Original Article

The Relationships between Erythrocyte Membrane n-6 to n-3 Polyunsaturated Fatty Acids Ratio and Blood Lipids and C-reactive Protein in Chinese Adults: An Observational Study*

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Abstract

Objective To investigate the relationships between erythrocyte membrane n-6:n-3 PUFAs ratio and blood lipids and high sensitivity C-reactive protein (hs-CRP).

Methods The observational study consisted of a population-based cross-sectional study of 456 Chinese and a subsequent 1-year follow-up study of 171 subjects with the fasting plasma total cholesterol of 5.13-8.00 mmol/L.

Results In the cross-sectional analysis, plasma low-density lipoprotein cholesterol (LDL-c) had a significant and negative association with the erythrocyte membrane n-6:n-3 PUFAs ratio (*P* for trend=0.019) after adjusting for sex, age and total PUFA percentage. In the follow-up study, 171 subjects were categorized into quartiles by the changes of n-6:n-3 ratio in erythrocyte membrane (△=month 12-month 0). In the top quartile whose ratios of n-6:n-3 increased by an average of 1.25 during the follow-up, the LDL-c-lowering extent was 3.3 times of that in the lowest quartile whose ratios of n-6:n-3 decreased by an average of 1.13 (-1.07 mmol/L *v.s.* -0.32 mmol/L). The hsCRP decreased by 0.11 mg/dL in the lowest quartile while increasing by 0.10 mg/dL in the top quartile (*P* for difference=0.052).

Conclusion Our results suggested that the balance between n-6 and n-3 fatty acids may optimize the cardiovascular benefits from dietary PUFAs.

Key words: Erythrocyte membrane; n-6:n-3 fatty acids ratio; Blood lipids; High sensitivity C-reactive protein; Observational study

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INTRODUCTION

Dietary changes could play an important role in the primary and secondary prevention of coronary heart disease (CHD)[1-2]. The standard dietary advice for persons with a high risk of CHD includes a decrease in intakes of total fat and replacement of saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFAs)[3]. The predominant PUFA in diet is the n-6 fatty acid

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linoleic acid (LA, 18:2)[4], which has increased over the last century from 3% of energy in the early 1900s and to the current 5%-7% of dietary energy and contributes 85%-90% of total PUFAs largely due to an increased consumption of LA-rich vegetable oils[5-7]. Another important series of PUFAs in diet is the n-3 fatty acids eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) derived from fish. An alternative source of n-3 PUFA is plant-derived α-linolenic acid (ALA, 18:3 n-3), which is more abundant than fish oil and can be elongated and desaturated to EPA and DHA although the extent and regulation of this conversion is unclear[8].

Although there is growing evidence suggesting that all of the above-mentioned n-6 or n-3 PUFAs, when substituted for isocaloric SFA, will have beneficial effects on cardiovascular health, it is controversial whether the ratio of n-6:n-3 PUFAs is of value in modifying CHD risk when energy from total amounts of dietary PUFAs is held constant[9]. A ten-week dietary intervention in 17 healthy subjects found that a decreased n-6:n-3 PUFAs ratio resulted in multiple, potentially favorable effects on tumor necrosis factor-α, low-density lipoprotein cholesterol (LDL-c), adiponectin and other metabolic and inflammatory profiles, but unfavorable effects on lipid oxidation[10], while OPTILIP study, a randomized trial conducted in UK, failed to find relevance of this dietary ratio with insulin sensitivity, lipoprotein size, postprandial lipemia and hemostatic factors[11-12]. Evidence from animal studies also supports that the concern on dietary n-6:n-3 fatty acid ratio should be raised. In the study of diet with a similar amount of PUFAs but varying in n-6:n-3 PUFAs ratio conducted by Zhang et al.[13] observed statistically significant low hepatic and aortic CRP expressions and low aortic surface lesions in apoE-/- mice fed with the low n-6:n-3 PUFA ratio diet compared with mice fed with the high n-6:n-3 PUFA ratio diet. A study *in vitro* showed that exposure of human hepatocytes to different mixtures of LA and ALA affects transcript levels of a portfolio of genes encoding regulating proteins involved in several stages of fatty acid metabolism, and the effects strongly depend on the ratio of n-6:n-3 fatty acids[14]. Kang et al.[15-16] used a genetic approach to modify the cellular n-6:n-3 fatty acid ratio by converting the endogenous n-6 to n-3 fatty acids and found that decreasing the ratio of n-6:n-3 fatty acids in these cells inhibited their adhesion, migration and proliferation, and down-regulated the expression of several adhesion/ invasion-related genes.

