Erythrocyte n-3 Fatty Acids and Metabolic Syndrome in Middle-Aged and Older Chinese

Geng Zhang, Qi Sun, Frank B. Hu, Xingwang Ye, Zhijie Yu, Geng Zong, Huaxing Li, Yunhua Zhou, and Xu Lin

Key Laboratory of Nutrition and Metabolism (G.Z., X.Y., G.Z., H.L., Y.Z., X.L.), Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate School of the Chinese Academy of Sciences, 200031 Shanghai, China; Departments of Nutrition (Q.S., F.B.H.) and Epidemiology (F.B.H.), Harvard School of Public Health and Channing Laboratory (Q.S., F.B.H.), Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115; and Department of Community Health Sciences (Z.Y.), Brock University, St. Catharines, Ontario L2S 3A1, Canada

Context: Few studies examined associations of circulating n-3 fatty acid levels with metabolic syndrome (MetS) among Chinese populations who have low consumption of these fatty acids and high risk of developing MetS.

Objective: The objective of the study was to determine associations between erythrocyte n-3 fatty acids and MetS as well as its components among middle-aged and older Chinese men and women.

Design and Participants: Erythrocyte levels of docosahexaenoic acid (DHA), docosapentaenoic acid, eicosapentaenoic acid, and \( \alpha \)-linolenic acid (ALA) were measured by gas chromatography among 2754 participants aged 50–70 yr living in Beijing and Shanghai. MetS was defined using the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian-Americans.

Results: After multivariable adjustment, higher levels of DHA, but neither eicosapentaenoic acid nor docosapentaenoic acid, were associated with lower odds of MetS as well as elevated blood pressure and triglycerides. Comparing extreme quartiles of DHA, odds ratios (95% confidence interval) were 0.75 (0.55, 1.01; \( P \) for trend \( <0.04 \)) for MetS; 0.70 (0.53, 0.92; \( P \) for trend = 0.01) for elevated blood pressure; and 0.64 (0.48, 0.87; \( P \) for trend = 0.005) for elevated triglycerides. In contrast, ALA concentrations were positively associated with MetS odds (odds ratio 4.06; 95% confidence interval 2.85, 5.80; \( P \) for trend <0.001).

Conclusions: Higher concentrations of erythrocyte DHA were associated with lower odds of MetS, whereas higher concentrations of ALA were associated with increased odds among middle-aged and older Chinese. These findings warrant replication in other populations. (J Clin Endocrinol Metab 97: E973–E977, 2012)
concerning the relationship between n-3 fatty acids and MetS is limited and inconclusive. Moreover, most previous studies used self-reported food frequency questionnaires to evaluate n-3 fatty acid intake, which may be affected by measurement error due to inaccurate recall, an incomplete food composition database, and likely regional variation in long-chain n-3 fatty acid contents in fish. On the other hand, tissue concentrations of n-3 fatty acids can serve as objective biomarkers reflecting dietary intake because human body cannot synthesize these fatty acids de novo. To date, only a few studies have investigated the relationship between blood n-3 fatty acid concentrations and MetS, as well as its components, and existing data remain inconsistent (3–7).

In this study, we aimed to determine the associations of erythrocyte n-3 fatty acids, including α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) with MetS and its components among a middle-aged and older Chinese population.

### Subjects and Methods

#### Study population

The present study was based on the Nutrition and Health of Aging Population in China project, which included 3289 men and women aged 50–70 yr living in Beijing and Shanghai, China, in 2005 (8). A total of 535 participants were excluded due to the following exclusion criteria: 1) missing fatty acid levels (n = 31); 2) with implausibly high (>4000 kcal/d for men or >3500 kcal/d for women) or low (<800 kcal/d for men or <500 kcal/d for women) energy intake (n = 151); and 3) with self-reported CVD or cancers (n = 353), leaving 2754 subjects in the final analyses. The study was approved by the Institutional Review Board of the Institute for Nutritional Sciences and written informed consent was obtained from each participant.

