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Erythrocyte PUFAs, circulating acylcarnitines, and metabolic syndrome risk: a prospective study in Chinese

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Abstract The effects of PUFAs on metabolic syndrome (MetS) remain to be characterized, particularly in Asians. We aimed to investigate the prospective associations of PUFAs with MetS and the role of acylcarnitines in these associations in Chinese individuals. Among 1,245 Chinese men and women aged 50–70 years who completed a 6 year follow-up, baseline erythrocyte FAs and plasma acylcarnitines were profiled using gas chromatography coupled with positive chemical ionization and liquid chromatography-tandem mass spectrometry, respectively. Total n-6 PUFAs and three 22-carbon n-6 PUFAs were significantly associated with lower MetS risk comparing extreme quartiles: relative risks (RRs) (95% CIs) were 0.75 (0.57, 0.97) for total n-6 PUFAs, 0.69 (0.56, 0.85) for 22:2n-6, 0.76 (0.59, 0.99) for 22:4n-6, and 0.74 (0.58, 0.94) for 22:5n-6, while 18:3n-3 and 18:3n-6 were positively associated with MetS risk. In a network analysis, a module mostly consisting of long-chain n-6 PUFAs and very-long-chain saturated FAs was inversely associated with incident MetS (RR per SD: 0.84; 95% CI: 0.76, 0.92), and this module was more strongly associated with lower MetS risk when a short- to medium-chain acylcarnitine (C5–C10) module score was lower ($P_{interaction}$ = 0.03). Our data suggested inverse associations of total n-6 and certain long-chain n-6 PUFAs with cardiometabolic disorders, and this association might be modified by certain acylcarnitines.—Yiwei, M., L. Sun, J. Li, Y. Hu, Z. Gan, G. Zong, H. Zheng, Q. Jin, H. Li, F. B. Hu, R. Zeng, Q. Sun, and X. Lin. **Erythrocyte PUFAs, circulating acylcarnitines, and metabolic syndrome risk: a prospective study in Chinese. *J. Lipid Res.* 2019.** 60: **421–429.**

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A higher consumption of PUFAs has been shown to be associated with reduced risks of type 2 diabetes and CVD in Western populations (1, 2). Recent dietary guidelines such as the 2015–2020 Dietary Guidelines for Americans also encouraged PUFA intakes (3). However, little is known about whether the guidelines are pertinent to Asians who have different dietary patterns (4) and variations in the genes involving PUFA metabolism, as indicated by a recent trans-ethnic genome-wide association study in Chinese and European-ancestry populations (5).

Although associations of objectively measured PUFA biomarkers with type 2 diabetes or CVD have been investigated in several cohorts, findings on specific PUFAs with these cardiometabolic outcomes were inconsistent (6–8). Metabolic syndrome (MetS), a constellation of multiple cardiometabolic conditions such as central obesity, dyslipidemia, and elevated levels of blood pressure and fasting glucose, is known as a precursor of type 2 diabetes or CVD, and it is considered as a critical condition for early intervention to reduce the onset of cardiometabolic diseases (9). However, studies regarding the relations between PUFAs and MetS are sparse, and a limited number of

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Abbreviations: CHD, coronary heart disease; CRP, C-reactive protein; FAO, fatty acid oxidation; HOMA-IR, homeostatic model assessment of insulin resistance; ME, module eigengene; MetS, metabolic syndrome; RR, relative risk.

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prospective studies conducted in Western populations have shown mixed results (10–12). Because of the much longer life span of red blood cells, erythrocyte FAs were indicated to be more reproducible over time than FAs from other blood fractions (13). We previously observed an inverse association between erythrocyte 22:6n-3 (DHA) and MetS prevalence by using baseline data of the current cohort (14); nevertheless, the prospective associations between PUFAs and MetS risk remain to be determined in Asians.

Another knowledge gap that needs to be filled is the effects of acylcarnitines on FA metabolism and related metabolic risk. Acylcarnitines are esterified forms of carnitine and play an essential role in transporting long-chain FAs across the mitochondrial inner membrane for β-oxidation (15). Several studies have shown alterations of circulating acylcarnitines in cardiometabolic conditions (16–18). In our previous study conducted in the same cohort population, a panel of plasma acylcarnitines was found to be significantly associated with 6 y incident type 2 diabetes and substantially improved the ability of the disease prediction beyond established risk factors (19). Owing to the complex acylcarnitine metabolism, the overall pattern of acylcarnitines may reflect the degree of mitochondrial stress and dysregulation of fatty acid oxidation (FAO) better than individual acylcarnitines (20). Meanwhile, it is also of interest to explore the potential impacts of FAO status, indicated by specific acylcarnitine profile, on the associations between PUFAs and cardiometabolic outcomes.

Therefore, this study aimed to test whether erythrocyte PUFAs were inversely associated with 6 y incident MetS and its components in middle-aged and elderly Chinese individuals. Moreover, the role of acylcarnitines in the associations of interest was also explored by using a network analysis in this cohort study.

MATERIALS AND METHODS

Study population

Study participants were from the Nutrition and Health of Aging Population in China, a population-based cohort study of 3,289 residents aged 50–70 years living in Beijing and Shanghai (21). Briefly, the study was initiated in 2005, and a follow-up survey was performed in 2011. Most participants were successfully followed up (77%; n = 2,529). The study protocol was approved by the Institutional Review Board of the Institute for Nutritional Sciences, Chinese Academy of Sciences, and abided by Declaration of Helsinki principles. Written informed consent was obtained from all participants.

