British Journal of Nutrition (2024), 131, 103–112 doi:10.1017/S0007114523001447

Erythrocyte membrane *n*-3 PUFA are inversely associated with breast cancer risk among Chinese women

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(Submitted 11 April 2023 – Final revision received 31 May 2023 – Accepted 23 June 2023 – First published online 29 June 2023)

Abstract

The relationship between erythrocyte membrane *n*-3 PUFA and breast cancer risk is controversial. We aimed to examine the associations of erythrocyte membrane *n*-3 PUFA with odds of breast cancer among Chinese women by using a relatively large sample size. A case–control study was conducted including 853 newly diagnosed, histologically confirmed breast cancer cases and 892 frequency-matched controls (5-year interval). Erythrocyte membrane *n*-3 PUFA were measured by GC. Logistic regression and restricted cubic spline were used to quantify the association between erythrocyte membrane *n*-3 PUFA and odds of breast cancer. Erythrocyte membrane *α*-linolenic acid (ALA), docosapentaenoic acid (DPA) and total *n*-3 PUFA were inversely and non-linearly associated with odds of breast cancer. The OR values (95 % CI), comparing the highest with the lowest quartile (Q), were 0·57 (0·43, 0·76), 0·43 (0·32, 0·58) and 0·36 (0·27, 0·49) for ALA, DPA and total *n*-3 PUFA, respectively. Erythrocyte membrane EPA and DHA were linearly and inversely associated with odds of breast cancer ((EPA: $OR_{Q4\ v.\ Q1}$ (95 % CI) = 0·59 (0·45, 0·79); DHA: $OR_{Q4\ v.\ Q1}$ (95 % CI) = 0·50 (0·37, 0·67)). The inverse associations were observed between ALA and odds of breast cancer in postmenopausal women, and between DHA and oestrogen receptor+ breast cancer. This study showed that erythrocyte membrane total and individual *n*-3 PUFA were inversely associated with odds of breast cancer. Other factors, such as menopause and hormone receptor status, may warrant further investigation when examining the association between *n*-3 PUFA and odds of breast cancer.

Key words: ***n*-3 PUFA: DHA: EPA: Breast cancer: Erythrocyte**

Breast cancer is one of the most common cancers affecting women worldwide, with an age-standardised incidence rate of 47·8 cases per 100 000 women[1]. Based on the estimate from the WHO, 416 371 women were newly diagnosed with breast cancer in China in 2020[2]. Several lifestyle factors, including a healthy diet, impact breast cancer risk[3,4]. Over the past few decades, interest has increased in the associations between fatty acids and carcinogenesis, particularly *n*-3 PUFA. Accumulated animal and *in vitro* experimental evidence has indicated that *n*-3 PUFA inhibit the pathogenesis and progression of breast cancer[5,6].

Erythrocyte membrane *n*-3 PUFA as a biomarker of *n*-3 PUFA status[7,8] reflects dietary intake over several months and represents an integrative measure of the interaction concerning dietary, metabolic and genetic factors[9]. Despite growing experimental evidence supporting the anti-tumourigenic effects of *n*-3 PUFA, the epidemiological evidence on the potential protective role of *n*-3 PUFA in breast cancer risk reduction has been inconclusive. Thus far, only two case–control studies[10,11] and three nested case–control studies[12–14] have evaluated erythrocyte membrane *n*-3 PUFA in relation to the risk of breast cancer and the results remain inconsistent. Inverse associations of erythrocyte membrane *n*-3 PUFA with the odds of breast cancer were observed in a case–control study from Japan[10], a case–control study from China[11] and a nested case–control study from Italy[12]. Other nested case–control studies from the USA and Europe found no significant relationships[13,14]. Considering the vast differences in the

Abbreviations: ALA, *α*-linolenic acid; DPA, docosapentaenoic acid; ER, oestrogen receptor; PR, progesterone receptor.

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dietary habits and intake pattern between the Chinese and Western populations, and the changes over time, the relationship between *n*-3 PUFA and risk of breast cancer in Chinese women still demands investigation.

We aimed to investigate the associations of individual and total erythrocyte membrane *n*-3 PUFA with the odds of being a breast cancer patient among Chinese women. We hypothesised that higher levels of erythrocyte membrane total *n*-3 PUFA and individual *n*-3 PUFA were inversely associated with odds of breast cancer.

