Plasma omega-3 polyunsaturated fatty acid and tocopherol statuses and their relationships with aging-related diseases

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Research

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Abstract

Background: The beneficial effects of omega-3 polyunsaturated fatty acids (PUFAs) and tocopherols remain controversial. This study was conducted to examine the plasma levels of different analogues of omega-3 PUFAs and tocopherols, and to evaluate their relationships with aging-related diseases.

Methods: 136 consecutive men with the median age of 70 (ranging from 50 to 97) years old were recruited. Plasma eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), α-, γ-, and δ-tocopherol were measured by liquid chromatography mass spectrometry.

Results: Plasma omega-3 PUFAs were positively correlated with α- and γ-tocopherol ($p<0.01$), while the relationships between omega-3 PUFAs and δ-tocopherol were not significant ($p>0.05$). Increasing age was the most profound risk factor for coronary artery disease (CAD), carotid atherosclerosis (CAS), hypertension and these comorbidities ($p<0.01$). Age had positive associations with certain atherosclerotic parameters, including carotid intima-media thickness ($p=0.046$), carotid artery plague area ($p<0.001$) and brachial-ankle pulse wave velocity ($p<0.001$). In these observed isoforms, only γ-tocopherol had a mild inverse correlation with age ($p=0.002$). High plasma α-tocopherol concentration served as a potential protective factor for CAD (odds ratio [OR] 0.65; 95% CI 0.48-0.89; $p=0.006$), and was inversely associated with maximum systolic internal carotid artery (ICA) velocity ($p=0.011$). Plasma DHA concentration was negatively associated with carotid artery plague area ($p=0.028$) and ICA velocity ($p=0.006$), while its correlations with atherosclerosis diseases, including CAD and CAS, were not significant ($p>0.05$).

Conclusions: Age is positively correlated with atherosclerosis, hypertension and comorbidities. Alpha-tocopherol may be a protective factor for atherosclerosis, while the correlations between omega-3 PUFAs and atherosclerosis were not significant.

Introduction:

Omega-3 polyunsaturated fatty acids (PUFAs) have four isoforms, including α-linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid. Omega-3 PUFAs are known to have several beneficial effects, such as alleviating oxidative injuries, relieving vascular inflammation, improving cognitive function and restraining tumor growth [1]. Previous intervention studies suggested that omega-3 PUFAs, especially EPA and DHA, benefit multiple cardiovascular diseases [2–3]. Recently, large-scale clinical trials and meta-analysis revealed that the cardiovascular protective effects of omega-3 PUFAs remain ambiguous [4–10].

Natural vitamin E comprises eight analogues, the α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. Vitamin E is considered a lipid-soluble antioxidant that protects low density lipoprotein cholesterol (LDL-C) from oxidation. The antioxidative efficacy of vitamin E is interrelated to cell senescence, immune responses and vascular inflammation, particularly in elderly [11]. Alpha-tocopherol is the main form in supplements which has been widely studied. Accumulating evidence suggests that γ-tocotrienol has potential cardiovascular and metabolic health-promoting properties [12], while the biological efficiencies of other isoforms are not established.

Possible complementary role for these two dietary components has been investigated: omega-3 PUFAs may enhance lipid peroxidation and cytotoxicity [13], while tocopherol could improve the role of omega-3 PUFAs through protection from lipid peroxidation [14–16]. Omega-3 fatty acids and α-tocopherol co-supplementation could enhance the antioxidant properties [17] and reveals favorable effects on multiple aging-related diseases [18–19]. Studies have shown that nutritional intervention may be a promising approach to alleviating impaired vascular function and aging process. However, controversy exists concerning every nutritional regimen tested to date [20].

Correlations between varied omega-3 PUFAs and tocopherols haven't been well illustrated, especially in Chinese individuals. Blood-based measurements of nutrients are objective and accurate to estimate their biological exposure. In the present study, liquid chromatography mass spectrometry (LCMS) was employed to measure plasma EPA, DHA, α-, γ-, and δ-tocopherol

levels, aiming to examine whether different analogues of omega-3 PUFAs and tocopherols are correlated with aging-related diseases in Chinese men aged 50 years or older.