Data collection

In both baseline and 6 year follow-up surveys, information on demographic variables, health status, lifestyle factors, and physical activities was obtained using a standard questionnaire by trained staff. After overnight fasting, a physical examination was conducted, and venous blood samples were collected from each participant. Body weight, height, waistline, and blood pressure were measured following a standard protocol by trained medical professionals (22). BMI was calculated as kg/m^2.

Laboratory measurements

Blood samples were collected using EDTA-containing vacuum tubes. After centrifuging at 1,400 *g* for 15 min, plasma was aliquoted and stored at −80°C before analysis. The measurements of plasma glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin, C-reactive protein (CRP), and adiponectin have been described previously (21, 23). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as insulin (μU/ml) × glucose (mmol/l)/22.5.

Erythrocyte FAs were measured using gas chromatography coupled with positive chemical ionization (Agilent 6890N-5975B; Agilent Technologies, Santa Clara, CA) (24). Percentages of the area under each peak in summed areas of all FAs were determined as relative amounts of each FA. A total of 28 FAs were quantified, including four n-3 PUFAs [18:3n-3 (α-linolenic), 20:5n-3 (eicosapentaenoic), 22:5n-3 (docosapentaenoic), and 22:6n-3 (docosahexaenoic)] and eight n-6 PUFAs [18:2n-6 (linoleic), 18:3n-6 (γ-linolenic), 20:2n-6 (eicosadienoic), 20:3n-6 (dihomo-γ-linolenic), 20:4n-6 (arachidonic), 22:2n-6 (docosadienoic), 22:4n-6 (docosatetraenoic), and 22:5n-6 (docosapentaenoic)].

Plasma acylcarnitines were measured using liquid chromatography-tandem mass spectrometry (19). Chromatographic separation was performed by using a 1260 HPLC system (Agilent Technologies), and an Agilent 6410B QQQ mass spectrometer was applied for the mass spectrometric analysis. Deuterium-labeled carnitine and acylcarnitines (NSK-B Set; Cambridge Isotope Laboratories, Inc., Tewksbury, MA) were purchased for internal standards. A total of 34 acylcarnitines were measured, including free carnitine, 3-dehydroxycarnitine, 3-dehydrocarnitine, short-chain acylcarnitines (C2, C3, C3DC, C4, C5, C5OH, C5:1, C6, C6OH, C6DC, and C7DC), medium-chain acylcarnitines (C8, C8:1, C10, C10DC, C12, C12OH, C12:1, C12DC, C14, C14OH and C14:1OH), and long-chain acylcarnitines (C16, C16:1, C16:2, C18, C18OH, C18:1, C18:2, C20, and C20:4).

Definition of MetS

MetS was defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian Americans (25). Participants with MetS were defined if they had any three or more of the following features: waistline ≥90 cm for men or ≥80 cm for women; triglycerides ≥1.7 mmol/l; HDL-cholesterol ≤1.03 mmol/l for men or ≤1.30 mmol/l for women; blood pressure ≥130/85 mmHg or current use of antihypertensive drugs; and fasting glucose ≥5.6 mmol/l, or taking oral antidiabetic agents (or insulin), or previously diagnosed type 2 diabetes.

Participants with the following conditions were excluded: MetS or type 2 diabetes at baseline, insufficient data to define incident MetS, and lacking baseline data of FAs. When a specific MetS component was analyzed, participants with a corresponding component at baseline were excluded. The final analyses included 1,245 participants for MetS, 1,153 for central obesity, 1,717 for triglycerides, 1,314 for HDL-cholesterol, 705 for blood pressure, and 1,350 for fasting glucose.

Statistical analyses

Variables with skewed distribution were natural log-transformed. Spearman correlation coefficients (r_s) among metabolites and between metabolites and clinical risk markers were calculated after adjusting for age, sex, region, and residence. Relative risks (RRs) for MetS or each component were calculated by the log-Poisson model after adjusting for age, sex, region, residence, current smoking status, current drinking status, years of education,

physical activity, family history of chronic diseases [a parent or first-degree sibling having coronary heart disease (CHD), stroke, hypertension, or diabetes], and BMI.

R package WGCNA version 1.51 (26) was used for the network analysis of all FAs and acylcarnitines at baseline, which were standardized (mean: 0; SD: 1) before analyses. A soft-thresholding power of five and minimum module size of three were chosen to create metabolite modules. As the module-representative variable, the module eigengenes (MEs) were derived corresponding to the first principal component of each identified module. Module membership strength was evaluated by Pearson correlation coefficients between metabolites and corresponding MEs. RRs for MetS according to quartiles of MEs or per SD increase in MEs were also estimated using log-Poisson models. *P* for interactions between modules was calculated by using likelihood ratio tests.

All analyses were conducted with Stata version 9.2 (StataCorp LP, College Station, TX) and R software version 3.4.0 (27). Subnetwork visualization was performed by Cytoscape version 3.5.1 (28). Two tailed $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of participants

Over a 6 year period, 433 of 1,245 (34.8%) eligible participants developed incident MetS. Compared with noncases, incident cases were more likely to be females, urban, current nonsmokers and nondrinkers, and have a family history of chronic diseases at baseline (**Table 1**). Incident cases also had adverse profiles of blood pressure, triglycerides, total cholesterol, LDL- and HDL-cholesterol, fasting insulin, HOMA-IR, CRP, and adiponectin, as well as higher BMI and waistline at baseline. Baseline characteristics of all participants who were followed up or lost in 2011 are presented in supplemental Table S1, while those for incident cases and noncases stratified by gender are shown in supplemental Table S2.