Methods

Study population

The detailed study methods for this hospital-based case–control study have been published elsewhere[15]. Briefly, eligible breast cancer cases were recruited from Sun Yat-sen University First Affiliated Hospital and Guangdong Women and Children Hospital from September 2011 to December 2019. The inclusion criteria of the breast cancer cases were as follows: (1) aged between 25 and 70 years, (2) histologically confirmed breast cancer and diagnosed within 3 months before the interview and (3) Guangdong natives or residing in Guangdong for at least 5 years. Participants who could not understand or speak Mandarin or Cantonese were excluded. Overall, 1675 of 1882 eligible cases were recruited, with a participation rate of 89·00 %. Among them, 869 provided blood samples. Finally, 853 cases were included in the analysis after excluding sixteen participants who had insufficient blood samples for analysis.

Control subjects were recruited from other departments of the same hospitals during the same period. The same inclusion criteria were applied for the control subjects, and they must have no history of cancer. Finally, we identified 1932 eligible control subjects, of whom 1749 were successfully interviewed (with a participation rate of 90·53 %) and 1169 provided blood samples. Among them, 892 control subjects were frequency matched to the cases by 5-year age intervals and completed the laboratory analysis.

The study was approved by the Ethical Boards of the School of Public Health, Sun Yat-sen University (approval number: 2011–18) and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Data collection

A face-to-face interview was conducted by trained interviewers to collect information on potential confounding factors, including demographic characteristics, prior history of disease, menstrual and reproductive history, anthropometric measurements and lifestyle factors. A FFQ with eighty-one food items[16] was used to collect information on dietary intake. Total energy intake was assessed according to the China Food Composition Table[17]. The BMI was calculated as the current body weight (kg) divided by the height squared (m^2). The metabolic equivalent (MET) hours per day was used to estimate physical activity, and the detailed methods of calculating MET have been described previously[18].

Information on the oestrogen receptor (ER) status was available for 803 cases (94·14 %); among these, 581 cases were ER+ and 222 cases were ER−. Information on the progesterone receptor (PR) status was available for 800 cases (93·79 %); among these, 494 cases were PR+ and 306 cases were PR−.

Laboratory analysis of erythrocyte membrane n-3 PUFA

Venous blood samples were obtained following an overnight fasting period (8–12 h) on the second day of the participants' admission and collected in EDTA tubes. The breast cancer cases and control subjects did not receive medication or surgery before blood collection. Erythrocytes were washed and separated within 2 h of collection and were stored at −80°C for subsequent analysis. The fatty acid compositions in the erythrocytes were measured by GC[19]. The extraction of fatty acids was conducted with 100 μl erythrocyte sample and 750 μl chloroform/methanol (2:1, v/v)[20] and transmethylation with 750 μl 14 % boron-trifluoride ether/methanol (1:3, v/v) solution[21] for 60 min at 90°C. The fatty acid methyl esters were analysed using an Agilent 7890A GC system (Agilent) equipped with a DB-23 capillary column (60 m × 0·25 mm internal diameter × 0·15 μm film; Agilent) and a flame ionisation detector. Considering nitrogen as carrier gas, the GC method included a split ratio of 5:1 with the injection temperature at 250°C. The oven temperature started at 50°C for 1 min and was programmed from 50 to 185°C at a rate of 25°C/min followed by a 5-min hold period, and the temperature was continuously increased to 230°C at a rate of 2·4°C/min followed by a 20-min hold period. Comparing the retention time of the samples with commercially available standards to identify individual fatty acids, the amount of each fatty acid was expressed as a percentage of the total erythrocyte membrane fatty acids (relative, %). The following *n*-3 PUFA was included in the analysis: *α*-linolenic acid (ALA), EPA, docosapentaenoic acid (DPA), DHA and total *n*-3 PUFA. Intra-assay and inter-assay CV were determined using replicate assays of samples on the same day (*n* 12–16) and different days (*n* 100), respectively. The intra-assay CV were 5·37 %, 6·42 %, 6·05 % and 4·56 % for ALA, EPA, DPA and DHA, respectively. The inter-assay CV were < 20 % for all measured fatty acids.

Statistical analysis

Differences in characteristics between the case and control groups were examined using the Wilcoxon rank-sum test for continuous variables with skewed distribution and χ^2 test for categorical variables. Logistic regression analysis was used to examine the association between erythrocyte membrane *n*-3 PUFA and odds of breast cancer, and then the OR and 95 % CI were estimated. We classified the participants' erythrocyte membrane *n*-3 PUFA into quartiles (Q) based on the proportion of *n*-3 PUFA in control subjects and assumed that the median values of each quartile were continuous variables to perform the *P*-value for trend test.