#### Data collection

Data on demographic variables, health status, lifestyle practice, physical activity, and anthropometry were collected following a standardized protocol (8). Dietary intake was assessed by a 74-item food frequency questionnaires modified from the one used in the China Health and Nutrition Surveys (9). The food-composition values were obtained from the Chinese Food Composition Table (10). All nutrient and food intakes were energy adjusted using the residual method (11).

#### Laboratory measurement

Overnight fasting blood samples were obtained and stored at −80 °C until analyses were conducted. The measurements of plasma high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, and C-reactive protein (CRP) were previously described (8).

Erythrocyte membrane fatty acid concentrations were determined by gas-liquid chromatography (12). Briefly, erythrocyte samples were first extracted by hexane and isopropanol and then trimethylated with methanol and sulfuric acid. Fatty acid methyl esters were subsequently analyzed by gas chromatography. Individual peaks were identified by comparison with known standards. Relative amount of each fatty acid was quantified by expressing the area under each peak as a percentage of total area under the peaks. Intraassay coefficients of variation (CV; percentages) were 2.7% for ALA, 25.8% for EPA, 3.6% for DPA, 3.4% for DHA, and 0.7% for linoleic acid. The corresponding interassay CV were 7.8, 9.3, 14.1, 10.0, and 7.7%, respectively, for these fatty acids.

### Definition of MetS

MetS was defined using the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian-Americans (1) as meeting at least three of the following criteria: 1) central obesity (waist circumference of ≥90 cm in men or ≥80 cm in women); 2) elevated blood pressure (≥130/85 mm Hg or using antihypertensive medications); 3) elevated fasting glucose (≥5.6 mmol/liter or previously diagnosed diabetes or using antidiabetic agents or insulin); 4) reduced HDL cholesterol (<1.03 mmol/liter in men or <1.30 mmol/liter in women); and 5) elevated triglycerides (≥1.7 mmol/liter).

#### Statistical analysis

Spearman partial correlation coefficients of individual n-3 fatty acids with food consumption levels were calculated and adjusted for age, sex, region (Beijing/Shanghai), residence (urban/rural), and total energy intake. Multivariable logistic regressions were applied to estimate the odds ratios (OR) for MetS and its components. P values for trend were determined by assuming median values of quartiles as continuous variables. All statistical analyses were performed using Stata 9.2 (Stata, College Station, TX) and P < 0.05 (two sided) was considered statistically significant.

### Results

#### Distribution of n-3 fatty acid concentrations and their associations with food intake

The median values of ALA, EPA, DPA, and DHA concentrations in erythrocytes were 0.25, 0.42, 1.72, and 4.40%, respectively. In general, female, Shanghai, or urban participants had significantly higher concentrations of total and most individual types of n-3 fatty acids than their male, Beijing or rural counterparts with a few exceptions (Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). Both EPA and DHA, but not DPA, were positively correlated with fish consumption. Spearman partial correlation coefficients were 0.16 for EPA and 0.22 for DHA. ALA was not correlated with fish intake but weakly correlated with plant oil consumption (correlation = 0.07).

#### Associations of n-3 fatty acids with MetS and its components

Overall, total long-chain n-3 fatty acids, EPA, or DPA were not significantly associated with MetS after multivariable adjustment (Supplemental Table 2). On the other hand, higher DHA concentrations were significantly as-
associated with a lower prevalence of MetS: the OR in the highest vs. the lowest quartile was 0.75 [95% confidence interval (CI) 0.55, 1.01; \( P \) for trend = 0.04] (Table 1).