The mean levels (percentage total FAs) of total n-6 and n-3 PUFAs at baseline were 32.4% and 7.1%, respectively. The incident cases had significantly higher proportions of

TABLE 1. Baseline characteristics of participants with and without incident MetS

	Without Incident MetS ($n = 812$)	With Incident MetS ($n = 433$)
Age, years	58.0 ± 5.9	57.9 ± 6.0
Men, *n* (%)	428 (52.7)	166 (38.3)
Northern residents, *n* (%)	331 (40.8)	187 (43.2)
Urban residents, *n* (%)	287 (35.3)	193 (44.6)
BMI, kg/m^2	22.1 ± 2.6	24.4 ± 3.0
Waistline, cm	76.5 ± 8.3	83.1 ± 8.8
Current drinking, yes (%)	258 (31.8)	112 (25.9)
Current smoking, yes (%)	279 (34.4)	111 (25.6)
Education, *n* (%)		
0–6 years	406 (40.0)	196 (45.3)
7–9 years	262 (32.3)	145 (33.5)
⩾10 years	144 (17.7)	92 (21.3)
Physical activity, *n* (%)		
Low	58 (7.1)	23 (5.3)
Moderate	271 (33.4)	167 (38.6)
High	483 (59.5)	243 (56.1)
Family history of chronic diseases, *n* (%)	357 (44.0)	239 (55.2)
Systolic blood pressure, mmHg	130.5 ± 20.7	137.7 ± 20.6
Diastolic blood pressure, mmHg	76.2 ± 10.1	79.9 ± 10.3
Triglycerides, mmol/l	0.8 (0.6, 1.0)	1.1 (0.8, 1.4)
HDL-cholesterol, mmol/l	1.4 ± 0.3	1.3 ± 0.3
LDL-cholesterol, mmol/l	3.0 ± 0.9	3.3 ± 0.9
Total cholesterol, mmol/l	4.5 ± 0.9	4.7 ± 0.9
Fasting glucose, mmol/l	5.2 ± 0.5	5.2 ± 0.4
RBC HbA_{1c}, %	5.6 ± 0.4	5.7 ± 0.4
Fasting insulin, mIU/l	11.6 (8.4, 15.0)	13.3 (9.7, 17.8)
HOMA-IR	2.6 (1.9, 3.5)	3.1 (2.2, 4.1)
Adiponectin, mg/l	17.4 (10.8, 26.6)	13.2 (8.5, 21.1)
CRP, mg/l	0.4 (0.1, 0.9)	0.6 (0.3, 1.2)
Total n-6 PUFAs, %	32.3 ± 4.2	32.5 ± 4.2
18:2n-6 (Linoleic acid)	13.6 ± 2.8	13.7 ± 2.9
18:3n-6 (γ-Linolenic acid)	0.079 (0.049, 0.123)	0.101 (0.066, 0.147)
20:2n-6 (Eicosadienoic acid)	0.40 ± 0.07	0.39 ± 0.06
20:3n-6 (Dihomo-γ-linolenic acid)	1.26 ± 0.28	1.30 ± 0.28
20:4n-6 (Arachidonic acid)	12.9 ± 1.9	12.9 ± 2.0
22:2n-6 (Docosadienoic acid)	0.082 ± 0.019	0.077 ± 0.018
22:4n-6 (Docosatetraenoic acid)	2.62 ± 0.67	2.55 ± 0.63
22:5n-6 (Docosapentaenoic acid)	1.51 ± 0.44	1.48 ± 0.42
Total n-3 PUFAs, %	6.85 (6.01, 7.85)	7.00 (6.25, 7.82)
18:3n-3 (α-Linolenic acid)	0.229 (0.183, 0.297)	0.241 (0.187, 0.314)
20:5n-3 (Eicosapentaenoic acid)	0.411 (0.304, 0.573)	0.424 (0.331, 0.575)
22:5n-3 (Docosapentaenoic acid)	1.76 ± 0.29	1.74 ± 0.30
22:6n-3 (Docosahexaenoic acid)	4.45 ± 1.02	4.56 ± 0.99

Values are means ± SDs for normal distributed variables or medians (interquartile ranges) for skewed distributed variables. Percentages may not sum to 100 as a result of rounding. Adiponectin data are missing for 33 participants. HbA_{1c}, glycated hemoglobin; RBC, red blood cell.

18:3n-6, 20:3n-6, and 18:3n-3 but lower proportions of 20:2n-6 and 22:2n-6 than those of noncases (Table 1). Meanwhile, incident MetS cases had higher levels of free carnitine and medium- and long-chain acylcarnitines but lower 3-dehydroxycarnitine and 3-dehydrocarnitine than those without incident MetS (supplemental Table S3). Moreover, PUFAs were intercorrelated, with r_s values ranging from −0.27 to 0.60 (supplemental Table S4). Correlations were weak to moderate between PUFAs and BMI, waistline, blood lipids, and blood pressure or insulin, with all r_s values <0.40. Weak correlations were also detected for PUFAs and acylcarnitines, with no r_s values >0.30 (supplemental Fig. S1).