It has been elucidated that fatty acid profile of blood lipids, blood cell membranes and adipose tissue not only has been indicators of dietary fat intake[17-20], but also contains metabolic information and serves as risk markers for CHD[21-23]. The triangular relationships among dietary fat, fatty acid composition in tissue and CHD risk are not always consistent because tissue fatty acid composition is not only determined by dietary intakes, but also can be affected by genetic influences, lifestyle factors, such as smoking, physical activity, or the intake of other nutrients[24-25]. Sun et al.[21] observed a significantly inverse association of docosapentaenoic acid (DPA) concentrations in plasma or erythrocyte with nonfatal myocardial infarction (MI) risk, despite the fact that DPA is not correlated with dietary consumption of n-3 fatty acids estimated by semi-quantitative food-frequency questionnaires, and conversely, DHA content in plasma and erythrocytes is correlated with estimated dietary consumption but is not significantly associated with the risk of nonfatal MI.

Considering the evidence that lipid abnormalities and chronic inflammation play multifactorial roles in the progression of CHD independently or synergistically[26], we designed the present study to investigate the relationship between the erythrocyte membrane n-6:n-3 ratio and blood lipid profile and C-reactive protein (hs-CRP), a high sensitivity inflammatory marker for Chinese adults.

SUBJECTS AND METHODS

Subjects and Design

An overview of the present study is shown in Table 1. Study subjects were recruited during December 2005 and April 2006 *via* community and clinic advertisement, publicity in health talks and subject referral. All subjects were required to be Guangzhou residents of Chinese origin aged 40-65 years old. Exclusion criteria included a self-aware of history of chronic diseases, such as diabetes, hypertension, CHD, stroke, dyslipidemia, and cancers, which might change their dietary habit or lifestyle; current use or a history of 3-month (or more) use of any drugs known to affect lipid metabolism; a body mass index $\geqslant$30 kg/m^2.

After initial screening for their eligibility using a short questionnaire which recorded gender, age and exclusion criteria, 739 potential subjects were then invited to the First or the Second Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China.

Investigators verified the subjects' eligibility *via* face-to-face interview. 127 subjects were further excluded for prior diagnosis of the diseases indicated in the exclusion criteria (29 for diabetes, 12 for CHD or stroke, 86 for dyslipidemia), 20 subjects were also excluded for incomplete socioeconomic information. 592 subjects completed body examination and provided 12-h fasting venous blood, and, among them, 11 subjects and 125 subjects had no adequate specimen for fatty acid or hsCRP measurement, respectively. Thus the present cross-sectional study included 456 subjects (162 males and 294 females).

After the above-mentioned screening, a total of 237 subjects with mild-to-moderate hypercholeste rolemia (fasting blood TC, 5.13 mmol/L-8.00 mmol/L) were invited to participate in the 1 year follow-up study. 39 subjects refused participation and 198 subjects agreed. During the following year, lipid lowering dietary advices based on the dietary guidelines for Chinese residents proposed by the standing board of the Chinese Nutrition Society as follows were given to them every 3 months: (1) eat a variety of foods, with cereals as the staple; (2) balance food intake with physical activity to maintain a healthy body weight; (3) consume appropriate amounts of fish, poultry, and lean meat; reduce fatty meat and animal fat in the diet with cooking oil less than 25g/d; (4) consume plenty of vegetables, fruit, tubers and beans or bean-products; (5) drink alcoholic beverages in limited amounts; (6) choose a light diet that is also low in salt[27]. 171 subjects completed the study and provided blood specimen at the final visit.

