ANNALS OF MEDICINE
2021, VOL. 53, NO. 1, 1744–1757
https://doi.org/10.1080/07853890.2021.1937693

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ORIGINAL ARTICLE

The associations of circulating common and uncommon polyunsaturated fatty acids and modification effects on dietary quality with all-cause and disease-specific mortality in NHANES 2003–2004 and 2011–2012

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ABSTRACT

Background: Associations of dietary or supplementary intake of several unsaturated fatty acids and mortality have been widely studied but the results were still hitherto inconsistent or limited. It is still need to explore the effects of these fatty acids by using the objective biomarkers.

Objective: We aimed to investigate the relevancy of several serum n-3 and n-6 fatty acids with all-cause and disease-specific mortality to confirm their health effects and effects on the associations between dietary quality and all-cause mortality.

Methods: A total of 4132 people from NHANES 2003–2004 and 2011–2012 and the mortality information was confirmed from the NDI. CPH models adjusted for known risk factors were conducted to explore the associations between circulating n-3 and n-6 fatty acids and all-cause or CVD or cancer mortality under complex sampling. We further evaluated their effects on association between dietary quality and all-cause mortality.

Results: A total of 437 deaths occurred during the mean follow-up of 83.34 months, including 157 CVD death and 100 cancer death. Serum LA, ALA, EPA and DHA were associated with all-cause mortality (HR in quintile5: LA:0.584, 95%CI: 0.387–0.882, $P_{trend} = 0.011$; ALA:0.626, 95%CI: 0.432–0.907, $P_{trend} = 0.008$; EPA:0.535, 95%CI: 0.375–0.764, $P_{trend} = 0.001$; DHA:0.669, 95%CI: 0.468–0.955, $P_{trend} = 0.031$). Additionally, serum EPA and ALA were respectively related to CVD and cancer mortality (Q5 HR: EPA:0.450, 95%CI: 0.23–0.854, $P_{trend} = 0.009$; ALA:0.387, 95%CI: 0.167–0.900, $P_{trend} = 0.022$). Serum AA, GLA, DGLA and SDA were not associated with any risk of mortality. The effect on all-cause mortality of the lower AHEI scores can be improved by adherence to a higher serum LA, EPA and DHA (in the lowest AHEI strata, LA in tertile3 compared to tertile1 HR:0.596, 95%CI: 0.366–0.970; EPA:0.660, 95%CI: 0.454–0.959; DHA:0.666, 95%CI; 0.444–1.000).

Conclusions: Our results support the recent dietary recommendations to increase the intake of plant-derived and marine-derived n-6 and n-3 to improve the ability of primary and secondary prevention.

ARTICLE HISTORY
Received 5 February 2021
Revised 8 April 2021
Accepted 26 May 2021

KEYWORDS
Mortality; CVD; cancer; circulating polyunsaturated fatty acids; cohort study; AHEI-2010

Introduction

Polyunsaturated fatty acids, such as linoleic acid (LA, n-6), alpha-linolenic acid (ALA, n-3), arachidonic acid (AA, n-6), eicosapentaenoic acid (EPA, n-6), and docosahexaenoic acid (DHA, n-3) have been widely reported to play an essential role in regulating several physiological processes, such as inflammation, glucose regulation, lipid metabolism and oxidative stress. All these physiological processes are closely related to the development of metabolic disorders. An increasing number of suggestions recommended the partial replacement of dietary saturated fat with polyunsaturated fat, especially n-6 and n-3 PUFAs, to lower the risk of some metabolic disorders or cardiovascular disease, which are the major cause of death [1,2]. The beneficial influence of the replacement is mostly based on serum LDL concentrations [3]. In addition, results from several different studies recently showed that PUFAs n-3 docosapentaenoic acid (DPAn3, n-3), which paid less attention before, could also benefit to cardiometabolic health in a different way to the other long-chain n-3 PUFAs2 [4]. Similarly, another fatty acid,

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Supplemental data for this article can be accessed here.

stearidonic acid (SDA, n-3), indicated that single-dose SDA-rich echium oil increases plasma EPA, DPAn3, and DHA concentrations, which might be a promising alternative plant source for long-chain n-3 PUFAs compared to ALA [5–7].

However, debates also have been raised about the influence of polyunsaturated fatty acids (PUFAs). A common hypothesis mechanism is that LA (The majority fatty acid of n-6 PUFAs) and its metabolites, particularly AA, are substrates for several proinflammatory mediators. Increased intake of LA may increase chronic low-level inflammation levels associated with several chronic diseases such as cancer, diabetes, and neurodegeneration [8–10]. On the other hand, in several studies, increasing dietary or supplementary intake of n-6 (LA and AA) and n-3 PUFAs (ALA, EPA and DHA) did not show beneficial effects [11–20]. Besides of mixed evidence of dietary or supplementary PUFAs, most have focussed on the association with cardiovascular outcomes, with relatively fewer studies paying their attention to the other endpoints [21], especially all-cause and disease-specific mortality.

Serum n-6 or n-3 fatty acids, as the objective biomarkers, did not merely reduce the recall bias and estimation errors inherent in subjective dietary assessment methods, it also could accurately reflect the circulating metabolic status compared with dietary researches. In addition, biomarkers could largely accelerate the study of effects of individual PUFAs. However, there were still a lack of confirmations of associations between serum n-6 or n-3 fatty acids biomarkers and any cause of mortality [20]. In other respect, AHEI-2010 index, which was used to describe the overall dietary quality, showed an association with the risk of mortality [22,23]. As a part of AHEI, whether n-6 or n-3 PUFAs could reverse the negative impact of poor-quality diet on all-cause mortality was also remained unclear. Uncommon n-6 and n-3 PUFAs (usually at low or not contained in usual foods) GLA, DGLA, SDA and DPAn3, which paid less attention before, were also need confirmation of their potential effects on any cause of mortality [4,20]. In consideration of mixed and limited evidence [2,13,24–26], we aimed to analyse the associations of several n-6 and n-3 PUFAs with the risk of all-cause and disease-specific mortality and their modification on associations between dietary quality and all-cause mortality over the age of 18 from National Health and Nutrition Examination Survey (NHANES) by using their circulating concentration as an exposure. We finally assessed PUFAs such as LA, AA, ALA, EPA and DHA, and uncommon n-6 and n-3 PUFAs such as GLA, DGLA, SDA and DPAn3 in serum to infer the potential effect of these fatty acids for health protection.

Materials and methods

Study population

National Health and Nutrition Examination Survey (NHANES) is conducted as a representative multistage stratified sampling health survey of the United States. It consists of both interviews and examination part including demographic, socioeconomic, dietary, health-related questions, physiological measurements, laboratory tests and other information administered by highly trained medical personnel. Details has been provided previously [27,28]. Briefly, adults (aged over 18) who have serum PUFAs measured in NHANES from 2003–2004, 2011–2012 were selected in our study ($n = 6227$). After excluding the participants who had missing values on any we focussed PUFAs and/or mortality information ($n = 2095$), 4132 participants were included in the study. The institutional review board approval of the National Centre for Health Statistics and written informed consent were obtained before data collection.