PUFAs and incident MetS risk

Of n-6 PUFAs, total n-6 PUFAs and three 22-carbon n-6 PUFAs were significantly associated with a 24% to 31% lower MetS risk comparing extreme quartiles: the RRs (95% CIs) were 0.75 (0.57, 0.97) for total n-6 PUFAs, 0.69 (0.56, 0.85) for 22:2n-6, 0.76 (0.59, 0.99) for 22:4n-6, and 0.74 (0.58, 0.94) for 22:5n-6 after multivariate adjustment (**Table 2**). On the other hand, 18:3n-6 showed positive association with incident MetS (RR per SD: 1.15; 95% CI: 1.06, 1.24).

Of n-3 PUFAs, higher 18:3n-3 was also positively associated with incident MetS, with a per SD RR of 1.09 (95% CI: 1.03, 1.15). No significant association was detected for total n-3 PUFAs or other n-3 PUFAs, including 20:5n-3, 22:5n-3, or 22:6n-3 (Table 2).

For associations with individual MetS components (supplemental Table S5), total n-6 PUFAs and 20:4n-6 were inversely associated with central obesity, while 18:3n-6 and 20:3n-6 were positively associated with central obesity. Lower levels of 20:4n-6, 22:2n-6, 22:4n-6, and 22:5n-6 but higher concentrations of 18:3n-3 and 20:5n-3 were significantly associated with elevated triglycerides. Moreover, 20:4n-6 and 22:4n-6 were positively associated with HDL-cholesterol levels, while 18:3n-3 was inversely associated with HDL-cholesterol levels. The levels of total n-6 PUFAs and 20:4n-6 were also inversely associated with elevated fasting glucose. Associations stratified by gender are shown in supplemental Table S6.

Network analysis

Six modules (three FA modules and three acylcarnitine modules) were generated by a network analysis performed on baseline values of FAs and acylcarnitines. When module membership strengths >0.8 were considered (supplemental Table S7), module 1 was determined by 22-carbon n-6 PUFAs (22:4n-6 and 22:5n-6) and very-long-chain saturated FAs (22:0 and 24:0); module 2 was driven by monounsaturated FAs (20:1n-9, 22:1n-9, and 24:1n-9); module 3 consisted of *trans*-FAs (18:2n-6 9c12t and 18:2n-6 9t12c); module 4 was mostly driven by long-chain acylcarnitines (C14OH, C14:1OH, C16:1, C16:2, C18, C18:1, C18:2, C20, and C20:4); module 5 was determined by medium-chain acylcarnitines (C12, C12OH, C12:1, and C14); and module 6 was contributed by short- to medium-chain acylcarnitines (C6, C8, and C10). **Figure 1** shows the network heat map and correlations of the six modules as well as the subnetworks of modules 1 and 4. The intercorrelations of FA modules ranged from −0.25 to 0.09, and the intercorrelations of acylcarnitine modules ranged from 0.38 to 0.76 (Fig. 1B, supplemental Table S8).

Modules 1 and 3 were both inversely associated with incident MetS after multivariable adjustment, with per SD RRs (95% CIs) of 0.84 (0.76, 0.92) and 0.90 (0.83, 0.98) ($P < 0.05$), respectively, whereas module 6 was marginally positively associated with incident MetS, with a per SD RR of 1.07 (95% CI: 0.99, 1.15) ($P = 0.08$) (**Table 3**).

We next focused on the interactions between FA modules that were significantly associated with MetS risk (modules 1 and 3) and acylcarnitine modules. Significant interaction was found between modules 1 and 6 ($P_{interaction} = 0.03$) (supplemental Fig. S2). Adjusted RRs (95% CIs) of MetS according to tertiles of module 1 were 1 (reference), 0.76 (0.57, 1.03), and 0.52 (0.35, 0.75) in the lowest module 6 tertile and 0.91 (0.69, 1.21), 0.86 (0.65, 1.14), and 0.63 (0.45, 0.88) in the highest module 6 tertile, respectively.

DISCUSSION

In this prospective study among Chinese men and women, we found that total n-6 PUFAs and three long-chain (22-carbon) n-6 PUFAs were inversely associated with a 6 year risk of developing incident MetS, while 18:3n-3 and 18:3n-6 showed positive associations. Using network analysis, we identified three FA modules and three acylcarnitine modules. Two FA modules consisting of long-chain n-6 PUFAs or *trans*-FAs were associated with a lower MetS risk. Although the FA modules and acylcarnitine modules were not strongly correlated with each other, data from an exploratory analysis suggested that the long-chain n-6 PUFA module was more strongly associated with lower MetS risk when the short- to medium-chain acylcarnitine (C5–C10) module score was also lower.

To our knowledge, this is the first prospective study demonstrating that total n-6 and long-chain n-6 PUFA biomarkers were associated with a reduced MetS risk in an Asian population. Our findings are in line with those from two earlier cohort studies in Finnish populations. One of the studies found an inverse association between serum total n-6 PUFAs and incident MetS among 665 Finnish men and women (10). Similar associations were also observed in another study that consisted of 661 middle-aged Finnish men (29). In addition, increased circulating levels of total n-6 PUFAs were associated with decreased incidence of type 2 diabetes or CHD in several European studies (30, 31).

