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Original article

Polyunsaturated fatty acids intake, omega-6/omega-3 ratio and mortality: Findings from two independent nationwide cohorts

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SUMMARY

Background & aims: Polyunsaturated fatty acids (PUFA) have been reported to exert pleiotropic protective effects against various chronic diseases. However, epidemiologic evidence linking specific PUFA intake to mortality has been limited and contradictory. We aim to assess the associations between specific dietary PUFA and mortality among adults in China and America, respectively.
Methods: Participants from China Health and Nutrition Survey (CHNS, $n = 14{,}117$) and National Health and Nutrition Examination Survey [NHANES ($n = 36{,}032$)] were prospectively followed up through the year 2011. Cox regression models were used to investigate hypothesized associations.
Results: A total of 1007 and 4826 deaths accrued over a median of 14 and 9.1 years of follow-up in CHNS and NHANES, respectively. Dietary marine omega-3 PUFA was robustly associated with a reduced all-cause mortality [Hazard ratio (HR) comparing extreme categories: 0.74, 95% CI: 0.61–0.89; $P < 0.001$ for trend] in CHNS. Nevertheless, this inverse relationship was not observed in NHANES. The overall mortality was positively associated with the intake of α-linolenic acid (ALA) (HR comparing extreme quartiles: 1.23, 95% CI: 1.01–1.50; $P = 0.054$ for trend) in CHNS, whereas weak inverse associations of ALA ($P = 0.035$ for trend) and LA ($P = 0.027$ for trend) with all-cause mortality were found in NHANES. Increased dietary intake of arachidonic acid was consistently linked with reduced all-cause mortality both in NHANES and CHNS. Importantly, consuming PUFA at an omega-6/omega-3 ratio of 6–10 was associated with a lower risk of death in CHNS.
Conclusions: Intakes of different specific PUFA show distinct associations with mortality and these relationships also vary between Chinese and US populations. These findings suggest maintaining an omega-6/omega-3 balance diet for overall health promotion outcomes (NCT03155659).

1. Introduction

Polyunsaturated fatty acids (PUFA) have been a topic of scientific interest worldwide for decades. Strong and consistent evidence demonstrated the cardioprotective and antihypercholesterolemic effects of PUFA [1,2]. Intake of omega-3 PUFA, especially marine long-chain omega-3 PUFA (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), has been linked with decreased risk of major chronic diseases, including cardiovascular disease (CVD), diabetes and Alzheimer's disease [3–6]. However, evidence linking omega-3 PUFA intake to mortality still remains inconsistent. Although some observational studies reported that higher circulating level or dietary intake of omega-3 PUFA was associated with lower overall mortality [7–9], such inverse association was not supported by a cohort of postmenopausal women [10] and a meta-analysis based on omega-3 PUFA intervention studies [11]. Moreover, inconsistent outcomes

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; CHNS, China Health and Nutrition Survey; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; LA, linoleic acid; NHANES, National Health and Nutrition Examination Survey; PUFA, polyunsaturated fatty acids.

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have been reported for the impact of marine omega-3 PUFAs and α-linolenic acid (ALA) on mortality [10,12,13].

The health effects of omega-6 PUFA and potential dose–response relationship have remained debatable so far. Public concern was also highlighted over theoretical pro-inflammatory and pro-thrombotic properties of omega-6 PUFA [14]. However, no quality data in human studies have supported this pro-inflammatory effect yet [15]. Some evidence linked the increasing omega-6/omega-3 ratio with the incidence of CVD, cancer and obesity [16–18]. Unfortunately, few studies have provided substantial insights into the association of omega-6 PUFA with mortality.

To our knowledge, the evidence of the association is still lacking for a nationwide investigation in both China and US. Notably, data on PUFA intake and mortality are particularly sparse in Chinese populations [19,20]. Here we comprehensively assessed the associations of specific PUFA intake with overall and cause-specific mortality in two nationally representative cohorts, the China Health and Nutrition Survey (CHNS) and U.S. National Health and Nutrition Examination Survey (NHANES).

2. Subjects and methods

2.1. Study population

We investigated the associations from CHNS for Chinese population and NHANES [NHANES (III), 1988–1994, and continuous circles 1999–2010] for US population, respectively. CHNS is a large-scale and household-based cohort established in 1989. The survey employed a multi-stage random-cluster model to enroll a sample of over 30,000 individuals in 9 provinces and municipal cities where the total population makes up approximately 50% of the Chinese population [21]. NHANES targets nationally representative individuals among the civilian non-institutional US population performed by the National Center for Health Statistics (NCHS) of the Center For Disease Control (CDC) [22]. We included individuals aged ≥20 y with complete dietary data and follow-up period among the two populations ($n = 14,469$ in CHNS and $n = 45,082$ in NHANES). After excluding those with CVD, cancer or pregnant women at baseline, the final analyses included 14,117 participants in CHNS and 36,032 participants in NHANES. A flow chart for describing the enrolled participants is shown in Supplemental Fig. 1.

