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Original article

Association of erythrocyte n-3 polyunsaturated fatty acids with incident type 2 diabetes in a Chinese population

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SUMMARY

Background & aims: The association between circulating n-3 polyunsaturated fatty acid (PUFA) biomarkers and incident type 2 diabetes in Asian populations remains unclear. We aimed to examine the association of erythrocyte n-3 PUFA with incident type 2 diabetes in a Chinese population.
Methods: A total of 2671 participants, aged 40–75 y, free of type 2 diabetes at baseline, were included in the present analysis. Incident type 2 diabetes cases (n = 213) were ascertained during median follow-up of 5.6 years. Baseline erythrocyte fatty acids were measured by gas chromatography. We used multivariable Cox regression models to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of type 2 diabetes across quartiles of erythrocyte n-3 PUFA.
Results: After adjustment for potential confounders, HRs (95% CIs) of type 2 diabetes were 0.68 (0.47, 1.00), 0.77 (0.52, 1.15), and 0.63 (0.41, 0.95) in quartiles 2–4 of docosapentaenoic acid (C22:5n-3) (*P*-trend = 0.07), compared with quartile 1; and 1.08 (0.74, 1.60), 1.03 (0.70, 1.51), and 0.57 (0.38, 0.86) for eicosapentaenoic acid (C20:5n-3) (*P*-trend = 0.007). No association was found for docosahexaenoic acid (C22:6n-3) or alpha-linolenic acid (C18:3n-3).
Conclusions: Erythrocyte n-3 PUFA from marine sources (C22:5n-3 and C20:5n-3), as biomarkers of dietary marine n-3 PUFA, were inversely associated with incident type 2 diabetes in this Chinese population. Future prospective investigations in other Asian populations are necessary to confirm our findings.

1. Introduction

Prospective associations between dietary n-3 polyunsaturated fatty acids (PUFA) and type 2 diabetes (T2D) have been inconsistent in the published literature [1–5]. Several prior meta-analyses have suggested that overall dietary intake of marine n-3 PUFA (including docosahexaenoic acid [DHA, C22:6n-3], docosapentaenoic acid [DPA, C22:5n-3] and eicosapentaenoic acid [EPA, C20:5n-3]) was not associated with incident T2D, but reflected substantial heterogeneity by geographic region: there was an inverse association in Asian populations (mainly Chinese), but a positive association in US populations and a null association in European populations [2,4,5]. Similar inconsistencies were found for the association between dietary alpha-linolenic acid (ALA, C18:3n-3), a plant-based n-3 PUFA, and T2D incidence [3,5].

Abbreviations: ALA, alpha-linolenic acid; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; PUFA, polyunsaturated fatty acids; Q, quartile; T2D, type 2 diabetes.

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Assessment of habitual n-3 PUFA intake from self-reported dietary questionnaires, as adopted by the majority of the previous observational studies, is known to be subject to measurement error and recall bias, with compromised accuracy [6]. Using objectively measured circulating biomarkers of n-3 PUFA to examine the association with T2D could overcome the above limitations of dietary measurement, although there are still very few studies linking objectively measured circulating n-3 PUFA with T2D incidence [7–12]. In a recent study, Forouhi et al. examined the prospective association between individual plasma phospholipid PUFA and T2D incidence in the EPIC-InterAct study and further conducted a comparative meta-analysis of the published literature [7]. The results suggested that ALA was inversely associated with incident T2D, while no association was found for EPA or DHA. Of note, all the above evidence was generated from observational studies in Western populations, including Australian, US, and European participants [7]. So far, to the best of our knowledge, there has been no study among Chinese populations examining the association between circulating n-3 PUFA and incident T2D.

The aim of the present study was to investigate the association between objectively measured individual n-3 PUFA in red blood cells (erythrocytes) and incident T2D in a community-based prospective cohort study in southern China. We hypothesized that erythrocyte marine n-3 PUFA were inversely associated with incident type 2 diabetes in the Chinese population.

2. Materials and methods

2.1. Study design and study population

Our study was based on the Guangzhou Nutrition and Health Study (GNHS), a community-based prospective cohort study in the urban area of southern China. Detailed study designs have been reported previously [13]. Briefly, between 2008 and 2013, 4048 participants, aged 40–75 years old, living in urban Guangzhou city for at least 5 years, were recruited into the GNHS; there were two waves of participant recruitment using the same criteria: between 2008 and 2010 (n = 3169), and between 2012 and 2013 (n = 879). All participants were followed up every 3 years, and up to May 31, 2017, two follow-up visits were performed for participants recruited between 2008 and 2010, and one follow-up visit for participants recruited between 2012 and 2013.