When individual n-6 PUFA biomarkers were considered, our study showed significantly inverse associations of 22-carbon n-6 PUFAs, including 22:2n-6, 22:4n-6, and 22:5n-6, with 6 year incident MetS, although the inverse association did not achieve statistical significance for 18:2n-6. Notably, existing results regarding the associations of specific n-6 PUFAs with cardiometabolic diseases remain controversial among individual studies. For instance, Yary et al. (29) found that both serum 18:2n-6 and 20:4n-6 were inversely associated with future MetS risk in Finnish men.

TABLE 2. RRs and 95% CIs for MetS according to baseline erythrocyte PUFAs

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	RR per SD	*P*
Total n-6 PUFAs						
Median (interquartile range)	26.95 (25.49, 28.32)	31.38 (30.39, 32.36)	34.57 (33.89, 35.23)	36.88 (36.43, 37.66)		
Case/noncase	101/210	116/196	111/200	105/206		
Model 1	1	1.02 (0.83, 1.27)	0.86 (0.67, 1.10)	0.74 (0.57, 0.97)	0.88 (0.80, 0.97)	0.009
Model 2	1	0.98 (0.80, 1.21)	0.89 (0.70, 1.14)	0.75 (0.57, 0.97)	0.90 (0.81, 0.99)	0.028
18:2n-6						
Median (interquartile range)	10.29 (9.55, 10.95)	12.38 (11.97, 12.92)	14.30 (13.85, 14.82)	16.96 (16.04, 18.09)		
Case/noncase	94/217	110/202	119/192	110/201		
Model 1	1	1.10 (0.88, 1.39)	1.07 (0.83, 1.38)	0.86 (0.65, 1.15)	0.93 (0.84, 1.04)	0.20
Model 2	1	1.11 (0.89, 1.38)	1.01 (0.79, 1.29)	0.85 (0.65, 1.13)	0.95 (0.86, 1.06)	0.37
18:3n-6						
Median (interquartile range)	0.038 (0.031, 0.046)	0.068 (0.060, 0.076)	0.106 (0.096, 0.119)	0.172 (0.148, 0.207)		
Case/noncase	76/235	103/209	114/197	140/171		
Model 1	1	1.31 (1.02, 1.68)	1.48 (1.16, 1.89)	1.70 (1.35, 2.15)	1.21 (1.12, 1.30)	<0.001
Model 2	1	1.22 (0.97, 1.54)	1.32 (1.05, 1.67)	1.48 (1.19, 1.85)	1.15 (1.06, 1.24)	<0.001
20:2n-6						
Median (interquartile range)	0.325 (0.301, 0.338)	0.370 (0.360, 0.380)	0.407 (0.398, 0.418)	0.462 (0.446, 0.498)		
Case/noncase	117/194	117/195	108/203	91/220		
Model 1	1	0.97 (0.79, 1.19)	0.91 (0.74, 1.12)	0.79 (0.63, 0.99)	0.92 (0.85, 1.00)	0.054
Model 2	1	1.08 (0.89, 1.31)	1.02 (0.84, 1.25)	0.89 (0.72, 1.11)	0.97 (0.90, 1.05)	0.42
20:3n-6						
Median (interquartile range)	0.968 (0.895, 1.025)	1.168 (1.126, 1.210)	1.331 (1.291, 1.379)	1.615 (1.516, 1.737)		
Case/noncase	90/221	114/198	97/214	132/179		
Model 1	1	1.28 (1.02, 1.61)	1.08 (0.85, 1.37)	1.47 (1.18, 1.82)	1.09 (1.01, 1.17)	0.024
Model 2	1	1.13 (0.91, 1.39)	0.92 (0.73, 1.15)	1.23 (0.998, 1.53)	1.03 (0.96, 1.11)	0.39
20:4n-6						
Median (interquartile range)	10.74 (9.94, 11.22)	12.41 (12.02, 12.72)	13.53 (13.25, 13.88)	15.13 (14.68, 15.71)		
Case/noncase	108/203	108/204	111/200	106/205		
Model 1	1	0.94 (0.76, 1.17)	0.94 (0.76, 1.16)	0.85 (0.67, 1.09)	0.92 (0.84, 0.996)	0.041
Model 2	1	0.95 (0.78, 1.16)	0.97 (0.79, 1.19)	0.87 (0.68, 1.11)	0.93 (0.86, 1.02)	0.11
22:2n-6						
Median (interquartile range)	0.060 (0.055, 0.064)	0.074 (0.071, 0.077)	0.084 (0.082, 0.087)	0.102 (0.095, 0.111)		
Case/noncase	132/179	115/197	103/208	83/228		
Model 1	1	0.86 (0.71, 1.04)	0.79 (0.65, 0.97)	0.65 (0.52, 0.81)	0.84 (0.77, 0.91)	<0.001
Model 2	1	0.86 (0.72, 1.02)	0.75 (0.62, 0.91)	0.69 (0.56, 0.85)	0.85 (0.78, 0.93)	<0.001
22:4n-6						
Median (interquartile range)	1.85 (1.68, 1.99)	2.31 (2.21, 2.41)	2.74 (2.62, 2.91)	3.44 (3.28, 3.66)		
Case/noncase	113/198	106/206	114/197	100/211		
Model 1	1	0.92 (0.75, 1.14)	0.93 (0.74, 1.18)	0.83 (0.62, 1.09)	0.89 (0.80, 0.99)	0.031
Model 2	1	0.87 (0.72, 1.06)	0.96 (0.77, 1.20)	0.76 (0.59, 0.99)	0.86 (0.78, 0.95)	0.003
22:5n-6						
Median (interquartile range)	0.96 (0.81, 1.09)	1.38 (1.30, 1.46)	1.66 (1.59, 1.73)	1.98 (1.88, 2.15)		
Case/noncase	112/199	109/203	114/197	98/213		
Model 1	1	0.88 (0.70, 1.10)	0.88 (0.69, 1.10)	0.75 (0.59, 0.97)	0.90 (0.82, 0.98)	0.016
Model 2	1	0.81 (0.66, 1.00)	0.82 (0.66, 1.03)	0.74 (0.58, 0.94)	0.88 (0.81, 0.97)	0.008
Total n-3 PUFAs						
Median (interquartile range)	5.55 (5.20, 5.85)	6.50 (6.32, 6.71)	7.36 (7.13, 7.60)	8.55 (8.12, 9.12)		
Case/noncase	93/218	113/199	120/191	107/204		
Model 1	1	1.19 (0.95, 1.48)	1.26 (0.99, 1.59)	1.12 (0.86, 1.44)	1.07 (0.99, 1.16)	0.073
Model 2	1	1.12 (0.91, 1.39)	1.21 (0.97, 1.52)	1.03 (0.81, 1.31)	1.05 (0.97, 1.13)	0.21