2.2. Dietary PUFA assessment

Dietary intake from participants in CHNS was assessed over three consecutive days by using daily interview in combination with a weighing technique to obtain at-home food consumption data and also record all food consumed outside home for individuals during the same days on the basis of 24-h recall. Dietary intake of various nutrients was calculated using corresponding versions of Chinese Food Composition Table (FCT) [23] for each round. Daily dietary data in NHANES were collected via a 24-h dietary recall administered by trained interviewers, while dietary nutrients and energy components were determined from the USDA Survey Nutrient Database. Since only a single 24-h recall was carried out in NHANES III and NHANES 1999–2002, we only utilized data from the first dietary recall in our current analysis to keep the dietary information consistent through the whole period of study. Other demographic and lifestyle covariates were also collected in CHNS and NHANES [21,22].

2.3. Cohort follow-up and ascertainment of deaths

Follow-up duration was calculated from baseline until death or censoring on 31 December 2010 depending on which came first. In CHNS, mortality status was obtained by the information reported in each round of survey. If a case of death was reported more than once, the initially reported date was used. In NHANES, mortality outcomes were measured using probabilistic record linkage with the National Death Index (NDI). Causes of death were classified into 9 leading causes and other causes according to International Classification of Diseases-10 cause-of-death codes.

2.4. Statistical analysis

We described dietary and lifestyle characters of the two cohorts by quartiles of total PUFA intake. Cumulative means of PUFA intake were calculated in CHNS to report long-term diet and minimize intra-individual difference. Hazard ratios (HRs) combined with 95% confidence intervals (CIs) were evaluated by using Cox proportional hazard regression methods with follow-up person-years for CHNS and person-months for NHANES as the time metric. Covariates of known or suspected risk factors for death were considered in the multivariate models. Tests for trend were calculated by considering quartiles of PUFA intake as continuous variables.

In sensitivity analyses, we excluded participants with extreme body-mass index (BMI; <18.5 or >40 kg/m^2) or extreme energy intake (<500 or >4000 kcal/day) to minimize their influence and excluded the first two years of follow-up to see whether our findings materially change. To further address the potential residual confounding factors by measured variables, we applied propensity-score adjustment [24] in the multivariate-adjusted model. Finally, the use of omega-3 supplements, and vitamin and mineral use in NHANES was included in the multivariate-adjusted model to further assess its influence on our findings for US population.

We also conducted several secondary analyses for specific PUFA, as well as separate analyses stratified by potential effect modifiers where the values of P for interactions were estimated by likelihood-ratio test.

We did not pool the HR of these two cohorts mainly considering the heterogeneity and discrepancies that we expected to observe. All analyses were conducted via the platform of SAS version 9.4 statistical package (SAS Institute Inc., Cary, NC). PROC Survey was used to estimate variance after assembling the sample weights for each participant in NHANES. Statistical analyses were all two-sided and $P < 0.05$ was considered statistically at a significant level.

3. Results

3.1. Population characteristics

Baseline characteristics of participants in CHNS and NHANES classified by quartiles of PUFA intake are shown in Table 1. At baseline, individuals who had higher total PUFA intake were married and younger men with higher BMI, education and more likely to drink alcohol and smoke; they also consumed higher calories, more red and white meat, more fruits and vegetables, and higher amounts of total and saturated fatty acids in both CHNS and NHANES. Moreover, participants with higher PUFA intake were more often Non-Hispanic Caucasian, tended to have a family history of cardiovascular disease whereas less likely to have hypertension in NHANES, and, in CHNS, more probable to reside in urban.

3.2. PUFA intake and total mortality

Overall 1007 deaths occurred during a median of 14 years of follow-up (199,091 total person-years) in CHNS, while 4826 deaths occurred during a median of 9.1 years of follow-up (378,359 total person-years) in NHANES. A weak reverse association of dietary

Table 1
Characteristics of the participants at baseline classified by quartiles of PUFA intake in CHNS (n = 14,117) and NHANES (n = 36,032).