Multivariable regression models were applied to adjust for confounding factors, and covariates were selected based on preliminary analyses and the literature[22,23], such as age, educational level, BMI, passive smoking, alcohol drinking, physical activity, age at menarche, history of breast cancer in

British Journal of Nutrition

first-degree relatives, history of benign breast disease and total energy intake. The level of erythrocyte membrane total *n*-6 PUFA was further adjusted as a covariate. In addition, we performed restricted cubic spline[24] with knots placed at the 5th, 35th, 65th and 95th percentiles to reveal the potential non-linear relationship between erythrocyte membrane *n*-3 PUFA and odds of breast cancer.

We conducted stratified analysis by menopausal status (pre- *v.* postmenopausal women). Stratified analysis by BMI ($< 24\ kg/m^2$ *v.* $\geq 24\ kg/m^2$) was also performed to examine the consistency of overall associations. Additionally, we considered the potential heterogeneity of tumour subtypes, defined by hormone receptors (ER + *v.* ER−; PR + *v.* PR−). The Wald test was used to test for heterogeneity, comparing the coefficients across cancer subtypes.

All the analyses were performed using SPSS version 22.0 (SPSS; IBM) and STATA version 15.0 (StataCorp). A two-sided *P* value < 0·05 was considered statistically significant.

Results

Participant characteristics

A detailed comparison of characteristics between the case and control subjects is shown in Table 1. Compared with the control subjects, the cases had higher BMI values, lower energy intake, lower educational level and participated in lower levels of physical activity. Additionally, the cases were more likely to drink alcohol, to be passive smokers, to be younger at menarche, to have a history of breast cancer in first-degree relatives and benign breast disease than the control subjects. The levels of erythrocyte membrane ALA, EPA, DPA, DHA and total *n*-3 PUFA were significantly lower in cases than in control subjects.

Associations between erythrocyte membrane n-3 PUFA and odds of breast cancer

As shown in Table 2, after adjustment for potential confounding factors, individual and total erythrocyte membrane *n*-3 PUFA were inversely associated with the odds of breast cancer (all $P_{trend} < 0{\cdot}001$). Compared with the lowest quartile (Q1) of erythrocyte membrane ALA, the highest quartile (Q4) had a 43 % reduction in the odds of breast cancer. Likewise, compared with Q1 of erythrocyte membrane EPA, DPA, DHA and total *n*-3 PUFA, Q4 had, respectively, a 40 %, 51 %, 47 % and 61 % reduction in the odds of breast cancer. These associations were unaltered after further adjusting for erythrocyte membrane total *n*-6 PUFA.

Dose–response relationships between erythrocyte membrane n-3 PUFA and odds of breast cancer

Figure 1 demonstrates the non-linear association between erythrocyte membrane *n*-3 PUFA and odds of breast cancer using restricted cubic spline. Non-linear associations of ALA, DPA and total *n*-3 PUFA with odds of breast cancer were observed ($P_{non\text{-}linear} < 0{\cdot}05$). Overall, odds of having breast cancer decreased with increased erythrocyte membrane EPA and DHA ($P_{non\text{-}linear} > 0{\cdot}05$). Specifically, the odds of breast cancer decreased rapidly as erythrocyte membrane ALA increased to 0·62 % and increased slowly above 0·62 %. However, the increased odds of breast cancer were not significant and may be driven by a wide 95 % CI when erythrocyte membrane ALA above 0·91 %. We also noted a similar pattern between odds of breast cancer and erythrocyte membrane DPA, with the cut-off point of DPA of 1·82 %.

Associations of erythrocyte membrane n-3 PUFA with odds of breast cancer stratified by menopausal status

Stratification by menopausal status showed that the associations of erythrocyte membrane total and individual *n*-3 PUFA with odds of breast cancer were similar to the overall association, except for erythrocyte membrane ALA. The decreased odds of breast cancer in association with increasing erythrocyte membrane ALA appeared in postmenopausal women ($OR_{Q4\ v.\ Q1} = 0{\cdot}30$; 95 % CI 0·18, 0·50; $P_{trend} < 0{\cdot}001$) but not in premenopausal women ($OR_{Q4\ v.\ Q1} = 0{\cdot}80$; 95 % CI 0·56, 1·13; $P_{trend} = 0{\cdot}094$; $P_{interaction} = 0{\cdot}015$; see Table 3).

Associations of erythrocyte membrane n-3 PUFA with odds of breast cancer stratified by BMI

Stratified analysis according to BMI showed that the levels of erythrocyte membrane ALA, EPA, DPA, DHA and total *n*-3 PUFA were consistently inversely associated with odds of breast cancer in BMI-stratified models (all $P_{interaction} > 0{\cdot}05$; see Fig. 2).