TABLE 2. *Continued.*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	RR per SD	*P*
18:3n-3						
Median (interquartile range)	0.153 (0.135, 0.171)	0.209 (0.197, 0.221)	0.264 (0.247, 0.282)	0.360 (0.326, 0.419)		
Case/noncase	102/209	96/216	110/201	125/186		
Model 1	1	0.99 (0.79, 1.24)	1.13 (0.90, 1.42)	1.24 (0.98, 1.57)	1.08 (1.01, 1.14)	0.015
Model 2	1	1.04 (0.84, 1.29)	1.18 (0.95, 1.46)	1.31 (1.06, 1.64)	1.09 (1.03, 1.15)	0.002
20:5n-3						
Median (interquartile range)	0.252 (0.218, 0.284)	0.361 (0.338, 0.387)	0.489 (0.454, 0.533)	0.693 (0.631, 0.805)		
Case/noncase	93/218	116/196	115/196	109/202		
Model 1	1	1.23 (0.99, 1.54)	1.31 (1.02, 1.67)	1.24 (0.95, 1.62)	1.10 (1.00, 1.20)	0.043
Model 2	1	1.27 (1.03, 1.58)	1.31 (1.03, 1.65)	1.16 (0.91, 1.49)	1.08 (0.99, 1.18)	0.065
22:5n-3						
Median (interquartile range)	1.43 (1.32, 1.50)	1.65 (1.60, 1.69)	1.82 (1.78, 1.87)	2.10 (2.01, 2.22)		
Case/noncase	112/199	113/199	95/216	113/198		
Model 1	1	1.09 (0.88, 1.34)	0.95 (0.76, 1.19)	1.19 (0.96, 1.48)	1.02 (0.94, 1.11)	0.61
Model 2	1	1.08 (0.89, 1.32)	0.90 (0.72, 1.12)	1.09 (0.87, 1.35)	0.98 (0.91, 1.07)	0.70
22:6n-3						
Median (interquartile range)	3.36 (3.02, 3.59)	4.13 (3.97, 4.27)	4.77 (4.60, 4.94)	5.66 (5.38, 6.08)		
Case/noncase	93/218	110/202	122/189	108/203		
Model 1	1	1.14 (0.91, 1.43)	1.24 (0.98, 1.56)	1.07 (0.83, 1.37)	1.03 (0.95, 1.12)	0.46
Model 2	1	1.11 (0.89, 1.37)	1.12 (0.89, 1.40)	1.00 (0.79, 1.26)	1.01 (0.93, 1.10)	0.77

Model 1 was adjusted for age, sex, region, and residence. Model 2 was further adjusted for current smoking status, current drinking status, years of education, physical activity, family history of chronic diseases, and BMI. *P* values were for RRs per SD change in exposures derived from log-Poisson models.

However, in a recent meta-analysis that consisted of 39,740 adults, only higher levels of 18:2n-6, but not 20:4n-6, were associated with a lower risk of type 2 diabetes (7). Moreover, inconsistent associations of 22:4n-6 or 22:5n-6 with CHD or type 2 diabetes were also reported in cohort studies among European populations (30, 32). The genetic differences might be one of the primary reasons underlying heterogenetic findings across ethnic groups. In a recent genome-wide association study of circulating PUFA levels, a genetic variant (rs174547) on *FADS1*, which encodes the Δ5 desaturase, showed different effect sizes of associations with the levels of several n-6 PUFAs between Chinese and European ancestries (5). Moreover, gene expression levels of *FADS1*/*FADS2* varied between Chinese and other ethnic populations (33). These lines of evidence suggested that differences in genetic architectures involving PUFA metabolism might partially explain the ethnic differences in the associations between PUFAs and cardiometabolic outcomes.