Table 1. Flow Diagram and Overview of the Visits and Measurements

Study design	Cross-sectional	Follow-up (month)			
Schedule		0	3rd	6th	9th	12th
Sample Size	456	198	—	—	—	171
Dietary Advice	-	√	√	√	√	-
Blood Lipids	√	-	-	-	-	√
hs-CRP	√	-	-	-	-	√
Fatty Acids Composition	√	-	-	-	-	√

All participants signed the written informed consent prior to the enrollment. The study protocol was approved by the Medical Ethics Committee of Sun Yat-Sen University.

Blood Lipids and hs-CRPt

12-h fasting venous blood was collected in vacuum tubes containing EDTA for lipid analysis. Plasma was separated after centrifugation at 1 500 g for 15 min at 4 ℃ within 2 h and then stored at -80 ℃ until analysis. Blood lipids were measured by Hitachi 7 600-010 automatic analyzer. The coefficients of variation for lipid measurements were 2.17% (at 5.03 mmol/L TC), 2.86% (at 1.14 mmol/L TG). 3.47% (at 1.70 mmol/L HDL-c), and 4.67% (at 2.65 mmol/L LDL-c).

Plasma hsCRP was measured by Hitachi 7170A automatic analyzer in clinical biochemistry laboratory, the First Affiliated Hospital of Sun Yat-Sen University. The coefficients of variation were 6.51% at 0.44 mg/dL and 2.06% at 3.06 mg/dL.

Erythrocyte Membrane Fatty Acids

RBC was thawed and hemolyzed in hypotonic Tris-HCL buffer, lipids were extracted with chloroform/methanol (2:1, v/v) with added 0.005% BHT, and the extract was dried in N2. Fatty acids methyl esters (FAMES) were obtained by incubation with 14% boron trifluoride ether/methanol (1:3, v/v) solution at 100 ℃ and analyzed by gas chromatography as described elsewhere[28].

Individual fatty acids were identified by comparison with known standards (Sigma-Aldrich Inc., St. Louis, MO) and expressed as a percentage of total fatty acids quantified from peak areas. Of 32 fatty acids identified in erythrocyte membranes, 19 fatty acids that have meaningful concentrations (mean concentration ＞0.10 %) are reported here, which together account for 96.9% of total identified fatty acids. The range of coefficient of variation (CV) for these samples was 5.9%-42.8%. The CVs for the most abundant fatty acids (＞10 %) were 5.9% for palmitic acid (16:0), 10.5% for stearic acid (18:0), 8.6% for oleic acid (18:1n-9), 10.8% for linoleic acid (18:2n-6), 11.3% for arachidonic acid (20:4n-6).

Statistical Analysis

Continuous variables were checked for normality before statistical analysis. Plasma lipids that were normally distributed were presented as mean±SD (standard deviation, unadjusted model) or

mean±SE (standard error of the mean, adjusted model). hsCRP was not normally distributed even after log transformation and therefore was presented as median (interquartile range). Individual fatty acids were expressed as a percentage of total fatty acids. In the cross-sectional study, subjects were categorized into quintiles by their erythrocyte membrane n-6 to n-3 fatty acids ratio. Comparisons of plasma lipids among the five groups were done by using either one-way ANOVA or analysis of covariance (ANCOVA) after adjusting for sex, age and total PUFA percentage. For hsCRP, the Kruskal-Wallis H test was used to test between-group comparison. Comparisons of count data among groups were performed by using χ^2 test. In the follow-up study, the relationships between changes in erythrocyte membrane n-6 to n-3 fatty acids ratio and plasma lipids and hsCRP were analyzed. 171 subjects were categorized into quartiles by the changes of n-6:n-3 fatty acid ratio in erythrocyte membrane (△=month 12-month 0). Comparisons of variables among groups were made by using the above descriptive methods. All analyses were conducted with SPSS for Windows (Version 11.5, SPSS, Inc., Chicago, IL). A two-sided *P*-value of less than 0.05 was considered as statistically significant.