Associations of erythrocyte membrane n-3 PUFA with odds of breast cancer stratified by oestrogen receptor and progesterone receptor status

Subgroup analysis by hormone receptor status (ER and PR) showed consistent associations with the overall association. Notably, women with higher proportions of erythrocyte membrane DHA had a lower likelihood of being ER+ breast cancer patient than ER− breast cancer (ER+: $OR_{Q4\ v.\ Q1} = 0{\cdot}46$; 95 % CI 0·33, 0·63; ER−: $OR_{Q4\ v.\ Q1} = 0{\cdot}68$; 95 % CI 0·44, 1·06; see Table 4), but the *P* value for the test of heterogeneity was not statistically significant ($P_{heterogeneity} = 0{\cdot}167$).

Discussion

This study aimed to evaluate the relationships between erythrocyte membrane *n*-3 PUFA and the odds of being a breast cancer patient. Higher erythrocyte membrane individual and total *n*-3 PUFA were inversely associated with the odds of breast cancer. Additionally, our findings suggested significant non-linear relationships between erythrocyte membrane ALA, DPA, total *n*-3 PUFA and odds of breast cancer.

Most previous studies used plasma[25–29] or serum[30–32] *n*-3 PUFA as biomarkers to evaluate the association of *n*-3 PUFA with breast cancer risk. Although one study from plasma[28] supports our findings, most studies using plasma[25–27,29] or serum[30–32] *n*-3 PUFA as biomarkers reported null findings on the relationship between *n*-3 PUFA and breast cancer risk. Compared with the plasma or serum fatty acid, the erythrocyte membrane fatty acid profile shows a longer time for fatty acid intake, approximately

British Journal of Nutrition

Table 1. Characteristics, erythrocyte membrane levels of *n*-3 PUFA of breast cancer cases and their matched controls (Numbers and percentages)

	Cases (*n* 853)		Controls (*n* 892)		*P**
Variables	*n*	%	*n*	%	
Age (years)					
Median		47·98		47·81	0·701
P_{25}, P_{75}		41·85, 55·52		41·56, 55·42	
BMI (kg/m^2)					
Median		22·89		22·14	< 0·001
P_{25}, P_{75}		20·95, 25·08		20·27, 24·23	
Physical activity (MET-h/week)					
Median		35·63		43·75	0·001
P_{25}, P_{75}		15·75, 52·50		25·22, 52·50	
Occupation					0·703
Administrator/other white-collar workers	177	20·75	197	22·09	
Blue-collar worker	233	27·32	249	27·91	
Farmer/other	443	51·93	446	50·00	
Educational level (years)					0·001
≤ 6	211	24·74	238	26·68	
7–9	257	30·13	206	23·10	
10–12	198	23·21	196	21·97	
≥ 13	187	21·92	252	28·25	
Household income (RMB yuan/month)					0·264
≤ 2000	50	5·86	52	5·83	
2001–5000	257	30·13	232	26·01	
5001–8000	308	36·11	335	37·56	
≥ 8001	238	27·90	273	30·60	
	Median	P_{25}, P_{75}	Median	P_{25}, P_{75}	
Energy intake (kcal/d)	1430·68	1209·87, 1673·82	1465·73	1263·68, 1693·87	0·038
Erythrocyte membrane *n*-3 PUFA (% of total fatty acids)					
ALA	0·38	0·28, 0·50	0·42	0·32, 0·55	< 0·001
EPA	0·73	0·55, 1·03	0·81	0·59, 1·16	< 0·001
DPA	0·97	0·67, 1·38	1·13	0·80, 1·50	< 0·001
DHA	3·28	2·70, 3·69	3·44	2·95, 3·83	< 0·001
Total *n*-3 PUFA	5·79	4·98, 6·55	6·26	5·44, 7·03	< 0·001
Erythrocyte membrane total *n*-6 PUFA (% of total fatty acids)	25·39	22·94, 27·72	25·24	22·17, 27·64	0·256
	n	%	*n*	%	
Regular smoker	12	1·41	10	1·12	0·593
Passive smoker	463	54·28	340	38·12	< 0·001
Regular drinker	76	8·91	47	5·27	0·003
Age at menarche (years)					
Median		14·10		14·32	< 0·001
P_{25}, P_{75}		12·82, 15·51		13·48, 15·67	
Age at first live birth (years)†					
Median		25·09		25·22	0·615
P_{25}, P_{75}		22·89, 27·59		23·26, 27·45	
Number of pregnancies†					0·257
0	38	4·45	48	5·38	
1–2	623	73·04	669	75·00	
≥ 3	192	22·51	175	19·62	
Menopausal status					0·711
Premenopausal	539	63·19	556	62·33	
Postmenopausal	314	36·81	336	37·67	
Breast-feeding history†	705	82·65	751	84·19	0·386
Oral contraceptive therapy	50	5·86	45	5·04	0·452
History of breast cancer in first-degree relatives	25	2·93	9	1·01	0·004
History of benign breast disease	308	36·11	217	24·33	< 0·001