With respect to individual component of MetS, participants in the highest DHA quartile had a 30% lower odds of elevated blood pressure (OR 0.70, 95% CI 0.53, 0.92; \( P \) for trend = 0.01) and a 36% lower odds of elevated triglycerides (OR 0.64, 95% CI 0.48, 0.87; \( P \) for trend = 0.005) compared with those in the lowest quartile. Unexpectedly, ALA was positively associated with MetS (OR 4.06, 95% CI 2.85, 5.80; \( P \) for trend <0.001) as well as reduced HDL cholesterol (OR 4.42, 95% CI 3.22, 6.06; \( P \) for trend <0.001) and elevated triglycerides (OR 11.48, 95% CI 7.75, 17.00; \( P \) for trend <0.001) in the fully adjusted model (Table 2).

### Stratified analysis

Potential modification effects of sex, geographic, and urban-rural differences on associations between n-3 fatty acids and MetS were also examined in stratified analyses (Supplemental Table 3 and 4). Most of the analyses confirmed the previous results except that the inverse association between DHA concentrations and MetS was observed only among women or participants living in Beijing (\( P = 0.08 \) for interaction of DHA and sex and \( P = 0.31 \) for interaction of DHA and region).

### Discussion

In this study, we observed significant sex and geographic differences in erythrocyte n-3 fatty acid concentrations among this Chinese population. A higher concentration of DHA in erythrocytes was significantly associated with lower odds of MetS and elevated blood pressure and triglycerides independent of conventional CVD risk and inflammatory factors, whereas an opposite association was found for ALA.

Unlike the well-established cardioprotective role, evidence regarding the relationship between tissue contents of n-3 fatty acids and MetS, a strong predictor for CVD and type 2 diabetes (1), is scarce, and existing results were controversial (3–5, 13). Plasma long-chain n-3 fatty acids were associated with lower odds of MetS in a Chinese study (3), whereas in other investigations using serum or erythrocyte specimens, null results were found (4, 5, 13).
Discrepancies among these studies and the current investigation might be due to a few reasons. First, in comparison with plasma phospholipids or serum cholesterol esters, erythrocyte concentrations may reflect a relatively longer-term dietary intake of n-3 fatty acids of marine origin. Second, various effects of individual n-3 fatty acids on metabolic outcomes might be concealed when all n-3 fatty acids were combined in the previous studies (14). Finally, different study design, sample size, characteristics of participants, and covariates adjusted may further explain the heterogeneous results.

It was unexpected that erythrocyte ALA was strongly associated with MetS odds, and this association was primarily driven by the strong associations with dyslipidemia in the current study. A meta-analysis on randomized control trials of ALA revealed that ALA intake might slightly decrease HDL levels and nonsignificantly increase low-density lipoprotein and triglyceride levels, although significant heterogeneity across the trials was also documented (15). Likewise, in observational studies intake or circulating levels of ALA were not consistently associated with risk of metabolic syndrome or CVD outcomes (5, 16–18). It is unclear whether the association of ALA with higher MetS odds in the current study was due to cooking method: stir-fry is a common Chinese cooking practice, and several deleterious trans-isomers and cyclic fatty acids could be generated during the high-temperature heating process (19).

Clearly, our results need to be replicated in other populations and examined further in experimental studies.

To the best of our knowledge, this is the first study to investigate the associations of individual n-3 fatty acid biomarkers in erythrocytes with MetS in a large population-based sample in Asia. Using nutritional biomarkers is particularly important for assessing diet in countries like China without a complete food composition database for fatty acid content and with a tradition of sharing dishes during meals. In addition, the detailed, standardized protocol used to identify outcomes and collect information on sociodemographic, lifestyle, and dietary factors and inflammatory marker made the comprehensive multivariable-adjusted statistical analysis possible.