Characters	CHNS (n = 14,117)					NHANES (n = 36,032)				
	Quartiles of PUFA intake (g/day)				P for trend[a]	Quartiles of PUFA intake (g/day)				P for trend[a]
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
	≤6.85	(6.85–9.50)	(9.50–13.17)	≥13.17		≤8.54	(8.54–14.00)	(14.00–21.54)	≥21.54	
Male (%)	33.6	40.9	48.9	56.5	<0.001	36.3	43.2	50.5	64.3	<0.001
Age[b] (years)	40.8	39.0	37.6	37.6	<0.001	48.0	45.0	42.0	40.0	<0.001
Body mass index (kg/m^2)	22.5	23.1	23.3	23.6	<0.001	27.0	27.0	27.0	27.1	0.002
Race (%)										
Hispanic	N/A	N/A	N/A	N/A	N/A	25.6	25.7	24.4	22.8	<0.001
Non-Hispanic Caucasian	N/A	N/A	N/A	N/A	N/A	39.1	42.6	44.2	45.0	<0.001
Non-Hispanic Black	N/A	N/A	N/A	N/A	N/A	25.0	22.7	22.9	25.1	0.82
Others	N/A	N/A	N/A	N/A	N/A	10.3	9.0	8.5	7.2	<0.001
Married (%)	85.2	88.0	88.0	87.1	0.02	56.0	60.1	62.3	63.0	<0.001
Education										
Greater than high school (%)	7.4	11.8	13.3	17.9	<0.001	32.1	39.4	42.3	46.1	<0.001
Vigorous activity (%)	46.0	36.9	31.9	24.7	<0.001	24.2	27.7	31.1	34.6	<0.001
Current smoker (%)	18.4	19.9	21.8	25.3	<0.001	24.4	23.8	24.0	27.7	<0.001
History of hypertension (%)	16.9	15.0	14.6	16.4	0.47	40.6	37.2	34.2	30.5	<0.001
Current drinker (%)	19.0	23.7	26.1	32.0	<0.001	48.4	56.3	60.7	66.0	<0.001
Diabetes (%)	2.7	3.1	3.3	3.6	0.03	14.3	13.5	10.9	9.7	<0.001
Family history of cardiovascular disease (%)	N/A	N/A	N/A	N/A	N/A	17.4	17.8	17.8	18.9	0.02
Urban site (%)	26.0	32.6	39.1	53.9	<0.001	N/A	N/A	N/A	N/A	N/A
Dietary intake, median										
Total energy (kcal/day)	1842.9	2029.2	2193.3	2394.4	<0.001	1227.0	1729.0	2161.0	2949.0	<0.001
Fruits[c] (cup equivalents or g/day)	31.5	40.9	45.4	48.3	<0.001	0.76	1.12	1.39	1.89	<0.001
Vegetables[c] (cup equivalents or g/day)	383.0	406.7	434.0	501.7	<0.001	0.45	0.52	0.50	0.48	<0.001
Red meat (g/day)	33.3	72.7	100.0	138.9	<0.001	37.5	52.7	60.1	74.1	<0.001
White meat (g/day)	12.8	33.3	44.4	61.2	<0.001	56.4	84.5	99.9	137.9	<0.001
Total fat (g/day)	47.6	58.2	67.0	74.8	<0.001	35.4	59.3	81.6	123.2	<0.001
Saturated fat (g/day)	7.2	12.9	17.4	25.5	<0.001	12.8	19.9	26.1	36.3	<0.001

[a] P-trend values for categorical variables were analyzed by Cochran–Armitage tests and the t test for slope was used for continuous variables in generalized linear models. N/A, not available (Covariates including race and family history of cardiovascular disease were only available in NHANES, and residence (urban vs. rural) was only available in CHNS).
[b] Median (all such values).
[c] The unit cup equivalents/day was used in NHANES and g/day was used in CHNS.

PUFA intake with all-cause mortality was found in NHANES after full adjustment for potential confounders in multivariate-adjusted model (P_{trend} = 0.05) (Table 2). Surprisingly, in CHNS, the increase of total omega-3 PUFA intake was marginally related to incremental total mortality for the fourth quartile when comparing with the first quartile (HR = 1.22 (95% CI: 1.00, 1.50)) (Table 2) in multivariate-adjusted analyses. Whereas in NHANES, we observed a modest borderline inverse association of either dietary omega-3 PUFA (P_{trend} = 0.03) or omega-6 PUFA (P_{trend} = 0.04) intake with mortality.