Material And Methods

Design and subjects

This was a cross-sectional, population-based study. We recruited consecutive participants who presented to our department for routine physical examination between September and December 2017. Inclusion criteria were asymptomatic male subjects who were aged fifty years or older with or without chronic diseases, including hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or had antihypertensive treatments), coronary artery disease (CAD, at least 50% stenosis of coronary artery confirmed by coronary computed tomography angiography or coronary angiography), carotid atherosclerosis (CAS, carotid artery plaque detected by ultrasound) and diabetes mellitus (DM, fasting blood glucose ≥ 7.0 mmol/l or blood glucose ≥ 11.1 mmol/L after oral glucose tolerance test within two hours or had antidiabetic treatments). Comorbidity was assigned a value according to the number of disease (s) that participants combined, including hypertension, CAD, CAS and DM. Exclusion criteria included age less than fifty years old, inpatients or outpatients who were recovering from severe diseases and failure to obtain informed consent. Briefly, 136 male subjects aged 50 years or older were enrolled in this study.

This study was performed in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from every participant, and the study protocol was approved by Hospital Institutional Ethics Committee.

Measurement of omega-3 PUFAs and tocopherols

Fasting plasma samples were collected and stored at -80℃ until analyses. Plasma EPA, DHA, α-, γ-, and δ-tocopherol were measured by LCMS as we declared in previous article [21] (Fan-Xing Biological Technology-Beijing Co., Ltd). The peak area of each component was linearly correlated with its concentration ($R^2 > 0.99$). Therefore, plasma concentrations of omega-3 PUFAs and tocopherols were calculated according to the equation of linear regression between peak area and concentration.

Peripheral atherosclerotic parameters

All the carotid parameters were obtained by B-mode ultrasonographic scanning we had described before [22] (ACUSON S2000, Germany). Carotid intima-media thickness (IMT) was measured between the intimal-luminal and the medial-adventitial interfaces of the carotid artery (CA) wall. CA plaque was defined as a focal thickening that encroached into the lumen, roughness or inconsistency in the boundaries. Internal CA (ICA) velocity was recorded as the maximum systolic velocity of bilateral ICA.

Brachial-ankle pulse wave velocity (BAPWV) and ankle-brachial index (ABI) were evaluated by an automatic noninvasive device (OMRON BP-203RPE Ⅱ, Japan). BAPWV was calculated by dividing the pulse wave transmission time in the distance between the brachial and ankle arteries on each side. ABI was defined as the ratio of simultaneous systolic blood pressure in the ankle and ipsilateral brachial arteries. BAPWV was recorded as the higher value while ABI was noted as the lower value of the two sides [23].

In addition, other biochemical parameters, including hemoglobin (Hb), alanine transaminase (ALT), triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting plasma glucose (Glu), hemoglobin A1c (HbA1c) and serum creatinine (Scr) were measured by standard analytical methods with routine laboratory testing at our hospital.

Statistical analyses

The normality of each continuous variable was examined by the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation (SD) and were tested by the student t-test between two groups. Non-normally

distributed variables were presented as median with interquartile range (IQR) and were tested by the nonparametric test between two groups. Potential differences in categorical variables between two groups were estimated by the chi-square test or Fisher's exact test. Bivariate correlation analyses were employed to estimate the associations between varied nutrition ingredients, and also the relationships between every analogue and aging-related parameters, including age, comorbidity and peripheral atherosclerotic parameters. Ordinal logistic regression analysis was used to evaluate the associations of different isoforms and comorbidity. Binary logistic regression model was used to evaluate the associations of different isoforms and aging-related diseases. P values less than 0.05 were regarded as statistically significant. All statistical analyses were performed using SPSS 20.0 software (Statistical Package for the Social Sciences, SPSS Ins., Chicago, IL).

Results

Study participants

136 male subjects aged 50 years or older were selected for this study. Main characteristics of participants are shown in Table 1. Age was a negative-skewed variable in this study and the median age of all the participants was 70 (58-81) years old, ranging from 50 to 97. Distributions of involved nutrition analogues were non-normal. In the present study, the median concentrations of plasma EPA, DHA, α-, γ- and δ-tocopherol were 18.35 (11.13-32.55) ug/dL, 81.10 (55.43-115.75) ug/dL, 304.00 (213.75-499.50) ug/dL, 77.85 (56.55-112.25) ug/dL and 3.59 (2.21-5.32) ug/dL, respectively. Due to the extremely low concentration of plasma β-tocopherol (median 0.027, 0.021-0.037 ug/dL), we did not include this analogue for further analysis. The average levels of other biochemical parameters, including Hb, ALT, lipid profiles, Glu, HbA1c and Scr were in normal ranges (Table 1).

