration and lung function samples to minimise bias, the results of which were consistent with each other, making our results more robust. Furthermore, the IVW, MR-Egger, weighted median and weighted mode were also employed to examine the causal relationship between the two samples. The MR findings, based on a general population, have expanded our understanding of this issue that lifelong higher circulating *n*-3 PUFA concentration is unlikely to have a causal relationship with lung function. Circulating concentration of *n*-3 PUFA is considered a biochemical marker of long-term dietary intake, providing valuable insight into patterns of *n*-3 PUFA consumption. Therefore, our study suggests that an increase in circulating *n*-3 PUFA concentration caused by *n*-3 PUFA intake would not result in improved lung function.

Based on the analysis of the relationship between dietary intake of *n*-3 PUFA intake and lung function in NHANES, and consistent with the MR analysis conducted on the entire study population, it was concluded that *n*-3 PUFA have no significant effect on lung function. However, we also recognise several limitations in our findings. First, the data on *n*-3 PUFA intake were collected from two 24-h dietary recall interviews, which may not accurately represent the long-term average intake. Despite this, some large epidemiological studies have demonstrated the validity of 24-h recall dietary assessments. Second, the effect size of the GWAS was based on circulating *n*-3 PUFA concentrations rather than membrane concentrations, while considering the role of fatty acid receptors in cell signalling and immune responses, the association at membrane level may be of more significance. Third, we cannot completely rule out the presence of unmeasured confounding in this cross-sectional study, even though we have already adjusted for some known factors related to lung function. Additionally, stratified analyses based on sex, age, height and ethnicity were not possible due to the limited availability of only summary-level statistics from the general population of both sexes in the MR analysis. Lastly, given the availability of data sources, the findings from the two studies were based on a multi-ethnic US population and a population of European ancestry, respectively, so may not be generalisable to other ethnic populations.

Conclusion

In summary, while our analysis generally found no association between *n*-3 PUFA intake and improved lung function, observed significant findings at the highest quartile point to a potential dose-dependent effect. Our findings do not support a generalised role for *n*-3 PUFA in lung health enhancement, yet they pave the way for future studies to explore the threshold levels of *n*-3 PUFA required to impact lung function, potentially leading to new insights into diet-based interventions for respiratory health.

Acknowledgements

The authors thank the participants and researchers for their participation in the NHANES and MR study.

This research was funded by the Medical Science and Technology Project of Zhejiang Province (2023RC289, 2022RC272).

Z. X. L. and J. L. L. conceived and designed the study and interpreted the results of the data analyses. J. S. and L. J. L.

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

performed the data analyses. J. L. L., J. S. and C. Y. Z. wrote the manuscript. All authors have read and approved the final manuscript.

The authors declare no conflict of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524000266