Measurements

Blood samples were collected and analysed by the Centres for Disease Control and Prevention. Detailed information of the quantitative method of serum lipids, lipoproteins and self-reported details of sex, age, race, education level, smoking status, regular exercise, annual income, dietary supplement usage, history of disease, history of specific disease controlling has been reported previously [27,28]. BMI was calculated as the ratio of weight(kg) to the square of height(m). Dietary intakes were measured by a 24-h dietary recall in two times. The later 24-h dietary recall was conducted 3–10 days after the first one. Dietary nutrients and energy intake were estimated by using the guidelines of the U.S. Department of Agriculture's Food and Nutrient Database for Dietary Studies.

Serum fatty acids

Esterified fatty acids are hydrolysed primarily from triglycerides, phospholipids and cholesteryl esters using sequential treatment with mineral acid and base in the presence of heat. The extract is derivatized with pentafluorobenzyl bromide (PFBBr) in the presence of triethylamine to form pentafluorobenzyl esters. The reaction mixture is injected onto a capillary gas

chromatograph column to resolve individual fatty acids of interest from other matrix constituents. Quantitation is accomplished by comparing the peak area of the analyte in the unknown with the peak area of a known amount in a calibrator solution. Calculations are corrected based on the peak area of the internal standard in the unknown compared with the peak area of the internal standard in the calibrator solution [29].

AHEI-2010 index

In brief, Alternate Healthy Eating index 2010 (AHEI-2010), which based on the original Healthy Eating index and subsequent comprehensive review of studies, included 11 food components(fruit, vegetables, whole grains, sugar-sweetened beverages and fruit juice, nuts and legumes, red and processed meat, trans fat, long-chain n-3 fats, polyunsaturated fatty acids(PUFAs), sodium, and alcohol) and additionally showed an association with the risk of chronic diseases even related to total and cause-specific mortality [22,23]. Each component of food ranges from 0(unhealthy) to 10(healthiest) with the total scores ranges from 0 to 110. The score for each item is calculated by dividing the actual intake by the criteria for maximum and multiplying by the full score of the item. Finally, we combined the scores of each 11 item to get the final AHEI score. Higher total scores indicate healthier diet condition.

Main outcome

The outcome was the final mortality status ascertained by the National Death Index (NDI) [30]. The NDI is a highly reliable resource for death identification. The ICD-10 was used to determine disease-specific death. Death due to CVD was defined as ICD-10 codes I00–I09, I11, I13, I20–I51, or I60–I69. Cancer mortality was defined as ICD-10 codes C00-C97. In our study, finally, there were 437 death, including 157 death of CVD and 100 death of cancer documented.

Statistical analysis

According to the NHANES analytic guidelines, all analyses were considered the sample weights, stratification and clustering because of the complex survey design. Two-year Mobile Examination Centre exam weight and cluster and strata variables from demographic datasets were used in our weighted analysis to account for survey design [31,32]. Baseline characteristics were presented as mean (95%CI) for continuous variables and percentage (95%CI) for categorical variables. Univariate relations among groups were assessed by general linear models (for continuous variables) adjusting for age and chi-square tests (for categorical variables). Cox proportional hazard (CPH) models were used to evaluate the associations of PUFAs with CVD, cancer and all-cause mortality. Survival time was months between NHANES interview date and death or census date. The assumption of the Cox models was tested by conducting correlation test between Schoenfeld residuals and survival time rank (all were $p > .05$ and in line with the assumption, Supplemental Table 18). Potential confounders we selected were well-established or biological interest factors. All CPH models included age(years), sex(male/female), BMI (kg/m^2), race, current smoking(yes/no), current drinking(yes/no), education level, family annual income(dollars), leisure-time physical activity (yes/no), prevalent diabetes or cardiovascular disease or cancer (yes/no), ever controlled blood pressure, blood cholesterol or blood glucose (yes/no), serum triglycerides (mmol/L), serum total cholesterol (mmol/L), and intakes of SFAs (percentage of energy), USFAs (percentage of energy), fibre (g/d), total energy (kcal/day), carbohydrate (g/d), protein (g/d) and AHEI-2010 as confounders. Because of the dietary source and intercorrelation, we also conducted mutual adjustment for the PUFAs subtypes (LA, AA, ALA and LCn-3 were mutual adjusted) in all analysis. Serum high-density lipoprotein cholesterol and low-density lipoprotein cholesterol as potential effect mediators, were also further adjusted. Each fatty acid level was expressed as the form of percentage of total serum fatty acids and evaluated in quintiles as a categorical variable. We used median values for each subgroup of fatty acid variable and treat it as a continuous variable to test the linear trend. Nonlinear associations of death and intake of PUFAs with serum PUFAs were conducted by using restricted cubic splines (RCS) to visualise the shape of the dose-response relation of concentrations of USFAs in serum for all-cause mortality, cardiovascular mortality, and cancer mortality, and to investigate whether the relations should be judged linear or not. CPH models were also conducted to explore whether serum PUFAs could modify the impact on associations between dietary quality and all-cause mortality by categorising serum PUFAs and AHEI score into tertiles respectively and finally 9 strata were in consideration. Participants in the lowest tertile of serum PUFAs and lowest tertile of AHEI score were used as the reference. We did not analyse their modification effect on the association

between dietary quality and CVD or cancer mortality due to little amount of death data after stratified.

Missing values were imputed (most factors < 5%, except for current smoking (6.1%), current drinking (6.0%), leisure-time physical activity (11.3%)) by multiple imputation using chained equations (5 imputations). We also performed sensitivity analyses with excluding the deaths within the beginning of the first two years ($n = 75$) and using the complete data in original dataset to minimise the unrecognised influence. All data were analysed by using R (version 3.5.3), and P values were two-tailed ($\alpha = 0.05$). Accordingly, 95% CIs of hazard ratios (HRs) were provided.

Results

Table 1 shows the baseline characteristics of participants separately described by gender. Compared to men, women were more likely to be younger, less smoking and drinking, less dietary supplement intake, better serum lipid indexes, higher concentrations of serum n-6 and n-3 PUFAs and lower intake of energy, macronutrient and dietary fibre, lower AHEI-2010 score and higher proportion of dietary PUFA intake. Other variables were not significantly different.

The demographic, serum lipid parameters and nutrition characteristics in terms of each PUFAs of our

Table 1. Baseline characteristics of the 4132 people from the NHANES in 2003–2004 and 2011 2012[a].