According to the correlation analyses between varied nutrition ingredients (Table 2), positive correlations were found between most two nutrition isoforms, including EPA and DHA ($p<0.001$), α- and γ-tocopherol ($p<0.001$), α- and δ-tocopherol ($p=0.004$), δ- and γ-tocopherol ($p<0.001$), EPA and α-tocopherol ($p<0.001$), EPA and γ-tocopherol ($p=0.010$), DHA and α-tocopherol ($p<0.001$), DHA and γ-tocopherol ($p=0.010$). While the relationships between omega-3 PUFAs and δ-tocopherol were not significant (EPA and δ-tocopherol, $p=0.193$; DHA and δ-tocopherol, $p=0.313$).

Correlations between age and plasma nutrition ingredients (Table 3) showed that every analogue tended to be negatively correlated with age, merely γ-tocopherol had a significant inverse correlation with age ($p=0.002$).

Relations of plasma nutrition ingredients and aging-related diseases

Comparisons between participants with and without comorbidities (Table 4) showed that participants with comorbidities were older than those without comorbidities (75 vs. 56 years, $p=0.001$). There were no differences in plasm omega-3 PUFAs and tocopherols between the two groups ($p>0.05$). Both correlation (Table 5) and regression (Table 6) analyses suggested that age was positively correlated with comorbidity ($p<0.001$). Except for δ-tocopherol, every nutrition analogue had a mild inverse correlation with comorbidity ($p<0.05$, Table 5), however, the correlation was not significant in regression analysis ($p>0.05$, Table 6).

The enrolled participants were divided into different groups according to their median concentrations of each analogue. As shown in Table 7, no significant differences were found in the observed diseases between the low and high EPA subgroups ($p>0.05$). In the DHA subgroups, CAD proportion was lower in the high DHA group compared with the low DHA group (30.9% vs. 51.5 %, $p=0.015$). Similarly, the proportions of certain diseases, such as CAD (26.5% vs. 56.1 %, $p<0.001$) and hypertension (55.9% vs. 72.7 %, $p=0.042$) were significantly lower in the high α-tocopherol group compared with the low α-tocopherol group. The hypertension proportion in the high γ-tocopherol group was lower compared with the low γ-tocopherol group (54.4% vs. 74.2 %, $p=0.017$), and the same result was found in the δ-tocopherol subgroups (54.4% vs. 74.2 %, $p=0.017$).

Binary logistic regression analysis was conducted to figure out the independent influence factors of certain disease (Figure 1). Results showed that age was the most outstanding risk factor for the majority of observed diseases in this study,

including CAD (odds ratio [OR] 1.69; 95% CI 1.21-2.36; p=0.002), CAS (OR 2.68; 95% CI 1.77-4.04; p<0.001) and hypertension (OR 1.71; 95% CI 1.22-2.40; p=0.002). High plasma α-tocopherol concentration was found to be the single protective factor for CAD among all the measured analogues of omega-3 PUFAs and tocopherols (OR 0.65; 95% CI 0.48-0.89; p=0.006).

Relations of plasma nutrition ingredients and peripheral atherosclerotic parameters

Table 8 provides the comparisons of peripheral atherosclerotic parameters between different concentrations of plasma nutrition ingredients. The only significant differences were detected in carotid IMT between the high and low γ-tocopherol subgroups (1.00 mm vs. 1.10 mm, p=0.018), and ICA velocity between the high and low α-tocopherol subgroups (0.55m/s vs. 0.65 m/s, p=0.035).

Correlation analyses of peripheral atherosclerotic parameters (Figure 2) revealed that age had significant positive associations with certain atherosclerotic parameters, including carotid IMT (Spearman coefficient=0.19, p=0.046), CA plague area (Spearman coefficient=0.55, p<0.001) and BAPWV (Spearman coefficient=0.60, p<0.001). Among all the measured analogues of omega-3 PUFAs and tocopherols, both plasma EPA (Spearman coefficient=-0.20, p=0.043) and DHA (Spearman coefficient=-0.21, p=0.028) concentrations had mild inverse correlations with CA plague area. Similarly, plasma DHA (Spearman coefficient=-0.255, p=0.006) and α-tocopherol (Spearman coefficient=-0.235, p=0.011) concentrations were negatively associated with ICA velocity.

Discussion

The antioxidant properties and cardiovascular protective effects of omega-3 PUFAs (mainly EPA and DHA) and α-tocopherol have been investigated for decades. The present study was conducted to address the associations of different plasma nutrition ingredients and certain elderly issues, such as certain chronic diseases and peripheral atherosclerosis. According to our data, both plasma omega-3 PUFA (EPA and DHA) and tocopherol (α-, γ-, and δ-tocopherol) concentrations were lower than that in other reports, which may suggest that the Chinese elderly may have a lower nutrition baseline. Besides, these results may be secondary to LCMS, a more sensitive and precise separating method than traditional detection of high-performance liquid chromatography (HPLC) [24].