References

1. Burney PG & Hooper R (2011) Forced vital capacity, airway obstruction and survival in a general population sample from the USA. *Thorax* **66**, 49–54.
2. Agustí A, Noell G, Brugada J, *et al.* (2017) Lung function in early adulthood and health in later life: a transgenerational cohort analysis. *Lancet Respir Med* **5**, 935–945.
3. Burchfiel CM, Marcus EB, Curb JD, *et al.* (1995) Effects of smoking and smoking cessation on longitudinal decline in pulmonary function. *Am J Respir Crit Care Med* **151**, 1778–1785.
4. Adam M, Schikowski T, Carsin AE, *et al.* (2015) Adult lung function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-analysis. *Eur Respir J* **45**, 38–50.
5. Sunyer J (2009) Lung function effects of chronic exposure to air pollution. *Thorax* **64**, 645–646.
6. Patel AR, Patel AR, Singh S, *et al.* (2019) Global initiative for chronic obstructive lung disease: the changes made. *Cureus* **11**, e4985.
7. Mickleborough TD & Rundell KW (2005) Dietary polyunsaturated fatty acids in asthma- and exercise-induced bronchoconstriction. *Eur J Clin Nutr* **59**, 1335–1346.
8. Ng TP, Niti M, Yap KB, *et al.* (2014) Dietary and supplemental antioxidant and anti-inflammatory nutrient intakes and pulmonary function. *Public Health Nutr* **17**, 2081–2086.
9. McKeever TM, Lewis SA, Cassano PA, *et al.* (2008) The relation between dietary intake of individual fatty acids, FEV1 and respiratory disease in Dutch adults. *Thorax* **63**, 208–214.
10. Kim W, Moll M, Qiao D, *et al.* (2021) Interaction of cigarette smoking and polygenic risk score on reduced lung function. *JAMA Netw Open* **4**, e2139525.
11. Davey Smith G & Ebrahim S (2005) What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ* **330**, 1076–1079.
12. Bowden J, Davey Smith G & Burgess S (2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* **44**, 512–525.
13. Haycock PC, Burgess S, Wade KH, *et al.* (2016) Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr* **103**, 965–978.
14. Centers for Disease C, and Prevention & National Health and Nutrition Examination Survey Survey Methods and Analytic Guidelines. https://www.cdc.gov/nchs/nhanes/index.htm (accessed 7 February 2023).
15. Centers for Disease C, and Prevention NCHS Research Ethics Review Board (ERB) Approval. https://www.cdc.gov/nchs/nhanes/irba98.htm (accessed 7 February 2023).
16. Miller MR, Hankinson J, Brusasco V, *et al.* (2005) Standardisation of spirometry. *Eur Respir J* **26**, 319–338.
17. Hankinson JL, Odencrantz JR & Fedan KB (1999) Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* **159**, 179–187.
18. Centers for Disease C, and Prevention The Examination Protocol and Data Collection Methods. –https://wwwn.cdc.gov/nchs/data/nhanes/2011–2012/manuals/mec_in_person_dietary_procedures_manual_jan_2012.pdf (accessed 7 February 2023).
19. Navaneethan SD, Mandayam S, Arrigain S, *et al.* (2016) Obstructive and restrictive lung function measures and CKD: National Health and Nutrition Examination Survey (NHANES) 2007–2012. *Am J Kidney Dis* **68**, 414–421.
20. Chen F, Du M, Blumberg JB, *et al.* (2019) Association among dietary supplement use, nutrient intake, and mortality among U.S. adults: a cohort study. *Ann Intern Med* **170**, 604–613.
21. Menke A, Casagrande S, Geiss L, *et al.* (2015) Prevalence of and trends in diabetes among adults in the United States, 1988–2012. *JAMA* **314**, 1021–1029.
22. Whelton PK, Carey RM, Aronow WS, *et al.* (2018) 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* **138**, e426–e483.
23. VanderWeele TJ, Tchetgen EJ, Cornelis M, *et al.* (2014) Methodological challenges in Mendelian randomization. *Epidemiology* 25, 427–435.
24. Borges MC, Haycock PC, Zheng J, *et al.* (2022) Role of circulating polyunsaturated fatty acids on cardiovascular diseases risk: analysis using Mendelian randomization and fatty acid genetic association data from over 114 000 UK Biobank participants. *BMC Med* **20**, 210.
25. Park JH, Wacholder S, Gail MH, *et al.* (2010) Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. *Nat Genet* **42**, 570–575.
26. Burgess S & Thompson SG (2011) Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* **40**, 755–764.
27. Shrine N, Guyatt AL, Erzurumluoglu AM, *et al.* (2019) New genetic signals for lung function highlight pathways and chronic obstructive pulmonary disease associations across multiple ancestries. *Nat Genet* **51**, 481–493.
28. Higbee DH, Granell R, Hemani G, *et al.* (2021) Lung function, COPD and cognitive function: a multivariable and two sample Mendelian randomization study. *BMC Pulm Med* **21**, 246.
29. Thompson JR, Minelli C, Abrams KR, *et al.* (2005) Meta-analysis of genetic studies using Mendelian randomization–a multivariate approach. *Stat Med* **24**, 2241–2254.
30. Burgess S, Butterworth A & Thompson SG (2013) Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* **37**, 658–665.
31. Qian Y, Ye D, Huang H, *et al.* (2020) Coffee consumption and risk of stroke: a Mendelian randomization study. *Ann Neurol* **87**, 525–532.
32. Zhou H, Zhang Y, Liu J, *et al.* (2019) Education and lung cancer: a Mendelian randomization study. *Int J Epidemiol* **48**, 743–750.
33. Hemani G, Zheng J, Elsworth B, *et al.* (2018) The MR-Base platform supports systematic causal inference across the human phenome. *Elife* **7**, e34408.
34. Wei L, Wu Z & Chen YQ (2022) Multi-targeted therapy of cancer by *n*-3 fatty acids-an update. *Cancer Lett* **526**, 193–204.
35. Simopoulos AP (2008) The importance of the *n*-6/*n*-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med* **233**, 674–688.
36. Li J, Chen Y, Shi Q, *et al.* (2023) n-3 polyunsaturated fatty acids ameliorate PM2.5 exposure induced lung injury in mice