3.3. Marine omega-3 PUFA, ALA and mortality

We next conducted separate analyses according to various kinds of omega-3 PUFA. In multivariate-adjusted analyses, increased marine omega-3 PUFA intake was associated with a substantial decrease of total mortality in CHNS (Table 3). The multivariate HRs (95% CIs) across categories of marine omega-3 PUFA consumption were 1.0, 0.63 (0.52, 0.75), 0.72 (0.60, 0.87), and 0.74 (0.61, 0.89) (P_{trend} < 0.001). However, in NHANES, marine omega-3 PUFA intake was inversely related to total mortality only for participants consuming 33–100 mg/day of EPA and DHA when comparing with non-consumers (HR = 0.87 (95% CI: 0.77, 0.98); P_{trend} = 0.17) (Table 3). Nonetheless, higher marine omega-3 PUFA intake was associated with lower risk of death from vascular disease (P_{trend} = 0.002) in NHANES (Supplemental Table 1). Moreover, we also found a lower chronic pulmonary disease mortality (P_{trend} = 0.04) whereas a borderline higher kidney disease mortality (P_{trend} = 0.06) with higher intake of marine omega-3 PUFA (Supplemental Table 1). ALA consumption with more than 1.53 g/day was related to 23% higher risk of total mortality when comparing with the consumption with less than 0.65 g/day (HR = 1.23 (95% CI: 1.01, 1.50); P_{trend} = 0.05) in CHNS (Table 3). Conversely, higher ALA consumption was modestly associated with lower all-cause mortality when comparing with extreme quartiles (HR = 0.84 (95% CI: 0.70, 1.00); P_{trend} = 0.04) in NHANES (Table 3), which was mainly due to reduced stroke mortality ($HR_{Q4\ vs\ Q1}$: 0.54 (95% CI: 0.30, 0.98)) and pneumonia or influenza mortality ($HR_{Q4\ vs\ Q1}$ = 0.31 (95% CI: 0.12, 0.81); P_{trend} = 0.03) (Supplemental Table 1).

3.4. LA, AA and mortality

In separate analyses for omega-6 PUFA and mortality, we observed that LA intake was not significantly associated with all-cause mortality in CHNS (Table 4). However, in NHANES, we found LA intake was reversely associated with all-cause mortality ($HR_{Q4\ vs\ Q1}$ = 0.83 (95% CI: 0.69, 1.00); P_{trend} = 0.03) and Alzheimer's disease mortality ($HR_{Q4\ vs\ Q1}$ = 0.41 (95% CI: 0.17, 1.00); P_{trend} = 0.04). Besides, higher LA intake was significantly related to lower diabetes mortality ($HR_{Q4\ vs\ Q1}$ = 0.39 (95% CI: 0.17, 0.92)) (Table 4 and Supplemental Table 2). For the AA intake, a significant and reverse association with total mortality was observed in CHNS. The multivariable-adjusted HRs (95% CIs) across increasing categories were 1.0, 0.75 (0.62, 0.90), 0.70 (0.56, 0.86) and 0.76 (0.58, 0.99) (P_{trend} = 0.01). Participants in NHANES consuming higher AA only had a borderline lower all-cause mortality (P_{trend} = 0.06) (Table 4). For cause-specific mortality, higher intake of AA was associated with lower mortality due to heart disease (P_{trend} = 0.02) and chronic pulmonary disease (P_{trend} = 0.02) in NHANES (Supplemental Table 2).

Table 2
Multivariate-adjusted hazard ratios for associations between intakes of total PUFA, omega-3 PUFA and omega-6 PUFA and all-cause mortality in CHNS (n = 14,117) and NHANES (n = 36,032).

	Total PUFA intake, g/day					Total omega-3 intake, g/day[a]					Total omega-6 intake, g/day[b]				
	Q1	Q2	Q3	Q4	*P* for trend	Q1	Q2	Q3	Q4	*P* for trend	Q1	Q2	Q3	Q4	*P* for trend
	0–6.85	6.85–9.50	9.50–13.17	≥13.17		0–0.68	0.68–1.05	1.05–1.60	≥1.60		0–5.88	5.88–8.11	8.11–11.3	≥11.3	
CHNS (n = 14,117)															
No. of deaths (%)	300 (8.5)	198 (5.6)	236 (6.7)	273 (7.7)		267 (7.6)	190 (5.4)	235 (6.6)	315 (8.9)		304 (8.6)	210 (6.0)	229 (6.5)	264 (7.5)	
Age and gender-adjusted HR (95% CI)[c]	1.00	0.68 (0.57, 0.82)	0.81 (0.68, 0.96)	0.82 (0.69, 0.97)	0.11	1.00	0.74 (0.61, 0.89)	0.75 (0.62, 0.90)	0.95 (0.80, 1.12)	0.74	1.00	0.72 (0.60, 0.86)	0.79 (0.66, 0.94)	0.79 (0.67, 0.95)	0.03
Multivariate-adjusted HR (95% CI)[d]	1.00	0.81 (0.67, 0.98)	1.06 (0.87, 1.29)	1.19 (0.93, 1.52)	0.10	1.00	0.88 (0.72, 1.06)	0.96 (0.79, 1.16)	1.22 (1.00, 1.50)	0.05	1.00	0.86 (0.71, 1.04)	0.99 (0.81, 1.21)	1.14 (0.89, 1.47)	0.29
	0–8.54	8.54–14.00	14.00–21.54	≥21.54	*P* for trend	0–0.78	0.78–1.25	1.25–1.97	≥1.97	*P* for trend	0–7.55	7.55–12.50	12.50–19.34	≥19.34	*P* for trend
NHANES (n = 36,032)															
No. of deaths (%)	1746 (19.4)	1362 (15.1)	995 (11.1)	723 (8.0)		1671 (18.5)	1348 (15.0)	1028 (11.4)	779 (8.7)		1745 (19.4)	1357 (15.0)	990 (11.1)	734 (8.2)	
Age and gender-adjusted HR (95% CI)[c]	1.00	0.96 (0.88, 1.06)	0.84 (0.76, 0.93)	0.77 (0.68, 0.88)	<0.001	1.00	0.91 (0.82, 1.01)	0.82 (0.74, 0.92)	0.78 (0.69, 0.87)	<0.001	1.00	0.97 (0.87, 1.07)	0.84 (0.77, 0.93)	0.76 (0.66, 0.88)	<0.001
Multivariate-adjusted HR (95% CI)[e]	1.00	1.01 (0.91, 1.11)	0.90 (0.80, 1.02)	0.86 (0.71, 1.03)	0.05	1.00	0.94 (0.84, 1.06)	0.86 (0.74, 1.00)	0.85 (0.71, 1.01)	0.03	1.00	1.00 (0.90, 1.10)	0.90 (0.80, 1.01)	0.84 (0.70, 1.01)	0.04