	All ($n = 4132$)	Male ($n = 2037$)	Female ($n = 2095$)
Age, y	46.85 (45.87–47.84)	46.23 (45.18–47.28)	47.47 (46.29–48.64)*
BMI, kg/m^2	28.55 (28.17–28.93)	28.49 (28.16–28.83)	28.60 (28.08–29.13)
Non-Hispanic white, %	72.8 (67.0–77.9)	70.9 (65.5–75.8)	71.8 (66.5–76.7)
Current smoking, %	25.2 (22.5–28.0)	32.7 (28.8–36.9)	17.8 (15.4–20.5)*
Current drinking, %	72.5 (69.6–75.3)	82.6 (79.7–85.2)	72.5 (69.6–75.3)*
College graduate or above, %	27.9 (23.9–32.2)	27.1 (23.4–31.2)	28.6 (24.1–33.6)
>$100,000 annual household income, %	11.9 (8.3–16.8)	13.5 (9.0–19.7)	10.3 (1.7–14.3)
Regular exercise, %	44.6 (41.1–48.1)	44.2 (39.8–48.8)	45.0 (40.9–49.1)
Dietary supplement use, %	53.7 (51.3–56.1)	47.4 (44.6–50.1)	59.9 (56.5–63.2)*
Prevalent diabetes, %	11.0 (9.5–12.7)	11.8 (9.9–14.1)	10.1 (8.5–12.0)
Ever controlled diabetes, %	3.9 (3.2–4.8)	4.1 (3.1–5.5)	3.7 (2.9–4.6)
Prevalent high cholesterol level, %	38.9 (36.3–41.5)	41.0 (37.3–44.7)	36.9 (33.9–40.1)
Ever controlled cholesterol, %	22.1 (20.2–24.2)	22.8 (20.2–25.7)	21.5 (18.8–24.4)
Prevalent hypertension, %	37.0 (33.9–40.2)	38.7 (35.0–42.6)	35.3 (31.6–39.1)
Ever controlled hypertension, %	25.2 (22.4–28.3)	25.0 (21.5–28.8)	25.4 (22.4–28.7)
Prevalent cancer or malignancy, %	8.7 (7.5–10.2)	7.5 (6.1–9.2)	9.9 (7.8–12.5)*
Triglycerides, mmol/L	1.55 (1.48–1.62)	1.68 (1.61–1.75)	1.43 (1.34–1.51)*
HDL cholesterol, mmol/L	1.39 (1.36–1.42)	1.25 (1.23–1.28)	1.53 (1.49–1.56)*
LDL cholesterol, mmol/L	3.01 (2.97–3.06)	3.00 (2.95–3.05)	3.03 (2.97–3.09)
Total Cholesterol, mmol/L	5.10 (5.05–5.15)	5.00 (4.93–5.06)	5.21 (5.14–5.27)*
C-reactive protein, mg/dL	0.46 (0.40–0.51)	0.38 (0.29–0.46)	0.54 (0.45–0.62)*
LA, % in total serum fatty acids	31.38 (31.16–31.60)	31.01 (30.67–31.36)	31.74 (31.47–32.01)*
GLA, % in total serum fatty acids	0.48 (0.47–0.49)	0.49 (0.48–0.51)	0.47 (0.46–0.49)*
DGLA, % in total serum fatty acids	1.37 (1.35–1.39)	1.33 (1.30–1.35)	1.41 (1.39–1.43)*
AA, % in total serum fatty acids	7.22 (7.15–7.30)	7.13 (7.02–7.24)	7.32 (7.21–7.42)*
ALA, % in total serum fatty acids	0.68 (0.65–0.70)	0.68 (0.66–0.70)	0.67 (0.65–0.69)
SDA, % in total serum fatty acids	0.032 (0.031–0.034)	0.032 (0.030–0.035)	0.032 (0.030–0.034)
EPA, % in total serum fatty acids	0.51 (0.48–0.54)	0.51 (0.47–0.54)	0.52 (0.48–0.55)
DPAn3, % in total serum fatty acids	0.41 (0.40–0.42)	0.42 (0.41–0.43)	0.40 (0.39–0.41)*
DHA, % in total serum fatty acids	1.27 (1.21–1.33)	1.19 (1.14–1.25)	1.34 (1.27–1.41)*
Total n-6 fatty acids, % in total serum fatty acids	41.05 (40.83–41.28)	40.55 (40.22–40.89)	41.54 (41.27–41.81)*
Total n-3 fatty acids, % in total serum fatty acids	2.88 (2.79–2.98)	2.82 (2.73–2.91)	2.95 (2.84–3.05)*
Total serum n-6 / Total serum n-3	15.66 (15.18–16.13)	15.79 (15.29–16.29)	15.53 (15.02–16.03)
Dietary intakes			
Energy, kcal/d	2182.47 (2147.39–2217.56)	2549.56 (2496.42–2603.29)	1823.98 (1786.98–1860.99)*
Carbohydrate, g/d	263.73 (259.14–268.32)	302.52 (297.24–307.80)	225.88 (220.05–231.70)*
Protein, g/d	84.73 (83.00–86.45)	99.68 (97.09–102.28)	70.13 (68.46–71.80)*
Total fat, g/d	83.04 (81.04–85.04)	96.41 (93.46–99.37)	69.99 (67.89–72.09)*
Total sugars, g/d	118.83 (115.52–122.15)	134.71 (130.72.138.70)	103.34 (99.51–107.18)*
SFAs, % of energy	10.62 (10.39–10.86)	10.60 (10.38–10.83)	10.64 (10.32–10.97)
MUFAs, % of energy	12.17 (11.90–12.45)	12.24 (11.94–12.54)	12.11 (11.79–12.43)
PUFAs, % of energy	7.53 (7.39–7.67)	7.36 (7.21–7.51)	7.70 (7.49–7.90)*
Fibre, g/d	17.09 (16.46–17.72)	18.84 (18.09–19.58)	15.38 (14.76–16.01)*
AHEI-2010	55.15 (54.37–55.94)	59.71 (58.77–60.65)	50.70 (49.88–51.52)*

[a]Values are weighted means (95%CI) or weighted percentages (95%CI).
*$p < .05$. p was assessed with General linear models adjusting for age (continuous variables) or $\chi 2$ test (bivariate relationships).
All data analyses conducted in the current study were based on estimates with sample weights provided by NHANES.
LA: linoleic acid; GLA: gamma-linolenic acid; DGLA: dihomo-gamma-linolenic acid; AA: arachidonic acid; ALA: α-Linolenic acid; SDA: stearidonic acid; EPA: eicosapentaenoic acid; DPAn3: n-3 Docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

study in quintiles are presented in Supplemental Table 1–9. In baseline demographic characteristics, serum LA showed several similar trait as serum ALA, DGLA, EPA, DPAn3, DHA. For instance, higher LA and DGLA showed younger age or less likelihood of having high cholesterol level or hypertension; higher LA, DPAn3 and DHA were related to lower BMI; higher LA, ALA, EPA, DPAn3, DHA were more likely to less smoking; higher education level and family income are associated with higher ALA, EPA, DPAn3, DHA. About the beneficial serum lipid parameters, higher concentrations of LA, AA, EPA and DHA had a relation with lower triglycerides and higher HDL. In addition, higher GLA, AA, EPA, DPAn3, DHA were likely to intake fewer SFAs or MUFAs and those had more fibre intake were likely with higher concentrations of n-3 PUFAs.

As for relations in each series of PUFAs especially in n-6 and n-3 series in total study population, GLA showed moderate correlations with DGLA and AA ($r = 0.341$ and 0.362) but LA only showed weak relations with GLA and AA ($r < 0.15$) (Supplemental Table 10). In n-3 series, similarly, all were related to each other in varying degrees. ALA was weakly related to marine n-3 PUFAs yet uncommon fatty acids SDA and DPAn3 were strongly related to other marine n-3 PUFAs (Supplementary Table 11). We also estimate the association between the intake of each fatty acid and its own serum concentration. Intake of LA, AA, ALA, EPA and DHA were all related to its own objective biomarker serum concentrations (all P value for overall association $<$ .05; for nonlinearity $p < .05$, Supplemental Figure 1). We did not have information on dietary intake of GLA, DGLA, SDA and DPAn3.