Omega-3 fatty acids are sensitive to oxidation and α-tocopherol supplement can alleviate inflammation and oxidative injuries through decreasing the production of anti-inflammatory cytokines [25]. GISSI-Prevenzione trial is one of clinical trials focused on independent and combined effects of omega-3 PUFAs and α-tocopherol on morbidity and mortality after myocardial infarction [2, 18], and the results supported the anti-atherosclerotic benefits of these nutrition ingredients. However, correlations between plasma omega-3 PUFAs and tocopherols are seldom well illustrated. The present results demonstrated that plasma omega-3 PUFAs were positively correlated with α-tocopherol and had mild positive correlations with γ-tocopherol, while the relationships between omega-3 PUFAs and δ-tocopherol were not significant.

In this study, merely γ-tocopherol concentration had a significant inverse correlation with age. Some studies suggest that α- and γ-tocopherol are significantly lower in the older individuals [26], and γ-tocopherol level accounts for meaningful increases in biological aging [16], other studies state that the correlations between tocopherols and age are not significant [27]. All the published studies agreed that the circulating levels of tocopherols tend to be negatively correlated with age (correlation coefficient < 0). A possible explanation for these phenomena is that oxidative stress and inflammation are implicated in ageing process, resulting in an antioxidant depletion. As for omega-3 PUFAs, most studies found a regular increase in EPA and DHA with rising age [28–30], others were not [21]. In general, aging has been proven to be relevant to nutritional status [31] and the correlations maybe more apparent after adjustment for varied dietary intake.

The statistical results showed that age was the shared risk factor for CAD, CAS, hypertension and comorbidities, and had significant positive associations with carotid IMT, CA plaque area and BAPWV. These findings are consistent with previous evidence that aging compounds the pathophysiology of cardiovascular disease, mainly hypertension and atherosclerosis [11]. Correlations between comorbidities and the varied nutrition ingredients were not significant, when taking the diverse

analyses into account (Table 7 + Fig. 1 and Table 8 + Fig. 2), the comprehensive results demonstrated that high plasma α-tocopherol concentration served as a protective factor for CAD, and was inversely associated with maximum systolic velocity of ICA. Large observational studies also provided evidence of an association between a high intake of α-tocopherol and a lower risk of CAD [32–33]. Both antioxidative and anti-inflammatory ingredients have been reported to effectively lower the risk of toxicity caused by reactive oxygen species (ROS) and are important in preventing not just cardiovascular disease but many other aging-related diseases [34]. Alpha-tocopherol is the most active form of vitamin E and has been shown to be cardioprotective in numerous cell culture, animal model and human studies [34–36]. Daily supplementation with antioxidant vitamins (ascorbic acid and α-tocopherol) may help prevent myocardial infarctions [37] and increase the probability of healthy aging [38]. Some studies suggest that low concentration of vitamin E was significantly associated with CAS [39]. However, certain research reported conflicting results that high-dose tocopherol intervention had no significant effect on CAS [40].

Clinical trials evaluating the cardiovascular benefits of α-tocopherol have been equivocal to date [41–43]. Concerns of the anti-atherosclerotic effects of tocopherols decline recently. Similarly, although treatments with omega-3 PUFAs have been found to be effective in pre-clinical experiments of cardiovascular diseases [44–45] and certain clinical studies [2–3], data from large clinical trials, especially the recent VITAL trial [9] and meta-analysis from Cochrane database [8] have been disappointing. There are several possible explanations for this discrepancy. First, the benefits of nutritional supplementation may take many years to develop, and therefore the intervention doses and durations of recent trials maybe insufficient. Second, cardiovascular risk in individuals is not static, patients are likely to have lower risk of developing disease with better management of blood pressure, dyslipidemia and other risk factors for the past few years. Primary and secondary prevention of cardiovascular diseases has been aggressively used in patients at higher risk. Our previous study found that serum lipid levels, including TG, TC, and LDL-C, in the CAD group were markedly lower than that in the CAD-Risk group, which was mostly attributed to the widespread statin use (89.0% vs. 32.2%, $p < 0.001$) [21]. REDUCE-IT trial has manifested that patients with elevated triglyceride levels despite the use of statins, the risk of ischemic events was significantly lower among those who received high doses of pure EPA than those who received placebo [10]. Additionally, if a patient develops stable angina and receives more intense management and close follow-up, the chance of developing a cardiovascular event is lessened [46].