At baseline, we excluded those without valid questionnaire information on age or sex (n = 18), those with self-reported baseline cancers (n = 19), chronic renal dysfunction (n = 4), self-reported/diagnosed T2D (n = 323), or those without measurement of baseline erythrocyte membrane fatty acid compositions (n = 387). We also excluded those with missing covariates (n = 108) and those with extreme levels of total energy intake (men: <800 kcal or >4000 kcal; women: <500 kcal or >3500 kcal) (n = 47). We further excluded those without follow-up information (n = 471, 85% follow-up rate). Finally, 2671 participants were included in the present analysis, with a median 5.6 years of follow-up. A flow-chart showing detail of inclusion and exclusion criteria is shown in Supplemental Fig. 1.

Incident T2D cases (N = 213) were ascertained on the basis of fasting blood glucose ≥7.0 mmol/L or HbA1c ≥ 6.5% or currently under medical treatment for diabetes at either of the two follow-up visits, according to the American Diabetes Association criteria for the diagnosis of diabetes [14]. The study protocol was approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University, and all participants provided written informed consent.

2.2. Measurement of erythrocyte membrane fatty acids

Venous blood samples were collected after overnight fast (>12 h), and erythrocytes were washed and separated within 2 h of collection and stored at −80 °C. Erythrocyte membrane total fatty acid compositions were measured using gas chromatography (7890 GC, Agilent, California; DB-23 capillary column: 60 m × 0.25 mm internal diameter × 0.15 μm film, Agilent, California, USA) as described previously [15,16]. Commercially available standards (Nu-Chek Prep, Minnesota, USA) were used to identify individual fatty acids. Intra-assay coefficients of variation for DHA, DPA, EPA, and ALA were 11.4%, 8.1%, 14.6%, and 9.9%, respectively. Individual erythrocyte fatty acids were expressed as relative concentration (%) among the total fatty acids.

2.3. Measurement of dietary intake and other covariates

At baseline, socio-demographic factors, lifestyle and dietary factors, and medical history information were all gathered by questionnaire during face-to-face interviews. Habitual dietary intakes over the past 12 months were assessed by a validated food frequency questionnaire, as previously described in detail [17]. Dietary macronutrients (fat, protein and carbohydrate) were adjusted for total energy intake using the residual method [18]. Physical activity was assessed as total metabolic equivalent for task (MET) hours per day on the basis of a validated questionnaire for physical activity [19]. Anthropometric parameters, including weight, height, waist, and hip circumference, were measured by trained nurses at the site during the baseline interview.

Fasting venous blood samples were taken at each recruitment or follow-up visit. Serum low-density lipoprotein cholesterol and glucose were measured by colorimetric methods using a Roche cobas 8000 c702 automated analyzer (Roche Diagnostics GmbH, Shanghai, China). Intra-assay coefficients of variation (CV) were 3.1% for low-density lipoprotein cholesterol and 2.5% for glucose. High-performance liquid chromatography was used to measure glycated hemoglobin (HbA1c) using the Bole D-10 Hemoglobin A1c Program on a Bole D-10 Hemoglobin Testing System, and the intra-assay CV was 0.75%.

2.4. Statistical analysis

Statistical analysis was performed using Stata 14 (StataCorp, College Station, TX, USA). All erythrocyte fatty acid variables were winsorized using values representing the 1st and 99th percentiles of the distribution in the cohort. Difference in population characteristics between participants with and without follow-up information was examined by analysis of covariance (continuous variables) or chi-square test (categorical variables). Spearman correlation coefficients were calculated to examine the correlation between dietary intakes of fish and individual n-3PUFA with individual erythrocyte n-3PUFA.

As a primary analysis, we used Cox regression with age as the underlying timescale to estimate the HR and 95% CI for T2D comparing quartiles of each n-3 PUFA variable (total marine n-3 PUFA, DHA, DPA, EPA, and ALA) using three statistical models: model 1 included age (continuous, years), sex (men, women), BMI (continuous, kg/m^2) and ratio of waist to hip circumference (continuous); model 2, as model 1 + physical activity (quintiles 1–5, based on MET hours), education (middle school or lower, high school or professional college, university), alcohol drinking (current and non-current drinker), smoking (current and non-current smoker), household income (≤500, 501–1500, 1501–3000, >3000 Chinese Yuan/month/person), family history of diabetes (yes, no), total energy intake (continuous, kcal/d) and dietary intake (all diet variables: in quartiles) of dairy products, red and processed meat, fruits and

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vegetables; model 3, as model 2 + fasting serum glucose (continuous, mmol/L) and erythrocyte total n-6 PUFA (continuous, mmol/L). P-trend was estimated based on per-quartile increase in the corresponding fatty acid. We performed several sensitivity analyses based on the above model 3 to examine the robustness of the results (3a): excluded participants with less than one year of follow-up in order to assess the potential influence of reverse causality (3b); included serum LDL-C as an additional covariate as to assess the potential influence of blood lipids (3c); included additional dietary variables (total fat intake, coffee, fruit juice, and tea) as covariates.