After 83.34 ± 46.78(mean ± SD) months of follow-up, 437 death occurred in the whole study population, including 157 death of CVD, 100 death of cancer. Supplementary Table 12 and Figures 1 and 2 showed associations of the serum n-6 and n-3 PUFAs with all-cause, CVD and cancer mortality. After multivariable adjustment, total n-6 fatty acids showed no significance on association with mortality(all-cause mortality quintile 5 HR: 0.567, 95%CI: 0.358–0.897, P_{trend}=0.030; CVD mortality quintile 5 HR: 0.642, 95%CI: 0.275–1.498, P_{trend}=0.593; Cancer mortality quintile 5 HR: 0.710, 95%CI: 0.295–1.709, P_{trend}=0.292), whereas total n-3 fatty acids (all-cause mortality quintile 5 HR: 0.577, 95%CI: 0.404–0.824, P_{trend}=0.003; CVD mortality quintile 5 HR: 0.532, 95%CI: 0.279–1.013, P_{trend}=0.088; Cancer mortality quintile 5 HR: 0.387, 95%CI: 0.183–0.817, P_{trend}=0.009) and the ratios of total n-6 fatty acids to total n-3 fatty acids(all-cause mortality quintile 5 HR: 1.576, 95%CI: 1.088–2.284, P_{trend}=0.013; CVD mortality quintile 5 HR: 1.685, 95%CI: 0.865–3.283, P_{trend}=0.171; Cancer mortality quintile 5 HR: 2.493, 95%CI: 1.153–5.394, P_{trend}=0.004) were showed significance. When further studied specific n-6 or n-3 fatty acids, higher serum LA, ALA, EPA, DPAn3 and DHA were associated with a lower risk of all-cause mortality. As indicated by HR and 95%CI, those in the highest quintile of serum LA, ALA, EPA and DHA(quintile 5) had a lower risk of all-cause death(LA HR: 0.584, 95%CI: 0.387–0.882, P_{trend}=0.010; ALA HR: 0.626, 95%CI: 0.432–0.907, P_{trend}=0.013; EPA HR: 0.535, 95%CI: 0.375–0.764, P_{trend}=0.001; DHA HR: 0.669, 95%CI: 0.468–0.955, P_{trend}=0.027) than those in the lowest quintile. In addition, DPAn3 showed lower risk in their higher quintile (DPAn3 in quintile 4 HR: 0.600, 95%CI: 0.437–0.822) compared with their lowest quintile. Each 1-SD increase in serum LA, ALA, EPA and DHA was also associated with a multivariable-adjusted lower relative risk of all-cause death (LA HR: 0.835, 95%CI: 0.731–0.953; ALA HR: 0.821, 95%CI: 0.711–0.950; EPA HR: 0.859, 95%CI: 0.738–1.000; DHA HR: 0.880, 95%CI : 0.776–0.998, Figures 1 and 2). We also conducted restricted cubic splines to flexibly model the association of those fatty acids and all-cause mortality. The results showed that serum LA ($P_{linearity}$=0.0215, $P_{nonlinearity}$=0.7418, Figure 3), ALA ($P_{linearity}$=0.0208, $P_{nonlinearity}$=0.3154, Figure 3) and DHA ($P_{linearity}$=0.0500, $P_{nonlinearity}$=0.3051, Figure 3) had approximate linear associations with a lower risk of all-cause death after multivariable adjustment. By contrast, serum EPA showed a rapid decline of the risk of all-cause death before its approximate 0.41% proportion of total serum fatty acids concentrations and indicated protection effect may between 0.41%–1.50%($P_{linearity}$=0.0002, $P_{nonlinearity}$=0.0007, Figure 3). DPAn3 had a rapid decline risk before its approximate 0.40% proportion of total serum fatty acids concentrations and showed likely rising risk trend after approximate 0.54% proportion ($P_{linearity}$=0.1290, $P_{nonlinearity}$=0.0248, Figure 3).

When we analysed the association with the specific disease of death—CVD and cancer, serum EPA in its highest quintile(quintile 5) was associated with a lower risk of CVD death(HR: 0.450, 95%CI: 0.238–0.854, P_{trend}=0.014) compared with the lowest quintile after adjusted as well as each 1-SD increase of EPA was significant (HR: 0.685, 95%CI: 0.500–0.937, Figure 2). In addition, serum ALA showed a significant lower risk of cancer death in both highest quintile (quintile 5, ALA HR: 0.387, 95%CI: 0.167–0.900, P_{trend}=0.027, Figure 2) compared with the lowest quintile and each 1-SD increase of its serum concentration (HR: 0.701, 95%CI:

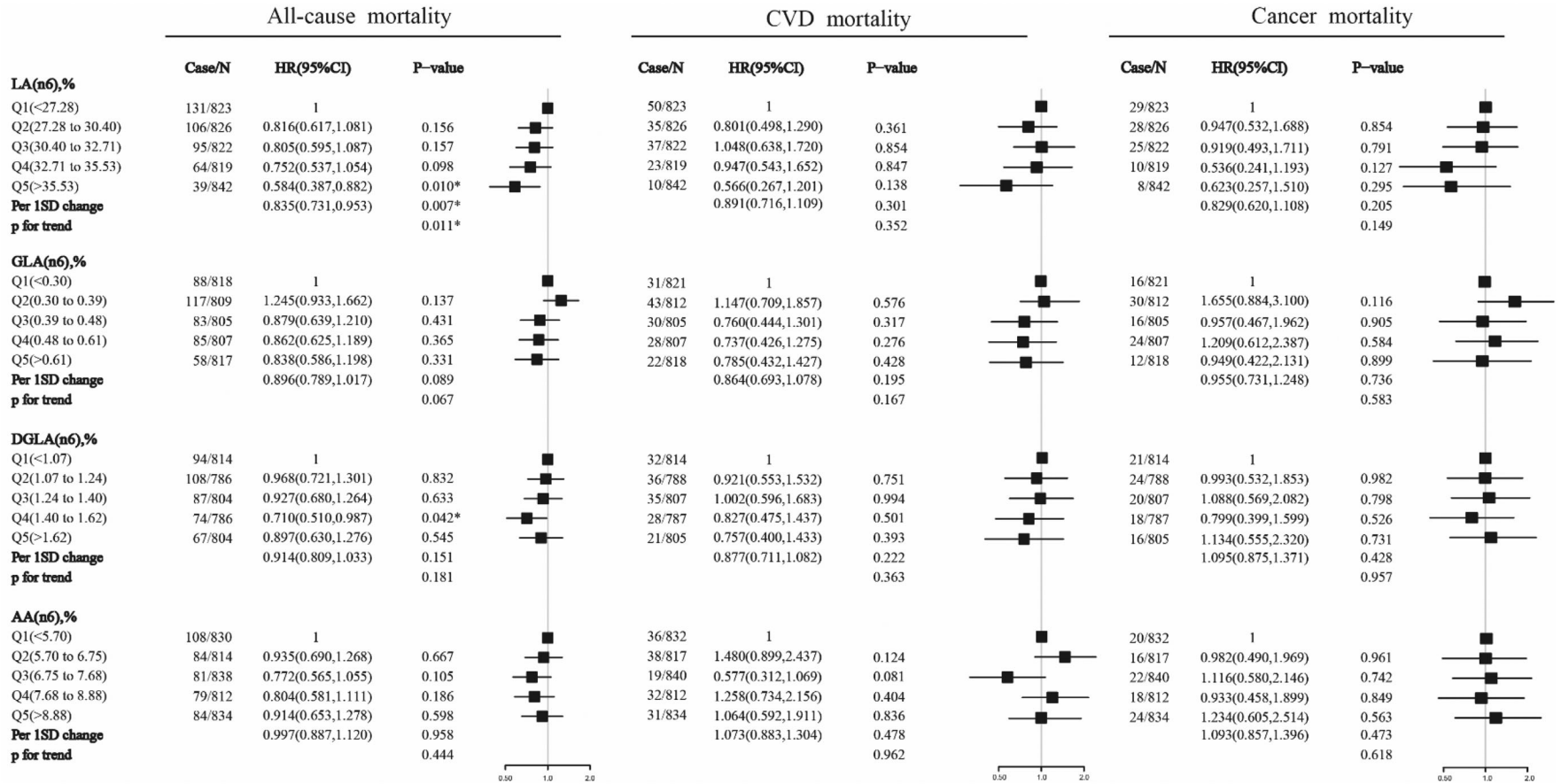

Figure 1. Adjusted HRs for associations between different n-6 PUFAs and all-cause, CVD and cancer mortality. The models were adjusted for age (years), sex (male/female), BMI (kg/m^2), current smoking (yes/no), current drinking (yes/no), education level, family annual income (dollars), leisure-time physical activity (yes/no), prevalent diabetes or cardiovascular disease or cancer (yes/no), ever controlled blood pressure, blood cholesterol or blood glucose (yes/no), serum triglycerides (mmol/L), serum total cholesterol (mmol/L) and intakes of SFAs (percentage of energy), USFAs (percentage of energy), fibre (g/d), total energy (kcal/day), carbohydrate (g/d),protein (g/d) and AHEI-2010. Serum LA, AA, ALA and LCn3 were mutually adjusted; %, ratio of each serum fatty acid to the total serum fatty acid; Q, quintile. X-axis tick marks was showed in logarithmic scale.

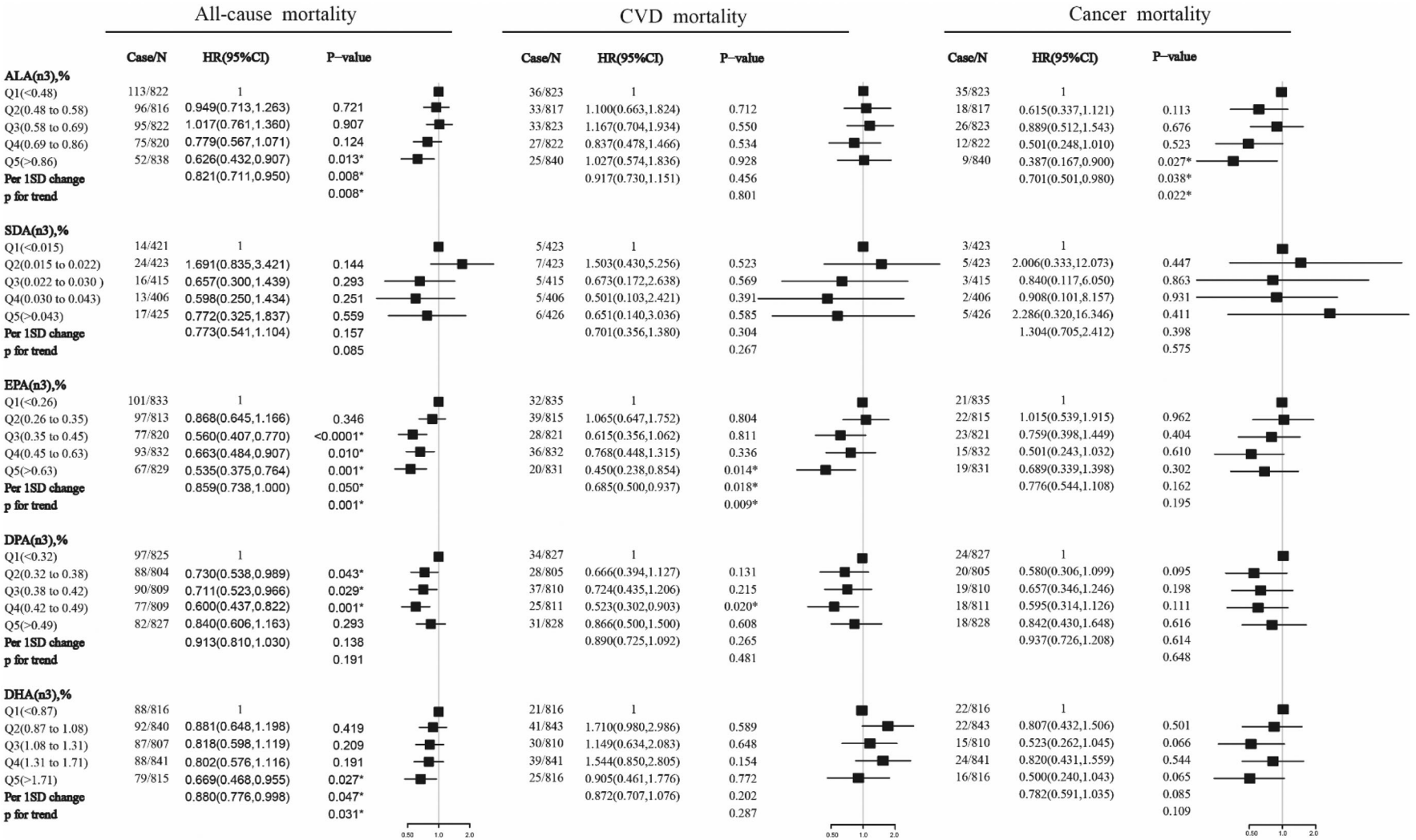

Figure 2. Adjusted HRs for associations between different n-3 PUFAs and all-cause, CVD and cancer mortality. The models were adjusted for age (years), sex (male/female), BMI (kg/m^2), current smoking (yes/no), current drinking (yes/no), education level, family annual income (dollars), leisure-time physical activity (yes/no), prevalent diabetes or cardiovascular disease or cancer (yes/no), ever controlled blood pressure, blood cholesterol or blood glucose (yes/no), serum triglycerides (mmol/L), serum total cholesterol (mmol/L) and intakes of SFAs (percentage of energy), USFAs (percentage of energy), fibre (g/d), total energy (kcal/day), carbohydrate (g/d), protein (g/d) and AHEI-2010; %, ratio of each serum fatty acid to the total serum fatty acid. Serum LA, AA, ALA and LCn3 were mutually adjusted; Q, quintile. X-axis tick marks was showed in logarithmic scale.