Several studies found that the treatment of each natural analogue of tocopherol often leads to different outcomes despite having similar ability to scavenge free radicals [47], and a mixture of tocopherols has a stronger inhibitory effect on lipid peroxidation induced in human erythrocytes than α-tocopherol alone [48]. In other words, although each tocopherol analogue has a similar antioxidant chemical “soul,” their different structural “bodies” may be responsible for their differential effects on signal transduction and gene expression [47]. Therefore, differential effects of varied analogues in elderly issues need increasing attention and prospective evidence.

Except injury of hepatitis transfer protein, anemia due to red blood cell damage or immune dysfunction, a deficiency of omega-3 PUFAs [49–51] or vitamin E [52–53] is rare. A relatively low dose, long-term intervention with the nutrient supplements may be beneficial to atherosclerosis and several age-related diseases [11, 20]. However, overdose of tocopherol (more than 400 IU/day) might interference with warfarin effects [54], and were associated with increased risk of all-cause mortality [55] and heart failure [56]. High dose purified omega-3 PUFA (4 g/day) would reduce plasma TG and ischemic events [10, 57], while the optimal dose, duration, and timing remain unclear [58]. Despite the inconsistent results obtained in previous studies, the American Heart Association (AHA) recommends patients with cardiovascular diseases, especially those with hypertriglyceridemia to take seafood or omega-3 PUFA supplements [59–62]. The use of nutrient supplements remains a decision involved a thoughtful discussion on the balance of cardiovascular benefits, possible cancer risk, preferences, cost, and other factors between a clinician and an individual.

There is no denying that some restrictions exist in this study. This was a cross-sectional study, which precluded us from obtaining a definite conclusion on the cause-effect relationship between these nutrition ingredients and aging-related diseases. Additionally, considering the limited sample size, we employed diverse statistical analyses to state every issue for the sake of possible bias. Larger-scale and more comprehensive studies are needed to elucidate our findings.

Conclusions

This is the first time to investigate the relationships between varied nutrition ingredients and several aging-related parameters, including comorbidities, carotid IMT, CA plague area, ICA velocity, BAPWV and ABI. Age is positively correlated with CAD, CAS, hypertension and comorbidities. Plasma omega-3 PUFAs were positively correlated with α- and γ-tocopherol, but merely γ-tocopherol declined with aging. Alpha-tocopherol may be a protective factor for atherosclerosis, while the correlations between omega-3 PUFAs and atherosclerosis were not significant. These findings suggest that differential effects of varied analogues of nutrients in elderly issues need increasing attention and prospective evidence.

Abbreviations

PUFAs: omega-3 polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LDL-C: low density lipoprotein cholesterol; LCMS: liquid chromatography mass spectrometry; CAD: coronary artery disease; CAS: carotid atherosclerosis; DM: diabetes mellitus; IMT: intima-media thickness; CA: carotid artery; ICA: internal carotid artery; BAPWV: brachial-ankle pulse wave velocity; ABI: ankle-brachial index; Hb: hemoglobin; ALT: alanine transaminase; TG: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; Glu: fasting plasma glucose; HbA1c: hemoglobin A1c; Scr: serum creatinine; SD: standard deviation; IQR: interquartile range; ROS: reactive oxygen species; AHA: American Heart Association

Declarations

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Authors' contributions

Wenwen. Liu developed an overall research plan, collected samples together with medical data, performed statistical analyses and wrote the paper. Meilin. Liu proposed project conception and had primary responsibility for the final content. Xiahuan. Chen and Bo. Huang provided essential medical records. Weimei. Ou assisted sample collection.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its Additional files].

Ethics approval and consent to participate

The study protocol was approved by Peking University First Hospital Institutional Ethics Committee. Written informed consent was obtained from every participant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Main characteristics of the participants

Variables	All participants(n=136)
Age(y)	70.00(58.00-81.00)
BMI(Kg/m^2)	25.16±2.90
Hypertension(%)	64.18%
DM(%)	36.57%
CAD(%)	41.04%
CAS(%)	61.90%
Hb(g/L)	145.00(134.00-152.50)
ALT(IU/L)	18.00(14.00-24.00)
TG(mmol/L)	1.21(0.97-1.78)
TC(mmol/L)	4.19±1.05
HDL(mmol/L)	1.08(0.93-1.27)
LDL(mmol/L)	2.20(1.74-2.91)
Glu(mmol/L)	5.46(5.00-6.27)
HbA1c(%)	6.00(5.68-6.40)
Scr(μmol/L)	88.01(80.58-96.28)
EPA(ug/dL)	18.35(11.13-32.55)
DHA(ug/dL)	81.10(55.43-115.75)
α-Tocopherol(ug/dL)	304.00(213.75-499.50)
γ-Tocopherol(ug/dL)	77.85(56.55-112.25)
δ-Tocopherol(ug/dL)	3.59(2.21-5.32)
Medication(%)	
Statins	58.95%
Antiplatelet Agents	75.00%
ACEIs	18.75%
ARBs	50.00%
β-Blockers	66.67%
CCBs	50.00%
Diuretics	16.67%
Nitrates	39.58%
Antidiabetics	26.00%
PPIs	41.67%