We used a restricted cubic spline model with 3 knots (at 10th, 50th, and 90th) [20] to explore the shape of the association between erythrocyte n-3 PUFA and incident T2D, adjusting for the covariates as in model 3. P-values for nonlinearity were calculated using a Wald test of the relevant parameter from the restricted cubic spline model. In order to allow comparison with the literature with per standard deviation (SD) estimation, HRs (95% CI) of T2D per SD increase in the individual erythrocyte n-3 PUFA were also estimated in Cox regression models, adjusted for potential confounders (i.e., model 3).

As secondary analyses, we examined the HRs (95% CI) comparing quartiles of dietary fish and n-3 PUFA intake with T2D risk using the same models (model 1 to model 3) used in the above primary analyses. We also examined the HRs (95% CI) of T2D by quartiles of total erythrocyte n-3/n-6 PUFA ratio and erythrocyte EPA/AA ratio to investigate the association of the ratio with the T2D risk.

3. Results

The mean age and BMI of the study participants was 58 y (SD: 5.7 y) and 23.2 kg/m^2 (SD: 3.0 kg/m^2), respectively. The mean levels of erythrocyte DHA, DPA, EPA, and ALA (% total fatty acids) were 4.5 (SD: 1.29), 1.52 (SD: 0.39), 0.77 (SD: 0.57), and 0.09 (SD: 0.03), respectively. The population characteristics by quartiles of total erythrocyte marine n-3 PUFA and by ALA are presented in Table 1. Supplemental Table 1 presents population characteristics among participants with and without follow-up information. Participants lost to follow-up tended to be less physically active, consumed fewer vegetables, had higher serum glucose levels, and were less educated. Dietary fish intake was significantly ($P < 0.001$) positively correlated with erythrocyte DHA ($r = 0.19$), DPA ($r = 0.08$) and EPA ($r = 0.11$) (Supplemental Table 2).

There was no association between total or individual erythrocyte marine n-3 PUFA and T2D incidence in model 1 or model 2, adjusting for baseline socio-demographic, lifestyle, and dietary factors (Table 2). After further adjustment for baseline circulating biomarkers (fasting glucose and n-6 PUFA) in model 3, HRs (95% CI) of T2D at Q2, Q3, and Q4 compared with Q1 for erythrocyte DPA were 0.68 (0.47, 1.00), 0.77 (0.52, 1.15), and 0.63 (0.41, 0.95) (*P*-trend = 0.007), respectively. In addition, the highest quartile (Q4) of erythrocyte EPA, compared with Q1, was inversely associated with risk of incident T2D (HR: 0.57, 95% CI: 0.38, 0.86) (*P*-trend = 0.007). Erythrocyte ALA was not associated with incident T2D in any of the statistical models. Sensitivity analysis did not materially change the above risk estimates (Supplemental Table 3).

Restricted cubic spline models did not identify evidence of nonlinearity, and the shapes of the association between levels of individual erythrocyte n-3 PUFA and total marine n-3 PUFA and incident T2D are presented in Fig. 1 and Supplemental Fig. 2. A linear inverse association between erythrocyte EPA and incident T2D was noted in the multivariable-adjusted model (model 3) with per SD HR 0.83 (95% CI 0.71, 0.98).

Dietary intake of fish, ALA, and total or individual marine n-3 PUFA were not associated with incident T2D in the multivariable-adjusted model 3 (Table 3). Ratio of erythrocyte n-3/n-6 PUFA was not associated with incident T2D, while ratio of EPA/AA was

Table 1
Population characteristics by quartiles of erythrocyte n-3 polyunsaturated fatty acids[a].