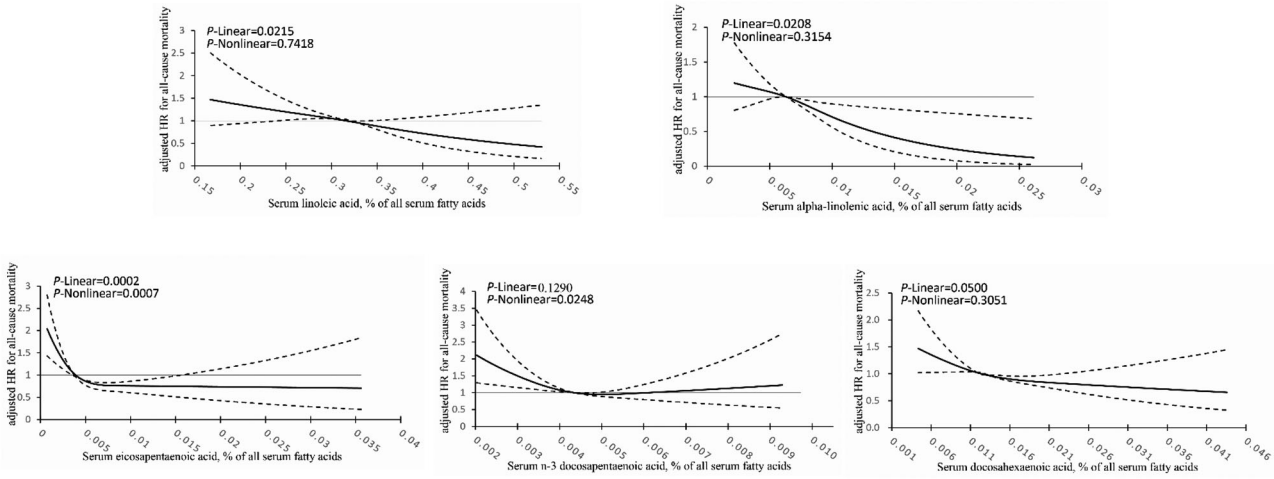

Figure 3. Associations between serum LA, ALA, EPA, DPAn3 and DHA with all-cause mortality. Assessed by multivariable-adjusted HRs using of Cox proportional hazard models and restricted cubic splines. The solid lines represent the central estimates and the dashed lines area the 95% confidence intervals. The models were adjusted for The models were adjusted for age (years), sex (male/female), BMI (kg/m^2), current smoking (yes/no), current drinking (yes/no), education level, family annual income (dollars), leisure-time physical activity (yes/no), prevalent diabetes or cardiovascular disease or cancer (yes/no), ever controlled blood pressure, blood cholesterol or blood glucose (yes/no), serum triglycerides (mmol/L), serum total cholesterol (mmol/L) and intakes of SFAs (percentage of energy), USFAs (percentage of energy), fibre (g/d), total energy (kcal/day), carbohydrate (g/d), protein (g/d) and AHEI-2010. Serum LA, AA, ALA and LCn3 were mutually adjusted. LA: linoleic acid; ALA: α-Linolenic acid; EPA: eicosapentaenoic acid; DPAn3: n-3 Docosapentaenoic acid; DHA: docosahexaenoic acid.

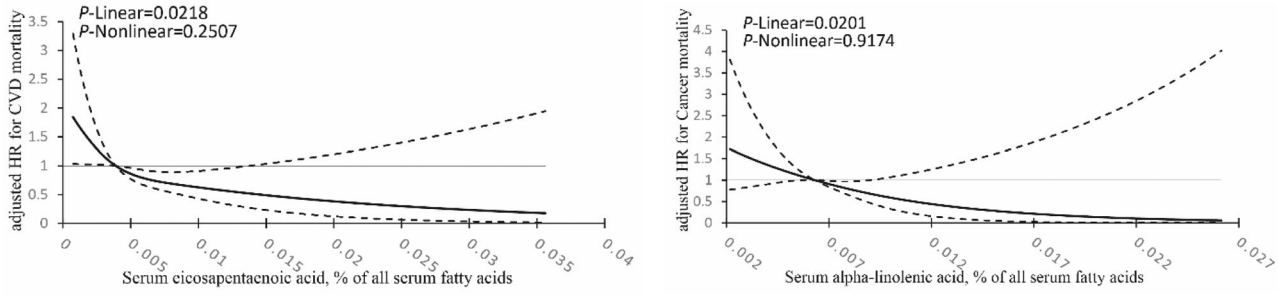

Figure 4. Associations between serum EPA and ALA with CVD and cancer mortality (left figure: association between EPA and CVD mortality; right figure: association between ALA and cancer mortality). Assessed by multivariable-adjusted HRs using of Cox proportional hazard models and restricted cubic splines. The solid lines represent the central estimates and the dashed lines area the 95% confidence intervals. The models were adjusted for The models were adjusted for age (years), sex (male/female), BMI (kg/m^2), current smoking (yes/no), current drinking (yes/no), education level, family annual income (dollars), leisure-time physical activity (yes/no), prevalent diabetes or cardiovascular disease or cancer (yes/no), ever controlled blood pressure, blood cholesterol or blood glucose (yes/no), serum triglycerides (mmol/L), serum total cholesterol (mmol/L) and intakes of SFAs (percentage of energy), USFAs (percentage of energy), fibre (g/d), total energy (kcal/day), carbohydrate (g/d), protein (g/d) and AHEI-2010. Serum LA, AA, ALA and LCn3 were mutually adjusted. ALA: α-Linolenic acid; EPA: eicosapentaenoic acid.

0.501–0.980, Figure 2). However, none of n-6 PUFAs were found associations with CVD and cancer death (Figure 1). Associations were generally the same as aforementioned results in RCS figures (EPA $P_{linearity} = 0.0218$, $P_{nonlinearity} = 0.2507$; ALA $P_{linearity} = 0.0201$, $P_{nonlinearity} = 0.9174$, Figure 4).

After further adjusted for the potential effect mediators serum HDL and LDL, serum LA, ALA, EPA and DHA in highest quintile (quintile 5) still remained lower risk of all-cause death (LA HR: 0.579, 95%CI: 0.379–0.886, $P_{trend} = 0.012$; ALA HR: 0.604, 95%CI: 0.412–0.886, $P_{trend} = 0.005$; EPA HR: 0.538, 95%CI: 0.357–0.772, $P_{trend} = 0.003$; DHA HR: 0.685, 95%CI: 0.477–0.985, $P_{trend} = 0.050$, Supplemental Figure 2 and 3) than those in the lowest quintile. Similar results with which not further adjusted, serum DPAn3 showed lower risk in higher quintile(DPAn3 in quintile 4 HR: 0.618, 95%CI: 0.448–0.854) compared with their lowest quintile. In the case of CVD death, serum EPA was associated with lower risk (Q5 HR: 0.451, 95%CI: 0.235–0.867, $P_{trend} = 0.021$, Supplemental Figure 3). As to the death of cancer, only serum ALA in the higher

quintile significantly lowered the risk than in lowest quintile (Q5 HR: 0.283, 95%CI: 0.116–0.690, $P_{trend} = 0.005$, Supplemental Figure 3). In another respect, n-6 PUFAs were still not associated with cancer and CVD death after further adjustment (Supplemental Figure 2). In sensitive analysis, when excluding the deaths within the beginning of the first two years and using the complete data in original dataset, results slightly attenuated the associations mentioned above except for serum LA, EPA, DPAn3 with all-cause mortality and EPA with CVD mortality and ALA with cancer mortality (Supplementary Figures 4 and 5).