Our study also evaluated the effects of n-6 PUFAs on each MetS feature and found that higher levels of 22:2n-6, 22:4n-6, and 22:5n-6 were associated with lower triglycerides and/or higher HDL-cholesterol levels. Although underlying mechanisms remain unclear, all of these long-chain n-6 PUFAs are the downstream products of 18:2n-6 (34). It is plausible that the processes of converting 18:2n-6 into these long-chain n-6 PUFAs via elongation and desaturation might also play a role in these findings. A previous study in hamsters showed that the administration of a high-cholesterol diet supplemented with 22:5n-6 significantly improved the blood lipoprotein profile and suppressed the gene expressions of SREBP-2 and 3-HMG-CoA reductase in the lipid metabolic pathway (35). On the other hand, 20:4n-6 is known to be the precursor of both pro- or antiinflammatory eicosanoids (36). Interestingly, in our study, 20:4n-6 was not correlated with CRP but associated with significantly reduced risks of central obesity, dyslipidemia, and elevated fasting glucose. Studies in vitro have also revealed that 20:4n-6 could alleviate chemical-induced cytotoxicity in insulin-secreting cells (37) and activate the PPAR-α pathway, which is critical in regulating the blood lipid profile (38). Overall, our study demonstrated that total and certain n-6 PUFAs exerted a favorable rather than harmful impact on cardiometabolic outcomes.

In addition, neither total nor marine-derived n-3 PUFAs such as 20:5n-3 and 22:6n-3 showed significant associations for incident MetS, which was similar to the finding from the Finnish cohort by Vanhala et al. (10) but not the finding from a Swedish study that showed an inverse association between n-3 PUFAs and MetS (12). It is noteworthy that, except for these two studies, most cohorts have focused on associations between n-3 PUFAs and CVD or type 2 diabetes and revealed inconsistent results. For instance, in a recent meta-analysis that included 10 trials and 77,917 participants, n-3 PUFA supplementation did not exert significant effects on reducing CVD risk (39). Additionally, in another systematic review and meta-analysis that included 18 independent cohorts and 540,184 individuals, Wu et al. (6) showed that neither intake nor biomarkers of n-3 PUFAs were significantly associated with incident type 2

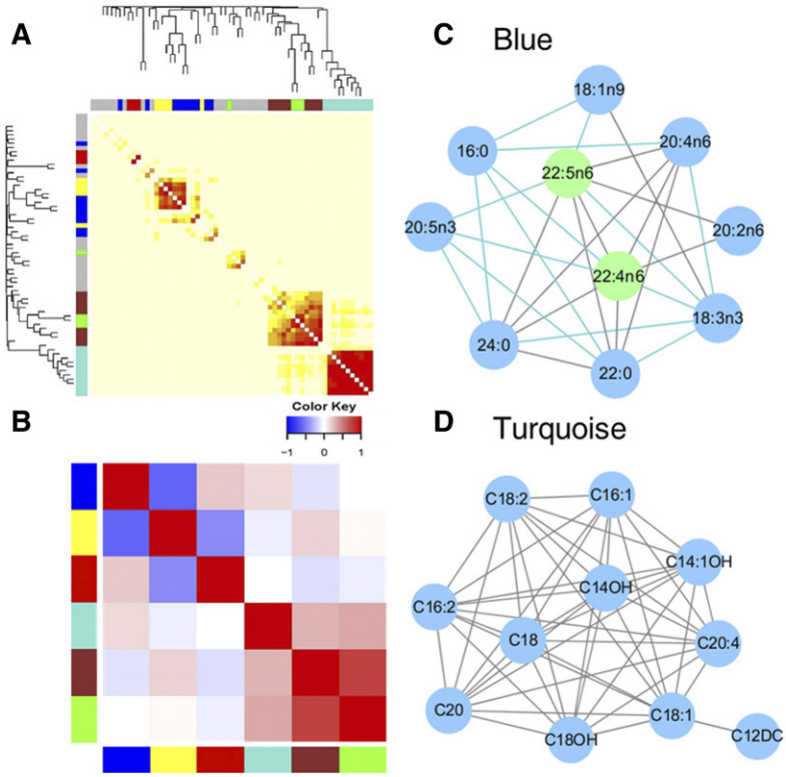

Fig. 1. Network analysis of baseline FAs and acylcarnitines. A: Network heat map, baseline FAs, and acylcarnitines were constructed into six modules (FAs: blue, yellow, and red; acylcarnitines: turquoise, brown, and green). B: Correlations among the six modules: color bars on the left and at the bottom indicate corresponding modules; grids represent Pearson correlation coefficients among MEs. C: Subnetwork plot of module 1 (blue in A): Pearson correlation coefficients >0.3 or <−0.3 were plotted; gray and blue edges indicate positive and inverse correlations, respectively. D: Subnetwork plot of module 4 (turquoise in A): Pearson correlation coefficients >0.5 were plotted.

diabetes. In the case of 18:3n-3 and 18:3n-6, findings from available studies are still inconclusive (32, 40, 41).

To explore biological networks from a system-scale perspective and to identify clusters of highly correlated metabolites (26), we also used a network analysis approach. The long-chain n-6 PUFA module derived by the network analysis was consistently associated with a lower MetS risk. Of note, the *trans*-FA module showed an inverse association with incident MetS risk. This finding was somewhat consistent with the observed inverse association of *trans*-18:1, a major *trans*-FA isoform as well as marker of dairy intake, with incident type 2 diabetes in the same Chinese population (42), who had a very low intake of *trans*-fat from partially hydrogenated oils (43).