	Erythrocyte marine n-3 PUFA (EPA + DPA + DHA)				Erythrocyte ALA			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Age, y	58.0 (5.4)	58 (5.7)	58.2 (6.1)	57.7 (5.7)	58.7 (6.1)	58.0 (6.0)	57.7 (5.6)	57.4 (5.2)
BMI, kg/m^2	23.3 (3.1)	23.3 (3.1)	23.1 (2.9)	23.0 (3.1)	23.4 (3.1)	23.4 (3.1)	23.1 (3.1)	22.9 (2.9)
Waist circumference, cm	83.0 (9.0)	83.0 (8.8)	82.5 (8.8)	82.4 (8.9)	83.5 (8.8)	83.2 (9.1)	81.9 (8.8)	82.1 (8.6)
Ratio of waist to hip circumference	0.89 (0.07)	0.89 (0.07)	0.89 (0.07)	0.89 (0.07)	0.90 (0.07)	0.89 (0.07)	0.88 (0.07)	0.89 (0.07)
Total energy intake, kcal/d	1832 (498)	1785 (502)	1742 (461)	1705 (458)	1789 (503)	1732 (474)	1744 (473)	1801 (475)
Physical activity, MET hours/d	42.7 (16)	41.4 (14.5)	40.7 (14.7)	41.5 (14.4)	41.0 (14.5)	41.1 (14.8)	42.4 (15.4)	41.7 (15)
Dairy intake, g/d	15.7 (13.2)	17.7 (15.2)	16.3 (14.5)	16.2 (14.7)	15.8 (14)	15.6 (12.8)	16.6 (15.4)	17.8 (15.3)
Red and processed meat intake, g/d	89.2 (55.7)	86.1 (59.0)	80.0 (48.0)	78.1 (48.5)	88.9 (54.8)	80.1 (49.9)	80.5 (51.4)	83.8 (55.6)
Vegetable intake, g/d	391.4 (277.5)	370.2 (172.3)	383 (257.7)	380.9 (165.4)	366.0 (183.0)	373 (252.5)	381.4 (197.4)	406.1 (253.5)
Fruit intake, g/d	149.6 (113.3)	149.9 (118.3)	143.5 (108.7)	150.7 (100.1)	140.2 (109.1)	146.3 (106.4)	147.2 (106.7)	160.2 (117.6)
Fish intake, g/d	51.4 (90.5)	52.7 (68.1)	52.1 (52)	65.3 (78.1)	55.7 (88.6)	56.2 (61.3)	56.8 (92.3)	53 (41.1)
Marine n-3 PUFA intake, g/d	0.06 (0.11)	0.06 (0.07)	0.07 (0.07)	0.09 (0.12)	0.07 (0.13)	0.07 (0.07)	0.07 (0.11)	0.07 (0.05)
Alpha-linolenic acid intake, g/d	0.84 (0.39)	0.86 (0.43)	0.81 (0.37)	0.81 (0.36)	0.80 (0.33)	0.83 (0.37)	0.84 (0.42)	0.85 (0.42)
Fasting blood glucose, mmol/L	4.6 (0.7)	4.8 (0.6)	4.7 (0.7)	4.7 (0.6)	4.7 (0.7)	4.7 (0.7)	4.6 (0.6)	4.6 (0.7)
Sex, % of women	67.8	69.4	68.1	74.8	59.9	71.0	72.4	76.8
Current alcohol drinking, %	6.8	5.8	6.8	6.6	9.0	4.1	7.4	5.6
Current smoking status, %	17.6	14.2	16.5	11.9	22.1	12.9	13.7	11.5
Family history of diabetes, %	10.0	10.6	10.1	10.6	8.7	11.0	10.2	11.4
Household income (Chinese Yuan/month/person), %[b]								
≤500	2.5	2.0	1.5	2.1	1.1	2.6	2.4	2.0
501–1500	26.6	28.0	26.3	24	25.2	25.5	26.8	27.3
1501–3000	51.6	56.8	56.8	60.6	58	57.2	55.9	54.6
>3000	19.4	13.2	15.5	13.3	15.8	14.7	14.9	16.1
Education, %								
Middle school or lower	30.1	27.5	27.9	28.3	28.7	29.3	27.4	28.5
High school or professional college	43.1	48.5	46.4	48.4	44.1	46.8	46.8	48.6
University	26.7	24.0	25.7	23.3	27.2	23.9	25.8	22.9

[a] Total number of participants included in the present study was 2671. Abbreviation: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; PUFA, polyunsaturated fatty acids; Q, quartile. The median levels for quartiles 1–4 of marine n-3 PUFA were 4.78%, 6.45%, 7.46%, and 8.72%, and were 0.06%, 0.07%, 0.09%, and 0.12% for quartiles 1–4 of ALA.

[b] 1 Chinese Yuan corresponds to around 0.16 US dollar.

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Table 2
Association of erythrocyte n-3 fatty acids with incident type 2 diabetes[a].