[a] Total omega-3 PUFA is the sum of eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid and α-linolenic acid.
[b] Total omega-6 PUFA is the sum of linoleic acid and arachidonic acid.
[c] Age and gender-adjusted model was only adjusted for age and gender.
[d] The adjusted variables in multivariable models used for CHNS included age, gender (male or female), BMI (in kg/m^2; <18.5, 18.5–23.9, 24–27.9, or ≥28), education (less than high school, high school, some college, or at least college), marital status (never married, married or living as married, widowed/divorced/separated, or unknown), residence (rural or urban), physical activity (no regular activity, low to moderate activity, or vigorous activity), smoking (never, former, current, or unknown), alcohol drinking status (abstainer or drinker), history of hypertension (yes, no, or unknown), history of diabetes (yes or no), intake of total energy, vegetables, fruits, red meat and saturated fat (all continuous variables).
[e] The adjusted variables in multivariable models used for NHANES included age, gender (male or female), race-ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other), BMI (in kg/m^2; <18.5, 18.5–24.9, 25–29.9, or ≥30), education (less than high school, high school, some college, or at least college), marital status (never married, married or living as married, widowed/divorced/separated, or unknown), physical activity (no regular activity, low to moderate activity, vigorous activity, or unknown), smoking (never, former, current, or unknown), alcohol drinking status (never, former, current, or unknown), history of hypertension (yes, no, or unknown), history of diabetes (yes or no), family history of cardiovascular disease (yes, no, or unknown), intake of total energy, vegetables, fruits, red meat and saturated fat (all continuous variables).

Table 3
Multivariate-adjusted hazard ratios for associations between specific omega-3 PUFA intake and all-cause mortality in CHNS (n = 14,117) and NHANES (n = 36,032).

	CHNS (n = 14,117)					NHANES (n = 36,032)				
	Q1	Q2	Q3	Q4	*P* for trend	Q1	Q2	Q3	Q4	*P* for trend
	0	0–21.4	21.4–68.6	≥68.6		0	0–33.0	33.0–100.0	≥100.0	
Marine long-chain n-3 PUFA, mg/day[a]										
No. of deaths (%)	520 (10.0)	162 (5.5)	164 (5.5)	161 (5.4)		2698 (21.4)	511 (6.5)	910 (10.4)	707 (10.4)	
Multivariate-adjusted HR (95% CI)[b]	1.00	0.63 (0.52, 0.75)	0.72 (0.60, 0.87)	0.74 (0.61, 0.89)	<0.001	1.00	0.90 (0.80, 1.02)	0.87 (0.77, 0.98)	0.96 (0.85, 1.09)	0.17
	0–0.65	0.65–1.00	1.00–1.53	≥1.53	*P* for trend	0–0.70	0.70–1.15	1.15–1.80	≥1.80	*P* for trend
α-linolenic acid, g/day										
No. of deaths (%)	253 (7.2)	205 (5.9)	229 (6.4)	320 (9.0)		1784 (19.0)	1258 (14.6)	1082 (11.7)	702 (8.0)	
Multivariate-adjusted HR (95% CI)[b]	1.00	0.93 (0.77, 1.13)	0.93 (0.76, 1.13)	1.23 (1.01, 1.50)	0.05	1.00	0.99 (0.89, 1.10)	0.90 (0.78, 1.04)	0.84 (0.70, 1.00)	0.04

[a] Marine omega-3 PUFA is the sum of eicosapentaenoic acid and docosahexaenoic acid.
[b] The adjusted variables in multivariable models are shown in the footnote of Table 2.