Who were in the lowest tertile of serum LA, ALA, EPA, DPAn3 and DHA respectively and in the lowest tertile of AHEI score concurrently was used as the reference, we found serum LA, EPA and DHA in their highest tertile within the group of lowest AHEI score showed protective effects on all-cause mortality compared with those in the lowest tertile after adjustment (LA HR: 0.596, 95%CI: 0.366–0.970; EPA HR: 0.660, 95%CI: 0.454–0.959; DHA HR: 0.666, 95%CI: 0.444–1.000, Supplementary Table 13–17). In contrast, within the lowest tertile of each serum fatty acid mentioned before, the highest group of AHEI showed no difference with the reference group on the risk of all-cause mortality (LA HR: 0.567, 95%CI: 0.314–1.061; ALA HR: 0.951, 95%CI: 0.494–1.832; EPA HR: 1.409, 95%CI: 2.757; DHA HR: 1.154, 95%CI: 0.567–2.350, Supplementary Table 13–17).

Discussion

In this prospective cohort study in NHANES from the United States, higher serum LA, ALA, EPA and DHA showed robust inverse associations with the risk of all-cause mortality. Simultaneously, serum EPA and ALA were respectively related to CVD and cancer death. No relations were found between n-6 PUFAs and CVD or cancer death. In addition, as for uncommon PUFAs, GLA, DGLA, AA, SDA and DPAn3, DPAn3 showed moderate protection of total mortality yet others showed no associations with all-cause and specific causes of death. In addition, LA, EPA, DHA seemed to modify the negative impact by lower AHEI score and lower serum LA, ALA, EPA and DHA could compensate for the positive impact by AHEI score. Serum LA, AA, ALA, EPA, DHA, as objective biomarkers, were all related to their own dietary intakes but most of them were not strongly associated with each other.

N-6 PUFAs, especially LA and AA, seemed to be the substrates to several pro-inflammatory substances, but both were also substrates to compounds with anti-inflammatory or pro-resolving properties, such as lipoxins, epoxy fatty acids, and nitrated LA [33–36]. However, even higher intakes of LA or AA have failed to show their pro-inflammatory properties in several experimental studies [37–39]. In addition, LA probably also decreased thrombosis [40], arrhythmias [41] and improve insulin sensitivity [42]. Some arguments focussed on the findings of dietary or supplementary n-6 PUFAs which seemed to fail to reduce all-cause mortality or CVD death [13,16,17,43–45]. Nevertheless, some of these trials were conducted before 1990 so that trans fatty acids which also contained in n-6-riched margarine of industry product were maybe the principal reason to cause death [46]. Indeed, a low compliance rate or events amount or other reasons could also affect the conclusions of the trials [44,47]. Several studies did confirm there are both cardioprotection and total mortality protection effect of LA even without replacement of saturated fat and carbohydrate in whichever biomarkers such as intake, circulating or adipose tissue [1,20,48–54]. Our study, using each serum n-6 PUFA as objective biomarkers, was largely consistent with the result of protection in all-cause death mentioned before. Yet in our result we failed to find any effect on CVD death. That might possibly due to the little amount of CVD death. Our result about circulating n-6 PUFAs not associated with cancer death was in line with few studies [51,54] although others showed higher or lower risk [34,55] which probably based on the evidence that some PUFAs-derived proinflammatory eicosanoids can change cell proliferation, apoptosis, angiogenesis, and migration [34,55]. As for uncommon n-6 fatty acids, GLA in its metabolism pathway firstly was converted from LA and soon rapidly elongated to DGLA, which finally convert to AA. Our findings were supported by a few prospective studies that GLA, DGLA seemed not associated with mortality and suggested that these uncommon n-6 PUFAs did not take an independence role in mortality risk, even DGLA has been reported that has both pro- and anti-inflammatory properties and antiproliferation effect [34,52–54,56]. Our present result of association with all-cause mortality and the relations between serum concentration and dietary intake, together with previous studies, indicate a higher intake of LA could provide benefits without increasing any risk of death.

Marine-derived n-3 fatty acids (long-chain n-3 fatty acids, EPA and DHA), as well as, plant-derived n-3 fatty acid (ALA) have been suggested to be beneficial for cardiovascular health. Dietary and circulating n-3 fatty

acids have been confirmed the reduction of total mortality and coronary artery disease in some cohort studies and several clinical trials. A prospective nested case-control study of Singapore Chinese showed higher circulating long-chain n–3 PUFAs and ALA were associated with a lower incidence of acute myocardial infarction [19]. But to date, there still remained an argument for protections of either long-chain n-3 fatty acid or ALA on total mortality or cardiovascular health. A recent high-quality meta-analysis including 79 RCTs (112059 participants) claimed little or no effect on both all-cause mortality (RR 0.98, 95% CI 0.90–1.03, 92,653 participants; 8189 deaths in 39 trails) and cardiovascular mortality(RR 0.95, 95% CI 0.87–1.03, 67,772 participants; 4544 CVD deaths in 25 RCTs) with long-chain n-3 fatty acids supplementation by mostly taking capsules. With ALA capsule supplementation, result was the same as long-chain n-3 supplementation with a relative risk of all-cause mortality from 0.84 to 1.20 in 95%CI (19,327 participants; 459 deaths, 5 RCTs) and relative risk of cardiovascular mortality from 0.74 to 1.25 in 95%CI (18,619 participants; 219 cardiovascular deaths, 4 RCTs) [57]. Another recent study aimed at assessing the impact of the Mediterranean diet pattern on primary prevention in population at higher CVD risk, and pointed out ALA could reduce the risk of all-cause mortality even in the context of higher intake of long- chain n-3 fatty acids (HR: 0.722, 95%CI: 0.558–0.933), but it probably failed to reduce CVD mortality (HR: 0.953, 95%CI: 0.579–1.566). Otherwise, long-chain n-3 fatty acids were associated with significant reductions in fatal CVD and CHD by 39% and 46%, respectively [18]. Our result about circulating n-3 PUFAs supported the positive effect of both plant-derived and marine-derived n-3 PUFAs. Cardiac membrane accretion of EPA and DHA improved myocardial oxygen consumption efficiency consequently to limit myocardial damage on ischaemia and ALA, which could improve endothelial dysfunction and plaque inflammation although this effect appeared little compared with DHA. This might be the reason why we did not detect a cardioprotective effect of ALA with significant correlations with long-chain n-3 PUFAs. In anti-cancer researches, a meta-analysis including 47 RCTs (108,194 participants) indicated that intake of long-chain n-3 has little or no effect on cancer death (RR0.97, 95% CI 0.90–1.06) [24] accorded with our serum n-3 PUFAs finding but we found serum ALA anti-cancer effects in its higher serum concentration. It might due to mediates mitochondrial apoptosis, curtailed hypoxic microenvironment along with inhibition of de novo fatty acid synthesis to impart anticancer effects [58]. In n-3 PUFAs series, ALA could convert to SDA, EPA, DPAn3 and DHA in a multistep of elongation and desaturation theoretically. But in fact, studies have shown the conclusion that the ability of conversion from ALA to long-chain n-3 fatty acids was limited (<5%) [59,60]. Addition with high intake of LA in typical Western diet, even large amount of ALA consumption could merely slightly increase in plasma EPA, DPAn3 and DHA [61–63]. SDA was the intermediate in the conversion of ALA to EPA. Because of skipping the process of ALA to SDA, which was regulated by rate-limiting enzyme delta-6-desaturase (D6D), it was more efficient in conversion. More researches have confirmed its high efficiency in conversion compared with ALA [5–7]. The unclear independent effects of serum SDA on death risk were in both present and our research. It might due to its high efficiency of conversion so that it could merely remained consistent low concentration in circulation. In consideration of associations between serum SDA and long-chain n3 PUFAs together with lower intake of marine-derived fish and environmental chemical pollutants, SDA-rich oil may safely and expediently increase the concentration of serum EPA or other long-chain n-3 PUFAs to play their beneficial roles [64]. DPAn3, the marine-derived long-chain n-3 PUFA intermediate between EPA and DHA, is mainly retro converted into EPA and slightly converted to DHA [65]. As for its beneficial effect, DPAn3 has been confirmed to play an important role in decreasing inflammation because of increasing the monocyte differentiation, the phagocytic activity of macrophages and the apoptosis of neutrophils [66,67]. Our study found that serum DPAn3 was stronger correlated with EPA than with DHA and the higher quintile of DPAn3 showed lower CRP concentrations. However, studies of DPAn3 on total mortality, cardiovascular or cancer mortality were limited or inconsistent. Some meta-analysis and observational studies indicated that circulation DPAn3 was inversely related to total mortality, cancer mortality and cardiovascular mortality especially CHD and stroke while some other studies showed negative effects of DPAn3 in erythrocytes, plasma, plasma phospholipids or adipose tissue [15,68–73]. We found evidence for decreased total mortality with higher serum DPAn3 exposure, but observed no association with mortality due to cardiovascular and cancer aspects. The discrepancy probably due to the independent study of long-chain n-3 PUFAs of pro-resolution of inflammation yet it remains difficult to differentiate the effects of DPAn3 itself compared to those of EPA and DHA as they are