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press

through remodeling the gut microbiota and modulating the lung metabolism. *Environ Sci Pollut Res Int* **30**, 40490–40506.
37. Farjadian S, Moghtaderi M, Kalani M, *et al.* (2016) Effects of *n*-3 fatty acids on serum levels of T-helper cytokines in children with asthma. *Cytokine* **85**, 61–66.
38. Mickleborough TD, Murray RL, Ionescu AA, *et al.* (2003) Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. *Am J Respir Crit Care Med* **168**, 1181–1189.
39. Shahar E, Folsom AR, Melnick SL, *et al.* (2008) Dietary *n*-3 polyunsaturated acids and smoking-related chronic obstructive pulmonary disease. *Am J Epidemiol* **168**, 796–801.
40. Tong H, Zhang S, Shen W, *et al.* (2022) Lung function and short-term ambient air pollution exposure: differential impacts of *n*-3 and *n*-6 fatty acids. *Ann Am Thorac Soc* **19**, 583–593.
41. Sharp DS, Rodriguez BL, Shahar E, *et al.* (1994) Fish consumption may limit the damage of smoking on the lung. *Am J Respir Crit Care Med* **150**, 983–987.
42. Shahar E, Folsom AR, Melnick SL, *et al.* (1994) Dietary *n*-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. Atherosclerosis risk in communities study investigators. *N Engl J Med* **331**, 228–233.
43. Leng S, Picchi MA, Tesfaigzi Y, *et al.* (2017) Dietary nutrients associated with preservation of lung function in Hispanic and non-Hispanic white smokers from New Mexico. *Int J Chron Obstruct Pulmon Dis* **12**, 3171–3181.
44. Miedema I, Feskens EJ, Heederik D, *et al.* (1993) Dietary determinants of long-term incidence of chronic nonspecific lung diseases. The Zutphen Study. *Am J Epidemiol* **138**, 37–45.
45. Smith GD, Lawlor DA, Harbord R, *et al.* (2007) Clustered environments and randomized genes: a fundamental distinction between conventional and genetic epidemiology. *PLoS Med* **4**, e352.
46. Jiang W, Li FR, Yang HH, *et al.* (2021) Relationship between fish oil use and incidence of primary liver cancer: findings from a population-based prospective cohort study. *Front Nutr* **8**, 771984.
47. Li ZH, Song WQ, Shen D, *et al.* (2022) Habitual fish oil supplementation and incident chronic obstructive pulmonary disease: data from a prospective cohort study. *Clin Nutr* **41**, 2651–2658.
48. Skilton MR, Raitakari OT & Celermajer DS (2013) High intake of dietary long-chain ω-3 fatty acids is associated with lower blood pressure in children born with low birth weight: NHANES 2003–2008. *Hypertension* **61**, 972–976.
49. Serra-Majem L, Nissensohn M, Øverby NC, *et al.* (2012) Dietary methods and biomarkers of *n* 3 fatty acids: a systematic review. *Br J Nutr* **107**, S64–S76.
50. Bønaa KH, Bjerve KS, Straume B, *et al.* (1990) Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromsø study. *N Engl J Med* **322**, 795–801.
51. Chilton FH, Murphy RC, Wilson BA, *et al.* (2014) Diet–gene interactions and PUFA metabolism: a potential contributor to health disparities and human diseases. *Nutrients* **6**, 1993–2022.
52. Glaser C, Lattka E, Rzehak P, *et al.* (2011) Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health. *Matern Child Nutr* **7**, 27–40.
53. Smith GD & Ebrahim S (2003) 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* **32**, 1–22.

British Journal of Nutrition

https://doi.org/10.1017/S0007114524000266 Published online by Cambridge University Press