Erythrocyte fatty acids		Multivariable-adjusted hazard ratio (95% CI)[b]				
		Q1	Q2	Q3	Q4	P-trend
Marine n-3 PUFA (DHA + DPA + EPA)	Median, %	4.78	6.45	7.46	8.72	
	No. of cases/person-years of follow-up	59/3568	49/3252	56/3285	49/3345	
	Model 1	1 (ref)	0.89 (0.61, 1.30)	1.04 (0.72, 1.51)	0.92 (0.63, 1.34)	0.87
	Model 2	1 (ref)	0.89 (0.61, 1.29)	1.04 (0.72, 1.50)	0.93 (0.63, 1.37)	0.93
	Model 3	1 (ref)	0.74 (0.49, 1.11)	0.89 (0.60, 1.32)	0.96 (0.63, 1.45)	0.92
DHA	Median, %	3.03	4.18	4.96	5.92	
	No. of cases/person-years of follow-up	53/3497	61/3338	46/3128	53/3486	
	Model 1	1 (ref)	1.17 (0.81, 1.70)	0.98 (0.66, 1.46)	1.13 (0.77, 1.66)	0.75
	Model 2	1 (ref)	1.16 (0.80, 1.68)	0.98 (0.66, 1.46)	1.15 (0.78, 1.68)	0.69
	Model 3	1 (ref)	1.15 (0.77, 1.73)	1.05 (0.68, 1.61)	1.52 (0.99, 2.34)	0.10
DPA	Median, %	1.11	1.45	1.65	1.9	
	No. of cases/person-years of follow-up	64/3554	53/3416	52/3189	44/3290	
	Model 1	1 (ref)	0.88 (0.61, 1.26)	0.91 (0.63, 1.31)	0.76 (0.52, 1.11)	0.19
	Model 2	1 (ref)	0.85 (0.59, 1.22)	0.94 (0.65, 1.36)	0.78 (0.53, 1.14)	0.29
	Model 3	1 (ref)	0.68 (0.47, 1.00)	0.77 (0.52, 1.15)	0.63 (0.41, 0.95)	0.07
EPA	Median, %	0.26	0.44	0.7	1.61	
	No. of cases/person-years of follow-up	55/3525	55/3414	56/3479	47/3032	
	Model 1	1 (ref)	1.02 (0.70, 1.48)	1.03 (0.71, 1.50)	0.84 (0.57, 1.25)	0.44
	Model 2	1 (ref)	1.00 (0.69, 1.46)	1.01 (0.69, 1.47)	0.83 (0.56, 1.23)	0.39
	Model 3	1 (ref)	1.08 (0.74, 1.60)	1.03 (0.70, 1.51)	0.57 (0.38, 0.86)	0.007
ALA	Median, %	0.06	0.07	0.09	0.12	
	No. of cases/person-years of follow-up	52/3245	57/3368	53/3392	51/3445	
	Model 1	1 (ref)	1.08 (0.73, 1.58)	1.07 (0.73, 1.57)	1.04 (0.70, 1.55)	0.85
	Model 2	1 (ref)	1.04 (0.70, 1.53)	1.05 (0.71, 1.54)	1.07 (0.71, 1.61)	0.74
	Model 3	1 (ref)	1.00 (0.67, 1.48)	1.17 (0.80, 1.72)	1.07 (0.72, 1.60)	0.57

[a] Abbreviations: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; PUFA, polyunsaturated fatty acids; Q, quartile.
[b] Multivariable-adjusted hazard ratios (95% CI) were calculated for quintiles 2 to 4 of the erythrocyte n-3 fatty acids, compared with quintile 1. Model 1 was adjusted for age, sex, BMI, and ratio of waist to hip circumference; model 2 included covariates in model 1 + physical activity, education, alcohol drinking, smoking, household income, family history of diabetes, total energy intake, and intake of dairy products, red and processed meat, fruits and vegetables; model 3 included covariates in model 2 + fasting serum glucose and erythrocyte total n-6 PUFA. P-trend was estimated based on per quartile increase in the corresponding fatty acid.

inversely associated with the risk with HRs (95% CI) 0.82, 0.93, and 0.49 at Q2, Q3, and Q4 (*P*-trend = 0.002), respectively (Supplemental Table 4).

4. Discussion

The results of the present prospective cohort study among a community-based Chinese population suggest that levels of erythrocyte marine n-3 PUFA: DPA and EPA were inversely associated with risk of incident T2D, while there was no association for erythrocyte DHA or ALA.

A few decades ago, ecological data consistently suggested low prevalence of T2D among populations with high consumption of fish and marine n-3 PUFA, which was especially true in Eskimos [21]. The beneficial role of marine n-3 PUFA in insulin secretion, insulin sensitivity, and T2D has been hypothesized [21]. However, the findings of studies examining a prospective association of dietary fish and marine n-3 PUFA with T2D have been inconsistent. For example, dietary marine n-3 PUFA was not associated with incident T2D in the Iowa Women's Health Study [22], the Cardiovascular Health Study [23], or the Rotterdam Study [24]. In the Shanghai Women's Health Study, higher intake of marine n-3 PUFA was associated with a lower risk of incident T2D [25]. In contrast, a positive association between marine n-3 PUFA intake and T2D was reported in the Nurses' Health Study and Nurses' Health Study 2 [26]. The above evidence was systematically reviewed by several independent groups between 2012 and 2013 [2,4,5], and the meta-analyzed results suggested that overall there was no association between marine n-3 PUFA intake and T2D, with huge heterogeneity by geographical region: an inverse association in Asian populations, null or positive associations in US or European populations, and the results regarding fish intake and T2D were consistent with those of marine n-3 PUFA.