Table 4
Multivariate-adjusted hazard ratios for associations between specific omega-6 PUFA intake and all-cause mortality in CHNS (n = 14,117) and NHANES (n = 36,032).

	CHNS (n = 14,117)					NHANES (n = 36,032)				
	Q1	Q2	Q3	Q4	*P* for trend	Q1	Q2	Q3	Q4	*P* for trend
	0–5.81	5.81–8.03	8.03–11.21	≥11.21		0–7.43	7.43–12.32	12.32–19.17	≥19.17	
Linoleic acid, g/day										
No. of deaths (%)	303 (8.6)	211 (6.0)	228 (6.4)	265 (7.5)		1754 (19.5)	1341 (14.9)	999 (11.1)	732 (8.1)	
Multivariate-adjusted HR (95% CI)[a]	1.00	0.85 (0.70, 1.02)	0.97 (0.79, 1.19)	1.16 (0.91, 1.49)	0.27	1.00	0.99 (0.89, 1.09)	0.90 (0.80, 1.01)	0.83 (0.69, 1.00)	0.03
	0–44.6	44.6–81.7	81.7–130.8	≥130.8	*P* for trend	0–55.0	55.0–100.0	100.0–200.0	≥200.0	*P* for trend
Arachidonic acid, mg/day										
No. of deaths (%)	327 (9.3)	201 (5.7)	206 (5.8)	273 (7.7)		1475 (16.3)	1601 (16.6)	996 (10.7)	754 (9.5)	
Multivariate-adjusted HR (95% CI)[a]	1.00	0.75 (0.62, 0.90)	0.70 (0.56, 0.86)	0.76 (0.58, 0.99)	0.01	1.00	0.97 (0.87, 1.07)	0.87 (0.77, 0.97)	0.93 (0.81, 1.06)	0.06

[a] The adjusted variables in multivariable models are shown in the footnote of Table 2.

3.5. Omega-6/omega-3 ratio and mortality

In CHNS, those who consumed dietary PUFA with an omega-6/omega-3 ratio ranging 6–10 had dramatically lower all-cause mortality compared with those who consumed PUFA with the ratio less than 6 (Table 5). HRs (95% CIs) across categories of omega-6/omega-3 ratio were 1.0, 0.79 (0.66, 0.95), 0.77 (0.64, 0.94) and 0.95 (0.80, 1.14) (P_{trend} = 0.54). Moreover, mortality due to all-cause and specific-causes was not related to omega-6/omega-3 ratio, except that mortality from Alzheimer's disease reduced with increased omega-6/omega-3 ratio in NHANES (Table 5 and Supplemental Table 3).

3.6. Subgroup analyses

In subgroup analyses, the documented inverse association of omega-3 PUFA consumption with all-cause mortality appeared significant among both genders in CHNS (Supplemental Table 4), whereas in NHANES the inverse correlation was only found in women (P_{trend} = 0.007; *P* for interaction = 0.03) (Supplemental

Table 5
Hazard ratios for associations between the omega-6/omega-3 ratio and all-cause mortality in CHNS (n = 14,117) and NHANES (n = 36,032).

	Ratio of omega-6 to omega-3 PUFA[a]				
	≤6	6–8	8–10	≥10	*P* for trend
CHNS (n = 14,117)					
No. of deaths (%)	329 (9.6)	201 (6.0)	204 (5.9)	273 (7.1)	
Age and gender-adjusted HR (95% CI)[b]	1.00	0.71 (0.59, 0.85)	0.74 (0.62, 0.89)	0.99 (0.84, 1.17)	0.79
Multivariate-adjusted HR (95% CI)[c]	1.00	0.79 (0.66, 0.95)	0.77 (0.64, 0.94)	0.95 (0.80, 1.14)	0.54
	≤8	8–10	10–12	≥12	*P* for trend
NHANES (n = 36,032)					
No. of deaths (%)	1536 (14.8)	1207 (12.4)	840 (12.8)	1243 (13.4)	
Age and gender-adjusted HR (95% CI)[b]	1.00	1.02 (0.93, 1.13)	0.99 (0.90, 1.10)	0.98 (0.88, 1.09)	0.64
Multivariate-adjusted HR (95% CI)[c]	1.00	1.03 (0.93, 1.14)	1.01 (0.90, 1.13)	0.99 (0.89, 1.11)	0.85

[a] Total omega-3 PUFA is the sum of eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid and α-linolenic acid. Total omega-6 PUFA is the sum of linoleic acid and arachidonic acid.
[b] Age and gender-adjusted model was only adjusted for age and gender.
[c] The adjusted variables in multivariable models are shown in the footnote of Table 2.