BMI, body mass index; DM, diabetes mellitus; CAD, coronary artery disease; CAS, carotid atherosclerosis; Hb, hemoglobin; ALT, alanine transaminase; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; Glu, fasting plasma glucose; HbA1c, hemoglobin A1c; Scr, serum creatinine; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ACEIs, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CCBs, calcium channel blockers; PPIs, proton pump inhibitors. Values were mean ± standard deviation (SD) or median (interquartile range, IQR).

Table 2. Correlations between varied omega-3 PUFAs and tocopherols

Pairs	Spearman's Coefficient	P value
EPA-DHA(ug/dL)	0.843	**<0.001**
α-γ Tocopherol(ug/dL)	0.514	**<0.001**
α-δ Tocopherol(ug/dL)	0.246	**0.004**
γ-δ Tocopherol(ug/dL)	0.713	**<0.001**
EPA-α-Tocopherol(ug/dL)	0.571	**<0.001**
EPA-γ-Tocopherol(ug/dL)	0.219	**0.010**
EPA-δ-Tocopherol(ug/dL)	0.112	0.193
DHA-α-Tocopherol(ug/dL)	0.501	**<0.001**
DHA-γ-Tocopherol(ug/dL)	0.219	**0.010**
DHA-δ-Tocopherol(ug/dL)	0.087	0.313

PUFAs, polyunsaturated fatty acids. P values < 0.05 were expressed in bold.

Table 3. Correlations between age and observed nutrition ingredients

Variables	Spearman's Coefficient	P value
EPA(ug/dL)	-0.147	0.088
DHA(ug/dL)	-0.110	0.202
α-Tocopherol(ug/dL)	-0.107	0.214
γ-Tocopherol(ug/dL)	-0.266	**0.002**
δ-Tocopherol(ug/dL)	-0.089	0.305

P values < 0.05 were expressed in bold.

Table 4. Comparisons between participants with and without comorbidities

variables	Without comorbidities(n=17)	With comorbidities(n=15)	P value
Age(y)	56.00(53.50-64.00)	75.00(68.00-85.00)	**0.001**
EPA(ug/dL)	26.20(12.15-39.00)	19.80(6.34-26.20)	0.189
DHA(ug/dL)	98.90(62.05-141.50)	80.50(52.60-102.00)	0.313
α-Tocopherol(ug/dL)	341.00(226.00-574.50)	243.00(203.00-343.00)	0.123
γ-Tocopherol(ug/dL)	91.80(64.15-117.00)	96.40(54.40-128.00)	0.970
δ-Tocopherol(ug/dL)	3.71(2.29-5.79)	3.81(2.76-5.25)	0.970

Without comorbidities was defined as participants had no combined disease. With comorbidities was defined as participants combined hypertension, CAD, CAS and DM. P values < 0.05 were expressed in bold.

Table 5. Correlations between comorbidity and observed nutrition ingredients

variables	Spearman's Coefficient	P value
Age(y)	0.496	**<0.001**
EPA(ug/dL)	-0.253	**0.004**
DHA(ug/dL)	-0.210	**0.018**
α-Tocopherol(ug/dL)	-0.259	**0.003**
γ-Tocopherol(ug/dL)	-0.194	**0.030**
δ-Tocopherol(ug/dL)	-0.031	0.734

Comorbidity was assigned a value according to the number of disease (s) that participants combined, including hypertension, CAD, CAS and DM. P values < 0.05 were expressed in bold.

Table 6. Ordinal logistic regression analysis of comorbidity

Covariates	Estimate	Wald	95% CI for Estimate	P value
Age(y)	0.049	28.741	0.031-0.067	**<0.001**
EPA(ug/dL)	-0.010	0.887	-0.031-0.011	0.346
DHA(ug/dL)	-0.001	0.043	-.009-0.007	0.835
α-Tocopherol(ug/mL)	0.038	0.004	-1.217-1.294	0.952
γ-Tocopherol(ug/dL)	-0.004	1.139	-.010-0.003	0.286
δ-Tocopherol(ug/dL)	0.058	0.996	-.056-0.171	0.318

P values < 0.05 were expressed in bold.