TABLE 3. RRs and 95% CIs for incident MetS according to MEs

		Quartile 1	Quartile 2	Quartile 3	Quartile 4	RR per SD	*P*
Module 1							
16:0, 18:1n-9, 18:3n-3, 20:2n-6,	Case/noncase	112/199	110/202	113/198	98/213		
20:5n-3, 20:4n-6, 22:0, 24:0,	Model 1	1	0.83 (0.67, 1.04)	0.77 (0.60, 0.99)	0.62 (0.46, 0.84)	0.85 (0.78, 0.94)	0.001
22:4n-6, 22:5n-6	Model 2	1	0.77 (0.62, 0.95)	0.76 (0.60, 0.97)	0.63 (0.47, 0.84)	0.84 (0.76, 0.92)	<0.001
Module 2							
18:1n-7, 18:2n-6, 20:1n-9,	Case/noncase	126/185	110/202	100/211	97/214		
22:1n-9, 24:1n-9	Model 1	1	0.94 (0.77, 1.15)	0.91 (0.72, 1.15)	0.96 (0.71, 1.30)	1.00 (0.90, 1.12)	0.96
	Model 2	1	0.93 (0.76, 1.13)	0.95 (0.76, 1.19)	1.10 (0.82, 1.46)	1.06 (0.96, 1.18)	0.25
Module 3							
18:1t isomers, 18:2n-6 9c12t,	Case/noncase	112/199	97/215	117/194	107/204		
18:2n-6 9t12c	Model 1	1	0.84 (0.67, 1.04)	0.94 (0.76, 1.16)	0.82 (0.65, 1.03)	0.92 (0.85, 0.996)	0.041
	Model 2	1	0.85 (0.69, 1.05)	0.93 (0.75, 1.14)	0.81 (0.65, 1.01)	0.90 (0.83, 0.98)	0.013
Module 4							
C12DC, C14:1OH, C14OH,	Case/noncase	94/206	98/202	109/191	117/182		
C16:2, C16:1, C18:2, C18OH,	Model 1	1	1.10 (0.87, 1.39)	1.21 (0.96, 1.52)	1.30 (1.04, 1.62)	1.09 (1.01, 1.18)	0.019
C18:1, C18, C20, C20:4	Model 2	1	1.01 (0.81, 1.26)	1.06 (0.85, 1.31)	1.09 (0.88, 1.35)	1.03 (0.96, 1.10)	0.46
Module 5							
C2, C6OH, C10DC, C12OH,	Case/noncase	106/194	116/184	92/208	104/195		
C12:1, C12, C13DC, C14, C16	Model 1	1	1.10 (0.89, 1.35)	0.93 (0.74, 1.16)	1.06 (0.86, 1.32)	1.06 (0.98, 1.15)	0.15
	Model 2	1	1.05 (0.86, 1.28)	0.87 (0.70, 1.08)	1.03 (0.84, 1.27)	1.04 (0.95, 1.12)	0.40
Module 6							
C5, C6, C8, C10	Case/noncase	103/197	101/199	97/203	117/182		
	Model 1	1	1.01 (0.81, 1.26)	1.02 (0.82, 1.28)	1.24 (1.01, 1.53)	1.13 (1.05, 1.21)	<0.001
	Model 2	1	0.96 (0.78, 1.19)	0.90 (0.72, 1.19)	1.14 (0.93, 1.39)	1.07 (0.99, 1.15)	0.08

Model 1 was adjusted for age, sex, region, and residence. Model 2 was further adjusted for current smoking status, current drinking status, years of education, physical activity, family history of chronic diseases, and BMI. Data of acylcarnitines are missing for 46 participants. *P* values were for RRs per SD change in exposures derived from log-Poisson models.

Our analyses showed a significant interaction between short- and medium-chain acylcarnitine module and the long-chain n-6 PUFA module, suggesting that the favorable effects of n-6 PUFAs could be more pronounced when these acylcarnitine levels are low. Elevated acylcarnitine concentrations were observed in obese individuals, and accumulated body fat might attribute to FA overload and stressed mitochondria with incomplete FAO (44). In our previous study, acylcarnitines substantially improved the ability to predict incident type 2 diabetes beyond conventional risk factors (19). Although the current study did not show significant associations of any acylcarnitine module with MetS, elevated short- and medium-chain acylcarnitine levels were reported to be independently associated with total CVD and stroke in a Spanish population (45). Overall, these data suggest a potential interplay between n-6 PUFAs and acylcarnitines in modulating cardiometabolic risk. More data are needed, however, to replicate our findings and elucidate relevant mechanisms.

Our study has some limitations that deserve to be discussed. First, approximately 23% of participants dropped out in the 6 year follow-up. Although this rate was similar to other cohort studies (46, 47), we cannot exclude the possibility that the loss to follow-up was linked to exposure or outcome ascertainments. Second, the metabolites were measured only once at baseline. Nevertheless, the erythrocyte FAs were demonstrated to be reproducible over time (13). Third, although PUFA biomarkers may reflect certain dietary fat intake more objectively than that of a questionnaire-based approach, levels of erythrocyte PUFAs could also be influenced by other factors such as genetic background, specific metabolic profile, and health status; thus, implications for dietary intake are limited. Finally, our study was conducted in middle-aged and elderly Chinese individuals, and the findings might not be generalized to other populations with different ages and ethnic backgrounds.

In conclusion, total n-6 PUFAs and 22-carbon n-6 PUFAs in erythrocytes are associated with a reduced 6 year incident MetS risk in Chinese men and women. More studies are merited to confirm our findings and to illuminate underlying mechanisms.

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