We did not find a significant association of dietary marine n-3 PUFA with T2D in the present study. However, the effect size of the present study was very similar to that of the Shanghai Women's Health Study (relative risk: 0.84 at quintile 5 versus quintile 1) [25], and both suggested an inverse association of dietary marine n-3 PUFA with T2D risk. In addition, the median dietary intake of marine n-3 PUFAs in the above study in Shanghai (0.07 g/d) was very close to that found in the present study (0.05 g/d). The non-significant results in the present study might be because of the relatively moderate sample size (corresponded to a wider confidence interval) compared with the previous study in Shanghai [25].

Objective measurements of n-3 PUFA in blood (including erythrocyte, plasma, serum and whole blood, or related lipid fractions) have been widely adopted by the scientific community as biomarkers of dietary n-3 PUFA intake [27]. However, due to the high cost and time-consuming nature of blood fatty acid measurement, only a few prospective studies have reported the associations between blood n-3 PUFA biomarkers and T2D incidence [8–12,23,28,29]. In the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct study and a comparative meta-analysis of studies in the literature, there was no significant association of DHA or EPA with incident T2D [7]. The above EPIC-InterAct study, as well as the studies identified in the systematic review within the same paper, was based exclusively on Western populations. Meanwhile the prospective evidence from Asia is rare, with only one nested case-control study (336 T2D cases) in Japan published very recently, reporting a null association [30]. Therefore,

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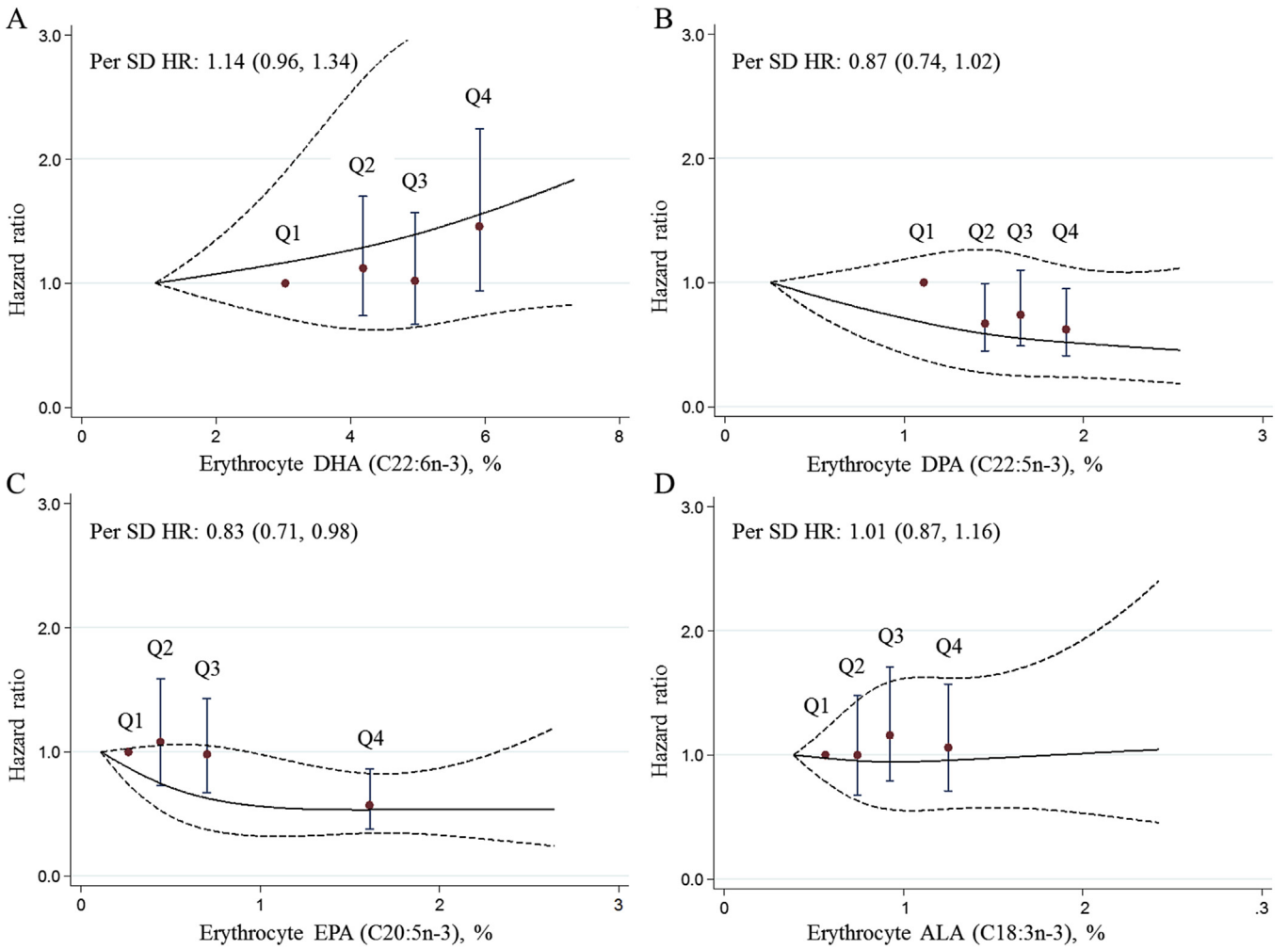