Table 7. Comparisons of observed diseases between different concentrations of plasma omega-3 PUFAs and tocopherols

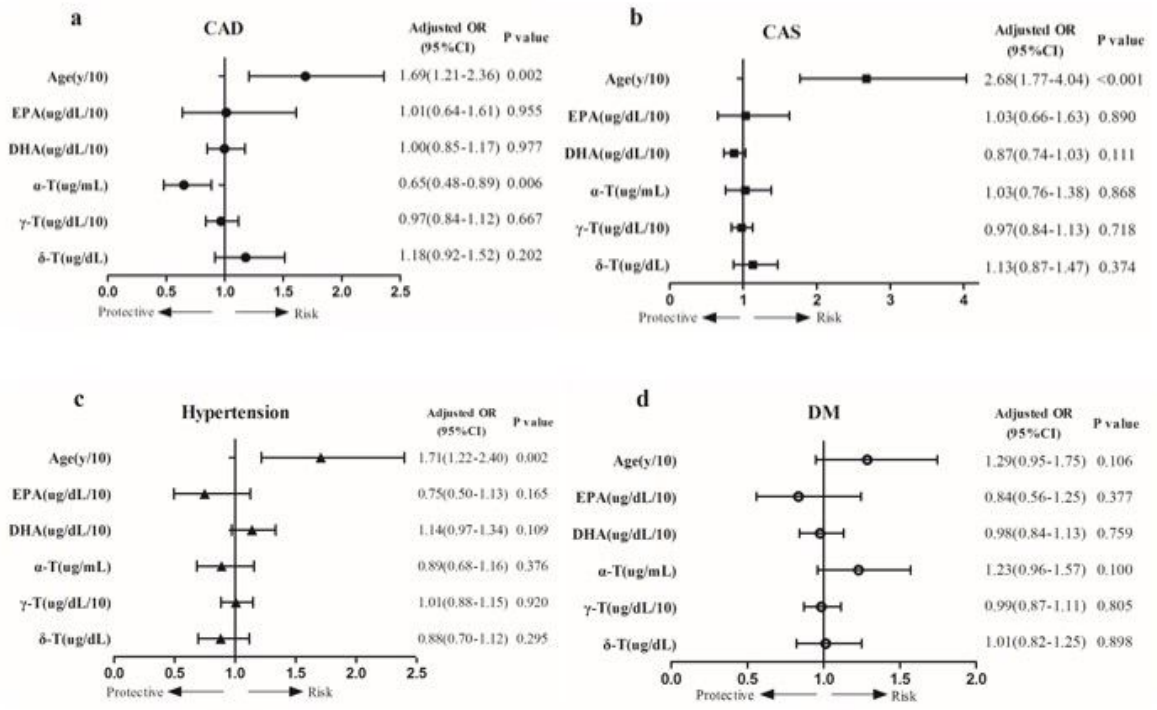
Variables	ω-3 PUFAs (ug/dL)						Tocopherol (ug/dL)								
	EPA(n=68/group)			DHA(n=68/group)			α-T(n=68/group)			γ-T(n=68/group)			δ-T(n=68/group)		
	EPA<18.35	EPA≥18.35	P value	DHA<81.10	DHA≥81.10	P value	α-T<304.00	α-T≥304.00	P value	γ-T<77.85	γ-T≥77.85	P value	δ-T<3.59	δ-T≥3.59	P value
CAD(%)	47.0%	35.3%	0.170	51.5%	30.9%	**0.015**	56.1%	26.5%	**<0.001**	43.9%	38.2%	0.502	40.9%	41.2%	0.975
CAS(%)	66.7%	57.6%	0.294	70.5%	53.9%	0.055	65.6%	58.5%	0.411	65.6%	58.5%	0.411	58.3%	65.2%	0.431
Hypertension(%)	68.2%	60.3%	0.341	68.2%	60.3%	0.341	72.7%	55.9%	**0.042**	74.2%	54.4%	**0.017**	74.2%	54.4%	**0.017**
DM(%)	39.4%	33.9%	0.503	37.9%	35.3%	0.756	36.4%	36.8%	0.962	31.8%	41.2%	0.261	31.8%	41.2%	0.261

α-T, α-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol. P values < 0.05 were expressed in bold.