RESULTS

Characteristics of Study Population

Table 2 shows the characteristics of the study population. There were 456 subjects in the cross-sectional study and 171 subjects in the follow-up study. These two populations had no significant differences in sex, monthly income, marital and smoking status, while the follow-up study included more subjects with a lower education level. Owing that subjects with mild-to-moderate hypercholesterolemia (fasting blood TC, 5.13 mmol/L -8.00 mmol/L) were selected to participate in the follow-up study, these subjects had higher serum TC, LDL-c and HDL-c (all $P \leqslant 0.001$).

Cross-sectional Study

Four types of n-3 (α-linolenic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic fatty acid)and six types of n-6 fatty acids (linoleic, γ-linolenic, eicosadienoic, dihomogammalinolenic, arachidonic, and docosatetraenoic fatty acid) were identified in erythrocyte membrane, which accounted for about 11% and 28% of total fatty acids, respectively. The mean of n-6:n-3 ratio in erythrocyte membrane for total 456 subjects was 2.79 and all of the subjects were categorized into quintiles by the ratio. Univariate analysis showed a statistically significant dose-respondent decrease in TC, LDL-c, and HDL-c associated with an increase in erythrocyte membrane n-6:n-3 fatty acids ratio (all *P* for trend <0.001). The negative associations were attenuated in the analysis of covariance adjusting for sex, age and total PUFA percentage (Table 3). Only LDL-c retained a significant negative association with the ratio of n-6:n-3 fatty acids (*P* for trend=0.019). In the adjusted model, mean LDL-c was 11.1%, which was lower among subjects in the top quintile of n-6:n-3 ratio, as compared with those in the lowest ratio group. No similar associations were observed between n-6:n-3 ratio and hsCRP (Table 3).

Follow-up Study

In the follow-up study, the erythrocyte membrane fatty acids, plasma lipids and hsCRP of 171 subjects were analyzed twice 1 year apart and the changes (△=month 12-month 0) were calculated. Total subjects were also categorized into quartiles by the changes of n-6: n-3 fatty acid ratio in erythrocyte membrane. The plasma lipids were improved in nearly all subjects, while the LDL-c-lowering effects were significantly related with the changes of the n-6:n-3 ratio (*P* for trend=0.001). In subjects whose ratios of n-6:n-3 in erythrocyte membrane increased by 1.30 averagely during the follow-up study, the LDL-c-lowering extent was 3.3 times of that of subjects whose ratios of n-6:n-3 in erythrocyte membrane decreased by 1.13 in average (-1.17 mmol/L *vs* -0.32 mmol/L, Table 4). Adjustment for sex, age and baseline data did not change the association. Table 4 also shows the changes in hsCRP level per quartile of changes in n-6: n-3 ratio. The hsCRP decreased by 0.11 mg/dL in the lowest quartile while increasing by 0.10 mg/dL in the top quartile (*P* for difference=0.052). The percentage of subjects with high hsCRP (>3mg/dL) also increased across the quartiles of changes in n-6: n-3 ratio though the trend was not significant (*P* for difference=0.086).