Table 5). Furthermore, in CHNS, the inverse relationship between marine omega-3 PUFA and all-cause mortality was stronger for rural residents than urban residents (P for interaction < 0.001) whereas the inverse association of AA was stronger among urban than rural residents (P for interaction < 0.001). For ALA and LA intake, those in the highest quartile were significantly and positively associated with incremental all-cause mortality for rural residents (ALA, HR = 1.53 (95% CI: 1.22, 1.92); LA, HR = 1.36 (95% CI: 1.02, 1.83)) compared with those in the lowest quartile (Supplemental Tables 4 and 6). Other differences across subgroups stratified by covariates like BMI, alcohol drinking, smoking and hypertension were also observed (Supplemental Tables 4–7).

3.7. Sensitivity analyses

The observed associations of specific types of PUFA were mostly essentially unchanged when we adjusted for propensity scores, the use of omega-3 supplements, vitamin and mineral (in NHANES only), and excluded those with diabetes, the first 2 years of follow-up, or persons with extreme energy intake, or those with extreme BMIs (Supplemental Table 8).

4. Discussion

In these two independent nationally representative cohorts with long follow-up durations, we showed that the associations of PUFA intake with mortality largely depended on specific subclasses of PUFA and were divergent between Chinese and US population.

We observed that higher marine omega-3 PUFA intake was dramatically associated with lower total mortality in CHNS and was modestly related to lower all-cause mortality for those consuming at 33–100 mg/day in NHANES. This finding was generally consistent with some previous studies [7–9,20], but not all [3,10,11]. In China, only one cohort study in Shanghai referred to the association of omega-3 PUFA intake with all-cause mortality and reported that dietary EPA and DHA was monotonously and reversely associated with total mortality ($HR_{Q5\ vs\ Q1}$: 0.79 (0.72, 0.87)) [20]. However, the participants were only restricted to those living in urban area in Shanghai. In subgroup analysis, we observed a stronger inverse association of omega-3 PUFA intake with all-cause mortality among rural residents than urban residents. Thus, our results revealed a more protective effect of marine omega-3 PUFA (EPA and DHA) intake on total mortality and were also more representative for Chinese population than previous outcomes. Therefore, the low level of marine omega-3 PUFA intake should be improved especially for Chinese population to meet the FAO/WHO recommendation (total amount of DHA and EPA: ≥250 mg/day) [25]. In NHANES, currently-documented reverse association of marine omega-3 PUFA intake with the death from vascular disease and chronic pulmonary disease was in agreement with considerable evidence [26,27]. However, we did not observe such a significant association in NHANES. The protective effect of marine omega-3 PUFA on vascular disease and chronic pulmonary disease mortality may be counterbalanced by its null or even adverse effects with mortality from other causes like kidney disease. Nonetheless, the unexpected borderline positive association with kidney disease mortality in NHANES needs further investigation [28]. Evidence linking marine omega-3 fatty acid intake with cancer risk have been mixed. In our study, we did not find any association between marine omega-3 PUFA intake and cancer mortality. In contrast, the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) reported that increased marine omega-3 PUFA intake after colorectal cancer diagnosis was associated with lower cancer mortality [29]. Thus, here we expect that marine omega-3 fatty acid could have an anti-cancer effect among patients with existing carcinogenesis or the therapeutical effect is restricted to colorectal cancer. Further researches are needed to investigate the effects of marine omega-3 PUFA on mortality from other subtypes of cancer, such as breast cancer and prostatic cancer.

The most significant differences between the two national populations were observed from ALA-related association outcomes. Dietary ALA intake (≥1.53 g/day) exhibited a positive association with total mortality in CHNS, whereas higher intake (≥1.80 g/day) was related to lower total mortality in NHANES. Previous evidence showed that dietary ALA was inversely related to total mortality in a trial on subjects at high CVD risk [13] and in a cohort encompassing postmenopausal women [10]. In addition, the investigated inverse association of ALA with stroke mortality and pneumonia or influenza mortality in NHANES was supported by other studies [30,31]. However, two recent studies found ALA intake was not related to total mortality but moderately associated with elevated CVD risk in old women [12] and a slightly increased cancer mortality in the NHS and HPFS [9]. Importantly, we observed that consuming high amount of ALA may elevate the risk of death in CHNS, especially among rural residents and ever-smokers. Therefore, further large-scale observational studies are warranted to formulate the recommended dose of ALA and avoid potential adverse effects for Chinese population.