Table 8. Comparisons of peripheral atherosclerotic parameters between different concentrations of plasma omega-3 PUFAs and tocopherols

Figures

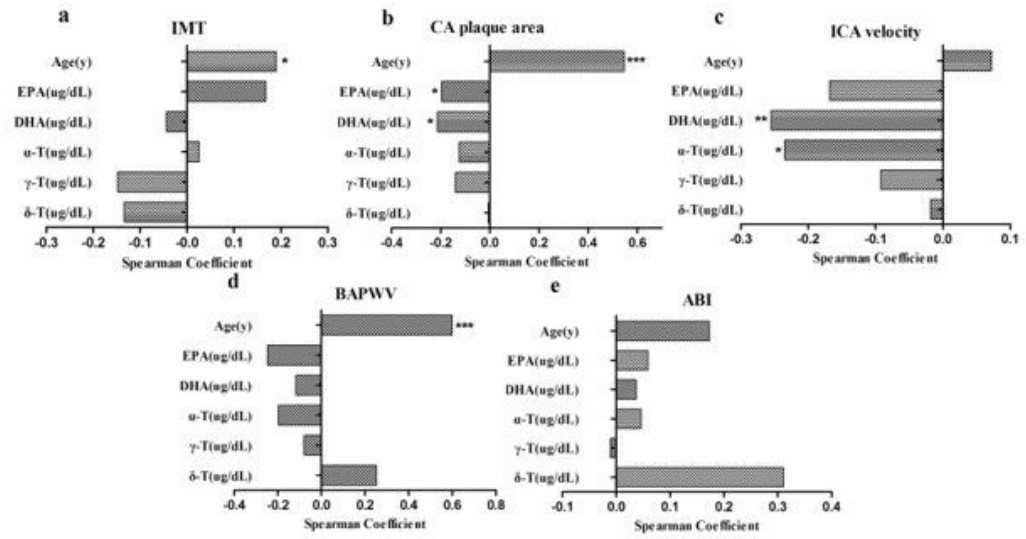
Variables	ω-3 PUFAs (ug/dL)						Tocopherol (ug/dL)							
	EPA(n=68/group)			DHA(n=68/group)			α-T(n=68/group)			γ-T(n=68/group)			δ-T(n=68/group)	
	EPA<18.35	EPA≥18.35	P value	DHA<81.10	DHA≥81.10	P value	α-T<304.00	α-T≥304.00	P value	γ-T<77.85	γ-T≥77.85	P value	δ-T<3.59	δ-T≥3.59	P value
IMT(mm)	1.00 (0.90-1.10)	1.10 (0.90-1.20)	0.112	1.00 (0.90-1.20)	1.05 (0.90-1.20)	0.938	1.00 (0.90-1.10)	1.10 (0.90-1.30)	0.396	1.10 (1.00-1.30)	1.00 (0.80-1.20)	0.018	1.10 (0.90-1.20)	1.00 (0.90-1.20)	0.259
CA plague area(mm^2)	3.38 (0-25.76)	0.30 (0-15.40)	0.232	6.27 (0-26.59)	0.21 (0-12.60)	0.083	0.52 (0-24.22)	0.25 (0-15.53)	0.277	1.40 (0-21.66)	0.10 (0-15.55)	0.145	0.32 (0-16.77)	0.32 (0-19.63)	0.917
ICA velocity(m/s)	0.64 (0.42-0.77)	0.54 (0.44-0.70)	0.089	0.64 (0.42-0.77)	0.55 (0.44-0.72)	0.222	0.65 (0.47-0.76)	0.55 (0.40-0.69)	0.035	0.60 (0.44-0.74)	0.57 (0.41-0.72)	0.443	0.55 (0.43-0.74)	0.63 (0.43-0.74)	0.573
BAPWV(cm/s)	1811 (1460-2170)	1662 (1427-1807)	0.251	1770 (1446-2158)	1662 (1380-2001)	0.413	1731 (1446-2074)	1819 (1286-2618)	0.852	1708 (1443-2152)	1786 (1383-2227)	0.871	1643 (1421-1984)	1805 (1593-2272)	0.239
ABI	1.104±0.091	1.125±0.096	0.398	1.100±0.088	1.138±0.101	0.155	1.110±0.093	1.117±0.095	0.984	1.110±0.101	1.112±0.078	0.897	1.090±0.104	1.132±0.076	0.226



a Influence factors of coronary artery disease (CAD). b Influence factors of carotid atherosclerosis (CAS). c Influence factors of hypertension. d Influence factors of diabetes mellitus (DM). OR, odds ratio; CI, confidence interval.

Figure 1

Binary logistic regression analyses of observed diseases



a Carotid intima-media thickness (IMT)-related factors. b Carotid artery (CA) plaque area-related factors. c Internal CA (ICA) velocity-related factors. d Brachial-ankle pulse wave velocity (BAPWV)-related factors. e Ankle-brachial index (ABI)-related factors. Significance: *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

Figure 2

Correlation analyses of peripheral atherosclerotic parameters