On the other hand, owing to the cross-sectional nature of the current study, a causal relation between n-3 fatty acid biomarkers and MetS cannot be inferred. Furthermore, greater measurement error for erythrocyte EPA (in-

### TABLE 2. Adjusted OR and 95% CI for MetS and its components according to quartiles of erythrocyte ALA concentrations

<table>
<thead>
<tr>
<th>Quartile of ALA concentration (%)</th>
<th>1 (lowest)</th>
<th>2</th>
<th>3</th>
<th>4 (highest)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MetS</strong></td>
<td>0.16 (0.14–0.18)</td>
<td>0.22 (0.21–0.24)</td>
<td>0.28 (0.27–0.30)</td>
<td>0.40 (0.36–0.48)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cases/n</td>
<td>228/688</td>
<td>252/689</td>
<td>275/689</td>
<td>368/688</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.50 (1.18, 1.89)</td>
<td>2.04 (1.60, 2.60)</td>
<td>3.84 (2.98, 4.94)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.41 (1.05, 1.89)</td>
<td>2.15 (1.58, 2.94)</td>
<td>4.06 (2.85, 5.80)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Central obesity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/n</td>
<td>295/688</td>
<td>312/689</td>
<td>318/689</td>
<td>368/688</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.33 (1.06, 1.67)</td>
<td>1.51 (1.20, 1.91)</td>
<td>2.08 (1.63, 2.65)</td>
<td>0.30</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.12 (0.78, 1.62)</td>
<td>1.35 (0.92, 1.99)</td>
<td>1.52 (0.98, 2.35)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Elevated blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/n</td>
<td>457/688</td>
<td>468/689</td>
<td>465/689</td>
<td>461/688</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.27 (1.00, 1.61)</td>
<td>1.42 (1.11, 1.82)</td>
<td>1.61 (1.25, 2.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.15 (0.89, 1.48)</td>
<td>1.21 (0.92, 1.60)</td>
<td>1.21 (0.89, 1.66)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Elevated fasting glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/n</td>
<td>316/688</td>
<td>281/689</td>
<td>228/689</td>
<td>249/688</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.09 (0.87, 1.37)</td>
<td>0.96 (0.76, 1.22)</td>
<td>1.28 (1.00, 1.63)</td>
<td>0.07</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.00 (0.78, 1.27)</td>
<td>0.83 (0.64, 1.08)</td>
<td>1.00 (0.74, 1.36)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Reduced HDL cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/n</td>
<td>208/688</td>
<td>252/689</td>
<td>295/689</td>
<td>392/688</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.35 (1.07, 1.71)</td>
<td>1.72 (1.36, 2.19)</td>
<td>2.91 (2.28, 3.72)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.53 (1.18, 1.98)</td>
<td>2.25 (1.71, 2.96)</td>
<td>4.42 (3.22, 6.06)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Elevated triglycerides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/n</td>
<td>66/688</td>
<td>111/689</td>
<td>162/689</td>
<td>300/688</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>2.29 (1.64, 3.19)</td>
<td>4.41 (3.18, 6.10)</td>
<td>11.93 (8.60, 16.57)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>2.29 (1.61, 3.26)</td>
<td>4.49 (3.14, 6.42)</td>
<td>11.48 (7.75, 17.00)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*a Model 1 is adjusted for age, sex, region, and residence. Model 2 is further adjusted for smoking, alcohol drinking, physical activity, educational attainment, family history of diabetes and CVD, total energy intake, red meat intake, fiber intake, body mass index, and CRP as well as EPA, DPA, DHA, and linoleic acid in erythrocytes.

b The significance of linear trends across quartiles was tested by assigning the median value within quartiles to each participant and modeling this value as a continuous variable.
tra assay CV 25.8%) might materially attenuate statistical power to detect the association between EPA and MetS.

In summary, we observed a significant inverse association between erythrocyte DHA and MetS among the middle-aged and older Chinese. Conversely, higher ALA concentrations were strongly associated with a higher prevalence of MetS. Further prospective studies are needed to confirm our findings.

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Address all correspondence and requests for reprints to: Dr. Xu Lin, Institute for Nutritional Sciences, Chinese Academy of Sciences, 294 Tai-Yuan Road, Shanghai 200031, China. E-mail: xlin@sibs.ac.cn.

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Disclosure Summary: The authors have nothing to disclose.

References


14. Anderson BM, Ma DW 2009 Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis 8:33