biologically interconverted [65]. Taken together these findings, although DPAn3 needs further confirmation of its benefit to death protection, it is still safe to increase the intake of DPAn3-rich food because of its ability of anti-inflammation and being a good source of EPA and DHA. Given that evidence of both recent studies and our serum n-3 PUFAs findings, suggestion still should be taken to increase marine-derived n-3 fatty acids and plant-derived n-3 fatty acid intake to prevent not only all-cause mortality risk but also cardiovascular or cancer mortality risk.

We also concerned the reverse causation particularly when study has participants with existing disease, because these people may have changed their dietary habits due to the disease. It is clear for those with a history of disease that they are at a higher risk of death than those health individuals. Changes in dietary habits may result in the false discovery that a healthier diet (e.g. higher intake of marine-derived PUFAs or LA) probably associated with increased risk of incident events of death. However, in our study, results were remained robust association after adjusting the disease history. Moreover, early death in follow-up also may due to the poor body condition or else. However, the results were just slightly attenuated when we excluded participants who were dead in first two years. Our findings thus support the beneficial effect of higher several PUFAs in the prevention of the prevalent disease outcomes today.

More and more researches focussed on dietary patterns rather than single nutrients or foods to estimate the association between diet status and health outcomes [74]. Dietary Approaches to Stop Hypertension (DASH), Alternate Mediterranean Diet score (AMED) and Alternate Healthy Eating Index–2010 (AHEI-2010), most used to evaluate the diet status, have been proved to be associated with 8%∼22% lower risk of any cause of death and 19%∼28% lower of cardiovascular death and 11%∼23% lower of cancer death [22]. Our study also confirmed the effect of AHEI-2010 score on the risk of all-cause death (HR per 1-SD change: 0.707, 95%CI: 0579–0.864, $p = .001$; HR in tertile 3 compared tertile 1:0.591, 95%CI: 0.390–0.894, $p = .013$, $P_{trend} = 0.006$) even when adjusted including several dietary factors. In comparison, obviously, lower AHEI score indicated more risk than higher AHEI score. Nevertheless, higher AHEI or lower AHEI score do not mean higher or lower intake of PUFAs and long-chain n-3 PUFAs, although these fatty acids were considered in AHEI-2010 evaluation process. In view of this, we paid attention to the effect of higher or lower AHEI score on all-cause death whether could be compensated by different concentrations of beneficial PUFAs confirmed in our study. To our surprise, we found serum LA, EPA, DHA seemed to play a role on reversing negative impact by lower AHEI score even though the composition of AHEI index is complex. We also found that lower serum PUFAs mentioned before could compensate for the positive impact by AHEI score no matter how high the score from diet evaluation (Figure 4 and Supplementary Table 13–17). In brief, even when we get high or low scores from AHEI evaluation, plant-derived LA and marine-derived long-chain n-3 fatty acids must be balanced or enhanced in our routine diet.

Strength and limitation

The strength of our study firstly is the use of objective biomarkers of PUFAs exposure using high-quality dietary data from a well-designed population-based study (NHANES). The associations reported in our study were also relatively robust with adjustment for a variety of established important confounders including potential effect mediators. Even though PUFAs were also calculated in the process of dietary quality evaluation because the composition of AHEI was complicated, another strength is we explored the modification effect from PUFAs on dietary quality and all-cause death. A major weakness is the single baseline fatty acids measurement because dietary habits may change during the long period of follow-up, this would increase random error and lead to attenuate the associations. In addition, several higher serum PUFAs were together with more favourable lifestyle and dietary habits so that residual confounding by unmeasured or imprecisely measured factors cannot be excluded.

Conclusion

In conclusion, we found higher serum LA, ALA, EPA and DHA showed robust inverse associations with the risk of all-cause mortality. Serum EPA and ALA were respectively related to CVD and cancer death. No relations were found between n-6 PUFAs and CVD or cancer death. In addition, uncommon PUFA, serum DPAn3 showed moderate protection of total death yet other serum uncommon PUFAs showed no associations with all-cause and specific causes of death. But on the other hand, in varying degrees, higher serum SDA and DPAn3 were related to higher beneficial marine n-3 PUFAs. Combined with these single findings, we also confirmed the positive and negative effect of

beneficial PUFAs on modifying associations between dietary quality and mortality. These findings for PUFAs confirm the results of previous studies and indicate overall benefits by higher intake of plant-derived and marine-derived n-6 or n-3 PUFAs. Frankly, our findings need to be confirmed largely and deeply in other cohorts as well. However, in the light of the existing evidence, we strongly recommend to increase the intake of polyunsaturated fatty acids such as LA or ALA-rich oil or marine fish to improve body health.

Acknowledgments

The authors thank the participants and staff of the National Health and Nutrition Examination Survey 2003–2004 and 2011–2012 for their valuable contributions.

Disclosure statement

All the authors declare no competing interests.

Funding

This research was supported funds from National Natural Science Foundation [82030100 to Ying Li, 81803227 to Tianshu Han], Young Elite Scientists Sponsorship Program by CAST [2019QNRC001 to Tianshu Han], National Key R&D Program of China [2017YFC1307401 to Changhao Sun].

Data availability statement

All data are available within the Article and Supplementary Files, or available from the authors upon request.

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