Fig. 1. Shape of the association between individual erythrocyte n-3 polyunsaturated fatty acids and incidence of type 2 diabetes. Restricted cubic spline functions were used to estimate the association between individual erythrocyte n-3 polyunsaturated fatty acids and incident type 2 diabetes in the Cox regression models, adjusted for potential confounders (i.e. Model 3). Panels A, B, C and D represented results for erythrocyte DHA, DPA, EPA and ALA respectively. None of the associations showed non-linearity ($P > 0.1$). Hazard ratios (95% CI) of type 2 diabetes per SD increase in the individual erythrocyte n-3 polyunsaturated fatty acids were also estimated in Cox regression models, adjusted for potential confounders (i.e., Model 3). In addition, to make the dose-response association comparable with the quartile results, we also plotted the hazard ratio (95% CI) of type 2 diabetes across quartiles 2–4 of the n-3 polyunsaturated fatty acids (quartile 1 as reference, location of each plot corresponds to the median levels within each quartile). EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; Q, quartile.

our present study makes a valuable contribution to the sparse literature investigating the prospective association between blood n-3 PUFA biomarkers and T2D in Asians. The reason for the inconsistencies between the results from our cohort and Western cohorts might be because levels of marine n-3 PUFA (median: DPA 1.54% and EPA 0.55%) in our cohort were lower than those found in Western cohorts using measurement of erythrocyte or erythrocyte phospholipid fatty acids (median: DPA >2.2%, EPA>0.75%) [8,10,12]. Though a potential threshold effect/non-linear association might exist, we did not observe such association in our study. Another possibility is the influence of covariate adjustment. The inverse association between T2D and DPA/EPA was observed after adjustment for circulating fasting glucose and n-6 PUFA. This suggests that baseline glycemic traits and n-6 PUFA context may have confounded the n-3 PUFA and T2D associations, which were rarely considered or adjusted for in prior studies [7].

Although we observed an inverse association between T2D and EPA/DPA, there was no association for DHA. In addition, the results for EPA were consistent with the ratio of EPA/AA. Indeed, compared with DHA, EPA has a stronger anti-inflammatory effect [31], through which EPA might be linked with a lower risk of T2D [32]. EPA competes with AA for access to cyclooxygenase and produces an alternative form of thromboxane (TXA_3) and prostaglandins (PGI_3), leading to reduced production of TXA_2 and PGI_2, important pro-inflammatory eicosanoids [31,33]. In contrast to DHA and EPA, DPA is a less well-investigated marine n-3 PUFA. Available evidence suggests that DPA could inhibit the production of inflammatory eicosanoids by competing with AA for the cyclooxygenase [34]. Nevertheless, the detailed mechanism underlying the effect of DPA and EPA on glucose metabolism and glycemic traits remains unclear and warrants further investigation.

We did not find a significant association between T2D and erythrocyte ALA. This result conflicts with previous findings from the EPIC-InterAct and its comparative meta-analysis that circulating ALA was inversely associated with T2D [7]. The reason for this inconsistency is unclear. Nevertheless, among studies using erythrocyte or erythrocyte phospholipid fatty acids as biomarkers [8,10,12], none has found a statistically significant association between ALA and T2D, which is consistent with our results.

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Table 3
Association of dietary fish and n-3 fatty acids with incident type 2 diabetes[a].