Table 2. Descriptive Socio-demographic Characteristics, Serum Lipids and hsCRP of Subjects

	Cross-sectional Study	Follow-up Study	P^3
n	456	171	
Age	52.5±7.1[1]	54.2±6.0	0.009
Sex(male/female)	162/294	54/117	0.865
Education (%)			＜0.001
Primary school or below	7.1	30.6	
Secondary school	26.4	37.2	
Senior high school	48.3	20.4	
College or above	18.1	11.7	
Monthly Income (%)			0.899
＜1000￥	26.4	28.9	
1000-1999￥	41.3	42.1	
2000-3999￥	28.0	23.7	
＞4000￥	4.2	5.3	
Marital Status (%)			0.953
Married	91.3	92.1	
Divorced or Separated	3.3	3.3	
Widowed	3.9	3.9	
Single	1.5	0.7	
Smoking (%)			0.880
Non-smoker	81.1	78.6	
Ex-smoker	4.4	4.5	
Current-smoker	14.5	16.9	
TC (mmol/L)	5.38±1.04	6.15±0.72	＜0.001
LDL-c (mmol/L)	3.68±1.12	4.34±0.95	＜0.001
HDL-c (mmol/L)	1.58±0.37	1.73±0.37	＜0.001
TG (mmol/L)	2.26±1.61	2.50±1.66	0.082
hsCRP (mg/dL)	0.95(0.42,1.80)[2]	1.00(0.42, .81)	0.505

Note. [1]Mean±SD, all such values. [2]Median (25th, 75th). [3]*P* for difference between subjects in cross-sectional study and those in follow-up study. χ^2 test for count data and *t* test for mean.

DISCUSSION

The present observational study includes two sections: a cross-sectional study and a subsequent 1-year follow-up study. The results of the two sections, to a large extend, were consistent. A significant and negative association were observed between erythrocyte membrane n-6:n-3 ratio and plasma LDL-c in the cross-sectional analysis and the increase of such ratio was significantly related to the decrease of plasma LDL-c level in the follow-up study. We also observed a marginally significant and positive relationship between the changes of erythrocyte membrane n-6:n-3 ratio and plasma hs-CRP (△=month 12-month 0).

It is well known that high blood LDL-c enhances the risk of CVD[29]. An analysis of data concluded that each reduction of 1.0 mmol/L in LDL-c that is sustained for 5 years may produce a proportional reduction in major vascular events of about 23%[30]. Meanwhile, hs-CRP is considered to be a most important inflammation marker and an independent valuable predictor for cardiovascular events in patients with atherosclerotic diseases[31-32] or in general populations[33]. A meta-analysis of prospective studies of general populations reported that a higher CRP level was related to a 58% increase in the incidence of cardiovascular diseases[33]. In view of the roles of LDC-c and hs-CRP in cardiovascular health, we concluded that the balance between n-6 and n-3 fatty acids may optimize the cardiovascular benefits from dietary PUFAs.

Table 3. Serum Lipids and hsCRP Levels of Subjects by Quintile of n-6:n-3 Ratio in Erythrocyte Membrane in the Cross-sectional Study[1]

	Quintile of n-6:n-3 Ratio						
	Group 1 (low)	Group 2	Group 3	Group 4	Group 5 (high)	P^3	P^4
	1.80	2.25	2.68	3.18	4.04 [2]		
Age	53.4±7.3	53.0±7.0	51.7±7.4	52.2±7.6	52.3±7.7	0.468	0.182
Sex (male, %)	33.0	30.5	35.4	30.3	36.3	0.842	0.675
TC (mmol/L)							
Model 1	5.62±1.10	5.60±1.16	5.56±1.11	5.24±1.07	5.00±0.98	＜0.001	＜0.001
Model 2	5.64±0.11	5.73±0.12	5.72±0.11	5.52±0.13	5.45±0.13	0.429	0.131
LDL-c (mmol/L)							
Model 1	4.02±1.14	3.89±1.10	3.91±1.20	3.50±1.16	3.22±1.01	＜0.001	＜0.001
Model 2	4.07±0.12	4.05±0.14	4.15±0.13	3.88±0.15	3.62±0.15	0.080	0.019
HDL-c (mmol/L)							
Model 1	1.63±0.32	1.63±0.36	1.67±0.45	1.53±0.37	1.49±0.36	＜0.001	＜0.001
Model 2	1.67±0.04	1.65±0.05	1.72±0.04	1.52±0.05	1.68±0.05	0.043	0.480
TG (mmol/L)							
Model 1	2.29±1.54	2.61±1.64	2.39±1.83	2.35±1.82	2.07±1.57	0.140	0.129
Model 2	2.39±0.18	2.53±0.20	2.18±0.18	2.29±0.21	1.99±0.22	0.407	0.104
hsCRP(mg/dL) [5]	1.10(0.47,1.72)	0.89(0.43,1.89)	1.00(0.45,1.83)	0.77(0.33,1.73)	0.95(0.45,1.69)	0.547	—
hsCRP＞3mg/dL (%) [6]	11.1	14.4	13.9	8.9	14.3	0.708	0.942