Limited data have involved the associations of omega-6 PUFA with mortality. Our results from NHANES are concordant with previous evidence showing decreased total mortality with increased circulating level of LA in US population [32,33]. However, recent cohort studies did not support such association [34–36]. In contrast, a clinical nutrition trial from the Sydney Diet Heart Study showed an elevated risk of mortality with increased level of dietary LA intake [37], which is in line with our finding that consuming higher amount of LA (>11.21 g/day) was associated with higher mortality among rural residents in CHNS. As a derivative of LA, dietary AA intake showed an inverse correlation with total mortality in both CHNS and NHANES, which is consistent with the results from NHS and HPFS [9]. We also found PUFA consumption with omega-6/omega-3 ratio ranging 6–10 was associated with reduced total mortality than the intake that the ratio ranging less than 6 in CHNS, while no significant association of the omega-6/omega-3 ratio with death was observed in NHANES. Previous clinical trials even demonstrated that PUFA intake with high omega-6/omega-3 ratio could lower the risk of CHD [38,39]. Therefore, the appropriate ratio of omega-6/omega-3 may range 6–10 for Chinese adults.

The two investigated studies between CHNS and NHANES have similar study design and almost synchronized duration of follow-up but we documented largely inconsistent findings between these two populations. This may result from gene polymorphisms and gene–diet interaction [40]. In the past few years, genome-wide association studies (GWAS) have demonstrated that genetic risk loci for common chronic diseases including CVD, diabetes and cancer were diverse between East Asian and Western people [41]. Given various disease-associated loci or risk alleles frequencies between Western and East Asian people, the association of the same intake levels of PUFA with related disease incidence may vary between these two populations via gene–diet interaction. Moreover, genetic variants at genes that affect PUFA uptake and metabolism *in vivo* may conduce to the variation in circulating levels of PUFA [42]. Therefore, racial difference in genetic architecture may contribute to different circulating PUFA levels and gene–diet interaction induced different incidences of disease. Another possible explanation may be the differences of dietary patterns

between Western and Asian populations. Typically, fried fish was common in Western countries but boiled or steamed fish was preferred in Asian countries. Thus, different dietary patterns may also be responsible for inconsistent associations of PUFA with mortality found in US and Chinese populations. Since the survey circle 2011–2012, NHANES included an oversample of Asian Americans. Future researches on this population may help elucidating whether the differences observed could be related to genetic profile or different lifestyles. Nevertheless, the heterogeneity between two cohorts including different age ranges of populations and different means of deaths ascertainment may also contribute to the discrepancies.

We also investigated different associations across several subgroups. The most significant difference was found for the residence in CHNS. This urban-rural disparity may be due to different environment, lifestyle and dietary patterns. The main dietary sources of ALA and omega-6 PUFA referred to soybean oil and salad oil for urban residents and rapeseed oil for rural residents, respectively. Besides, fish, the main source of marine omega-3 PUFA, consumed among rural residents may be higher quality and less contaminant than the fish consumed among urban residents. Thus, formulating recommendations of PUFA intake for Chinese rural and urban residents should be individually taken into consideration.

Strengths of our study included the use of two nationally representative samples of Chinese and US adult populations, long duration of follow-up and repeated assessment of diet in CHNS. In several sensitivity analyses, most of the documented associations remain materially unchanged, which reflects the robustness of our results. The associations did not alter when we further include the omega-3 supplements use in the model in NHANES. The omega-3 supplements use was not assessed in CHNS but should not substantially impact our results due to non-popular consumption of the supplements in Chinese. Our studies also have some limitations. First, the self-reported dietary intakes measured by 24-h dietary recall in NHANES were only at one time for the baseline investigation, which may not reflect the long-term dietary patterns. We also lacked data for cause-specific mortality in CHNS, which may provide more implications for health promotion. Furthermore, we did not adjust for *trans*-fatty acids (TFAs) intake in the model due to the lack of data. However, TFAs is unlikely to change our findings appreciably as the overall intake level of TFAs in China is quite low [43]. Last, reverse causation may be possible considering the observational nature. Thus, we excluded the patients with cancer, diabetes or CVD at baseline and also excluded initial two years of follow-up and observed similar results.

In conclusion, different subtypes of PUFA divergently are associated with mortality and the investigated associations also vary between Chinese and US populations. Marine omega-3 PUFA and AA may be more protective for Chinese population compared with US population. High consumption of ALA and LA may lower risk of death for US population whereas elevate mortality for Chinese population. Besides, PUFA consumption with an omega-6/omega-3 ratio ranging 6–10 may reduce risk of total mortality for Chinese population.

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Conflict of interest

None declared.

Author contribution

Study conception and design (Yu Zhang and Jingjing Jiao); data collection (Pan Zhuang, Wenqiao Wang, and Jun Wang); statistical analysis (Pan Zhuang); drafting the manuscript (Pan Zhuang); critical revision of the manuscript (Yu Zhang and Jingjing Jiao). All authors have read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.02.019.

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