Dietary intake		Multivariable-adjusted hazard ratio (95% CI)[b]				
		Q1	Q2	Q3	Q4	p-trend
Fish	Median, g/d	15.5	32.4	52.7	92.9	
	No. of cases/person-years of follow-up	59/3152	52/3196	49/3525	53/3577	
	Model 1	1 (ref)	0.90 (0.62, 1.31)	0.75 (0.51, 1.11)	0.84 (0.58, 1.22)	0.26
	Model 2	1 (ref)	0.91 (0.62, 1.35)	0.76 (0.51, 1.14)	0.85 (0.55, 1.30)	0.33
	Model 3	1 (ref)	0.85 (0.57, 1.28)	0.76 (0.51, 1.15)	0.94 (0.60, 1.45)	0.63
Marine n-3 PUFA	Median, g/d	0.021	0.042	0.068	0.12	
	No. of cases/person-years of follow-up	57/3356	50/3229	56/3388	50/3477	
	Model 1	1 (ref)	0.87 (0.59, 1.29)	0.98 (0.68, 1.42)	0.83 (0.57, 1.21)	0.46
	Model 2	1 (ref)	0.83 (0.56, 1.23)	0.97 (0.66, 1.42)	0.76 (0.51, 1.15)	0.32
	Model 3	1 (ref)	0.75 (0.50, 1.14)	0.87 (0.59, 1.29)	0.78 (0.52, 1.19)	0.39
DHA	Median, g/d	0.011	0.024	0.039	0.067	
	No. of cases/person-years of follow-up	58/3335	49/3265	58/3378	48/3472	
	Model 1	1 (ref)	0.86 (0.59, 1.27)	0.97 (0.67, 1.41)	0.80 (0.54, 1.18)	0.37
	Model 2	1 (ref)	0.82 (0.56, 1.22)	0.95 (0.65, 1.39)	0.73 (0.48, 1.10)	0.22
	Model 3	1 (ref)	0.77 (0.51, 1.17)	0.87 (0.59, 1.30)	0.74 (0.49, 1.13)	0.26
DPA	Median, g/d	<0.001	0.002	0.004	0.01	
	No. of cases/person-years of follow-up	51/3404	56/3402	39/3205	67/3440	
	Model 1	1 (ref)	1.14 (0.78, 1.66)	0.85 (0.56, 1.28)	1.36 (0.94, 1.95)	0.25
	Model 2	1 (ref)	1.10 (0.75, 1.62)	0.83 (0.54, 1.26)	1.34 (0.92, 1.95)	0.29
	Model 3	1 (ref)	1.01 (0.67, 1.50)	0.61 (0.39, 0.95)	1.08 (0.74, 1.57)	0.84
EPA	Median, g/d	0.008	0.016	0.025	0.042	
	No. of cases/person-years of follow-up	56/3354	50/3235	57/3388	50/3473	
	Model 1	1 (ref)	0.86 (0.58, 1.26)	0.97 (0.67, 1.41)	0.85 (0.58, 1.25)	0.57
	Model 2	1 (ref)	0.81 (0.55, 1.20)	0.93 (0.63, 1.37)	0.78 (0.52, 1.18)	0.36
	Model 3	1 (ref)	0.77 (0.51, 1.15)	0.85 (0.57, 1.27)	0.76 (0.50, 1.16)	0.30
ALA	Median, g/d	0.49	0.66	0.84	1.19	
	No. of cases/person-years of follow-up	40/3366	57/3333	50/3420	66/3330	
	Model 1	1 (ref)	1.38 (0.92, 2.07)	1.23 (0.80, 1.87)	1.62 (1.08, 2.41)	0.04
	Model 2	1 (ref)	1.40 (0.92, 2.12)	1.23 (0.80, 1.90)	1.67 (1.11, 2.52)	0.033
	Model 3	1 (ref)	1.37 (0.90, 2.09)	1.11 (0.72, 1.71)	1.53 (1.01, 2.33)	0.12

[a] Abbreviations: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; PUFA, polyunsaturated fatty acids; Q, quartile.
[b] Multivariable-adjusted hazard ratios (95% CI) were calculated for quintiles 2 to 4 of the dietary fish or n-3 fatty acid intake, compared with quintile 1. Model 1 was adjusted for age, sex, BMI, and ratio of waist to hip circumference; model 2 included covariates in model 1 + physical activity, education, alcohol drinking, smoking, household income, family history of diabetes, total energy intake, and intake of dairy products, red and processed meat, fruits and vegetables; model 3 included covariates in model 2 + fasting blood glucose and erythrocyte total n-6 PUFA. P-trend was estimated based on per quartile increase in the corresponding fatty acid.

There are several limitations in the present study. First, we measured erythrocyte fatty acids only in baseline samples and did not account for potential changes in n-3 PUFA composition over time. Second, given the nature of an observational study, we could not avoid the influence of residual confounders. Third, although this is the largest study among Chinese populations with fatty acid biomarkers and sufficient incident T2D cases, we included only 213 incident T2D cases over a relatively moderate follow-up time. Fourth, we measured erythrocyte fatty acids in a Chinese population, and the generalizability of our results to other lipid fractions or populations is limited. The strengths of the present study include the use of objective biomarkers for the measurement of n-3 PUFA, and adjustment for a variety of risk factors and several sensitivity analyses, which support the robustness of our findings.

5. Conclusions

We offer evidence that erythrocyte EPA and DPA, as biomarkers of dietary marine n-3 PUFA intake, are associated with lower incidence of T2D. The current results regarding erythrocyte biomarkers add to the evidence that dietary intake of marine n-3 PUFA is associated with lower risk of T2D in Asian populations. Detailed investigation of the potential causality of total and individual marine n-3 PUFA in T2D etiology is warranted, using a Mendelian randomization approach in observational studies or randomized controlled trials, especially among Asian participants.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.09.018.

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