Note. [1]model 1, mean±SD, one-way ANOVA; model 2, mean±SE, analysis of covariance(ANCOVA) adjusted for sex, age and total PUFA percentage. [2] Median values of the n-6: n-3 ratio in five groups. [3]*P* for differences between quintiles. [4]*P* for trend. [5]median (25^{th}, 75^{th}), Kruskal-Wallis H test. [6] χ^2 test.

Few studies have assessed the relationships between n-6:n-3 fatty acids ratio in erythrocyte membrane and CHD risk factors, although the roles of individual fatty acid have been fully studied. A meta-analysis by Harris et al.[23] showed that depressed levels of DHA in tissues were a consistent marker of increased risk for fatal or nonfatal CHD events. However, a recent study reported that higher plasma or erythrocyte concentrations of EPA and DPA were associated with a lower risk of nonfatal MI, but DHA or ALA were not significantly associated with the risk[21]. As for n-6 fatty acids, tissue LA content was also frequently inversely associated with the risk of CHD[23-34], while arachidonic acid (AA) appeared complicated. Harris et al.[23] found that increased AA content in phospholipid or triglyceride was not significantly associated with CHD events except when it was measured in adipose tissue; Block et al.[34] found a U-shaped relationship between AA and acute coronary syndrome in a case-control study. A recurrent confounding theme potentially contributed to the discrepancies: the simultaneous changes in different types of fatty acids[9]. The increase of n-3 fatty acids as a percentage of total fatty acids is always inadvertently accompanied by the decrease in the percentage of other fatty acids (e.g. n-6 fatty acids). As a consequence of this double shift, interpretation is compromised because one cannot discern whether the increase of n-3 fatty acids or the removal of other fatty acids contributes more to the observed biological effects. Therefore, the n-6: n-3 ratio in tissue may be a useful functional marker and disease indicator.

Our results had biological rationality. The primary mechanism by which n-6 PUFAs lower the risk of CHD is to lower LDL-c by up-regulation of the LDL receptor and increase the CYP7 activity[9,35]. On the contrast, one of the major mechanisms responsible for the observed effects of n-3 fatty acids on cardiovascular health is its role in retarding growth of atherosclerotic plaque through reducing adhesion molecule expression or anti-inflam mation[36-37]. On theoretical grounds, both n-3 and n-6 fatty acids are substrates for eicosanoid production. Competition occurs between the n-6 and the n-3 PUFA for the elongase and desaturase enzymes, yet n-3 PUFAs have greater enzyme- substrate

Table 4. Changes of Serum Lipids and hsCRP across 4 Categories of △n-6:n-3 Ratio in Erythrocyte Membrane during 1-year Follow-up[1,2]

		Categories of △n-6:n-3					
		Group 1	Group 2	Group 3	Group 4	*P*[4]	*P*[5]
		-1.03	-0.33	0.30	1.25[3]		
Age		54.7±6.1	55.3±6.5	53.6±5.5	55.6±5.4	0.352	0.633
Sex (male, %)		43.5	29.8	35.5	29.8	0.273	0.142
△TC (mmol/L)	Model 1	-0.70±0.86	-0.67±0.83	-0.74±0.79	-0.74±0.82	0.973	0.713
	Model 2	-0.71±0.15	-0.76±0.17	-0.66±0.17	-0.86±0.17	0.861	0.635
△TC (%)	Model 1	-10.9±13.5	-10.3±15.7	-11.4±10.6	-11.1±15.8	0.983	0.853
	Model 2	-11.1±2.3	-12.6±2.6	-10.1±2.5	-12.9±2.5	0.853	0.790
△LDL-c (mmol/L)	Model 1	-0.32±0.95	-0.51±1.04	-0.59±1.07	-1.07±1.12	0.004	0.001
	Model 2	-0.25±0.18	-0.74±0.20	-0.43±0.20	-1.26±0.20	0.002	0.002
△LDL-c (%)	Model 1	-6.1±20.5	-10.1±24.3	-10.0±23.6	-19.5±22.7	0.037	0.008
	Model 2	-4.1±3.9	-10.8±4.5	-6.7±4.4	-22.4±4.4	0.010	0.015
△HDL-c (mmol/L)	Model 1	0.00±0.35	-0.09±0.51	0.05±0.45	-0.14±0.44	0.142	0.596
	Model 2	-0.01±0.07	-0.12±0.08	0.03±0.08	-0.15±0.08	0.102	0.720
△HDL-c (%)	Model 1	2.4±21.4	0±27.2	3.4±28.4	-0.8±22.2	0.654	0.688
	Model 2	2.4±4.6	-3.3±5.2	6.2±5.1	-1.0±5.1	0.270	0.899
△TG (mmol/L)	Model 1	-1.25±1.65	-0.75±1.57	-1.09±1.50	-1.37±1.53	0.272	0.516
	Model 2	-0.98±0.27	-0.65±0.31	-1.00±0.30	-1.56±0.30	0.188	0.102
△TG (%)	Model 1	-25.4±65.6	-10.0±58.4	-26.2±60.8	-30.1±47.8	0.362	0.430
	Model 2	-16.5±10.5	-2.5±11.8	-20.6±11.6	-34.0±11.6	0.296	0.157
△hsCRP (mg/dL)[6]		-0.11(-0.57,0.31）	-0.18（-0.39,0.12）	0.02（-0.68,0.37）	0.10（-0.23,0.87）	0.052	—
hsCRP＞3 mg/dL(%)[7]		8.9	2.6	15.8	20.0	0.086	0.042

Note. [1]model 1, mean±SD, one-way ANOVA; model 2, mean±SE, analysis of covariance(ANCOVA) adjusted for sex and age. [2]△=mo 12-mo 0. [3]Median values of △n-6: n-3 ratio in four groups. [4]*P* for differences between quintiles. [5]*P* for trend. [6]Kruskal-Wallis H test. [7] Data at the end of follow-up, χ^2 test.

affinities than n-6 PUFAs. EPA can not only replace AA in phospholipid bilayers of cell membrane, but also act as a competitive inhibitor of cyclooxygenase and lipoxygenase, reducing the production of the 2-series prostaglandins, thromboxanes, and prostacyclins and the 4-series leukotrienes. The 3- and 5-series produced from EPA are generally less biologically active, then one of the net effects of n-3 fatty acids is to reduce inflammatory processes[38]. These theoretical hypotheses have been proven, at least partly, by a transgenic mouse, which is engineered to carry a *fat-1* gene from the roundworm *Caenorhabditis elegans* and can add a double bond into an unsaturated fatty-acid hydrocarbon chain and convert n-6 to n-3 fatty acids. This results in an abundance of n-3 and a reduction in n-6 fatty acids in the organs and tissues of these mice in the absence of dietary n-3[39]. The fat-1 mouse exhibits reduced pro-inflammatory response in multiple models of autoimmunity and inflammation[40-42]. In the present study, the n-6: n-3 PUFAs ratio in erythrocyte membrane had a significant negative association with LDL-c and a marginally significant positive association with hs-CRP, which reflected the preponderant roles of n-6 PUFAs in lipid metabolism and of n-3 in inflammation process.

Our findings had some implications for dietary guidelines. When individual fatty acid in erythrocyte membrane is compared with dietary intakes assessed by food frequency questionnaire (FFQ), the diet-erythrocyte correlations are not consistent for all fatty acids. Our previous study showed that the correlation coefficients between FFQ and erythrocyte membrane are weak or moderate for total n-6 fatty acids (r=0.10), ALA (r=0.19), EPA (r=0.37), and DHA (r=0.16), but poor for SFA, MUFA and total n-3 fatty acids[28]. The results are rather predictable because the fatty acid composition in erythrocyte membrane reflects the comprehensive information, including not only the amounts from dietary intakes, but also the metabolism and

utilization *in vivo*. In the absence of high intakes of oily fish and fish oil supplementation (e.g. the present study), LA makes up about 95% of n-6 fatty acid intake and ALA about 90% of n-3 fatty acid intake, while in erythrocyte membrane, LA and AA make up about 40% and 47% of n-6 fatty acid, and DHA nearly 80% of n-3 fatty acid, ALA and EPA less than 1% and 4% of n-3 fatty acid, respectively (data not shown). It is well known that n-6 fatty acids cannot be converted to n-3 fatty acids in human body, and vice versa. The balance of n-6 and n-3 in erythrocyte membrane lies on the optimal ratio in diet. Unfortunately, the present study failed to put forward an optimal n-6:n-3 ratio in diet due to lack of information from dietary intakes.

The critical drawback of using the n-6:n-3 ratio should also be addressed. Firstly, such concept ignores the respective individual role of n-6 and n-3 fatty acids and is likely to distract attention away from increasing absolute intakes of long-chain n-3 fatty acids which have been shown to have beneficial effects on cardiovascular health[43]. Secondly, if population variability of n-6 fatty acid is bigger than n-3 fatty acid, the ratio of n-6: n-3 would simply reflect variability of n-6 fatty acid, and vice versa. Then the use of ratio could mask such potential to induce an inappropriate conclusion that the balance may be important. While we did not find association between n-6 or n-3 fatty acids in erythrocyte membrane with serum lipids or hs-CRP in our study (data not shown), the meaning of our study is to emphasize that the balance of these n-6 and n-3 fatty acids in the diet is also a critical factor influencing cardiovascular health, although it is not the first consideration when we contemplate lifelong dietary habits affecting cardiovascular benefit. The absolute intake of PUFA expressed in terms of mass (% en or g/d) is more important than a simple ratio[9].

The study has other potential limitations. First, the small sample size limits the power to exclude random error. For example, it is probably due to small sample and wide variation of hs-CRP levels that we fail to find relationships between n-6:n-3 ratio and plasma hs-CRP in the cross-sectional study. Second, it is impossible for such an observational study to identify causal relationship. Third, we cannot entirely exclude the possibility that the observed associations are due to other biomarkers in erythrocyte membrane. Fourth, the population of the follow-up study is likely to involve regression to the mean, based on selection of adults with moderate hypercholesterolemia. Finally, these findings may not be generalized to populations with high fish intakes.

In conclusion, the results from this study suggest that a higher n-6:n-3 ratio in erythrocyte membrane is associated with a lower plasma LDL-c level, and also potentially with an increased plasma hsCRP level. These results indicate that the balance between n-6 and n-3 fatty acids may optimize the cardiovascular benefits from dietary PUFAs, and further studies are needed to determine the optimal ratio of n-6:n-3 in diet.

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