ALA, α-linolenic acid; DPA, docosapentaenoic acid.
* *P* value for between-group differences between cases and controls is based on Wilcoxon rank-sum test for continuous variables and χ^2 test for categorical variables.
† Among women with children.

4 months, and is not susceptible to the individual postprandial status[8,33]. Our observation of the inverse associations between erythrocyte membrane total and individual *n*-3 PUFA and breast cancer risk agreed with some previous study findings[10–12]. A case–control study of Japanese women showed that erythrocyte membrane EPA, DPA, DHA and total *n*-3 PUFA were inversely associated with odds of breast cancer[10]. Another case–control study from Shanghai, China noted that erythrocyte membrane EPA and total *n*-3 PUFA were inversely associated with odds of breast cancer[11]. By contrast, erythrocyte membrane total *n*-3 PUFA, EPA, DPA and DHA were not associated with odds of having breast cancer in two nested case–control studies within the Malm Diet Cancer cohort (Sweden)[13] and within the Nurses' Health Study II[14]. One study in Italy only observed a marginal

Table 2. Associations of the levels of erythrocyte membrane *n*-3 PUFA (% of total fatty acids) with odds of breast cancer by logistic regression (95 % confidence intervals)

Erythrocyte membrane *n*-3 PUFA	Cases/controls	Crude OR	95 % CI	Adjusted OR 1*	95 % CI	Adjusted OR 2†	95 % CI
ALA							
Q1 (< 0·32)	305/223	1·00 (ref.)		1·00 (ref.)		1·00 (ref.)	
Q2 (0·32–< 0·42)	217/223	0·71	0·55, 0·92	0·74	0·56, 0·96	0·74	0·56, 0·96
Q3 (0·42–< 0·55)	175/223	0·57	0·44, 0·75	0·56	0·42, 0·74	0·56	0·42, 0·74
Q4 (≥ 0·55)	156/223	0·51	0·39, 0·67	0·57	0·43, 0·75	0·57	0·43, 0·76
P_{trend}		< 0·001		< 0·001		< 0·001	
EPA							
Q1 (< 0·59)	262/223	1·00 (ref.)		1·00 (ref.)		1·00 (ref.)	
Q2 (0·59–< 0·81)	239/223	0·91	0·71, 1·18	0·93	0·71, 1·22	0·94	0·71, 1·23
Q3 (0·81–< 1·16)	192/223	0·73	0·56, 0·95	0·74	0·56, 0·97	0·73	0·56, 0·97
Q4 (≥ 1·16)	160/223	0·61	0·47, 0·80	0·60	0·45, 0·79	0·59	0·45, 0·79
P_{trend}		< 0·001		< 0·001		< 0·001	
DPA							
Q1 (< 0·80)	317/223	1·00 (ref.)		1·00 (ref.)		1·00 (ref.)	
Q2 (0·80–< 1·13)	206/223	0·65	0·50, 0·84	0·61	0·47, 0·80	0·57	0·43, 0·75
Q3 (1·13–< 1·50)	170/223	0·54	0·41, 0·70	0·50	0·38, 0·66	0·44	0·33, 0·58
Q4 (≥ 1·50)	160/223	0·50	0·39, 0·66	0·49	0·37, 0·65	0·43	0·32, 0·58
P_{trend}		< 0·001		< 0·001		< 0·001	
DHA							
Q1 (< 2·95)	287/223	1·00 (ref.)		1·00 (ref.)		1·00 (ref.)	
Q2 (2·95–< 3·44)	228/223	0·79	0·62, 1·02	0·77	0·59, 1·01	0·72	0·55, 0·95
Q3 (3·44–< 3·83)	177/223	0·62	0·47, 0·80	0·59	0·45, 0·79	0·55	0·42, 0·74
Q4 (≥ 3·83)	161/223	0·56	0·43, 0·73	0·53	0·40, 0·71	0·50	0·37, 0·67
P_{trend}		< 0·001		< 0·001		< 0·001	
Total *n*-3 PUFA							
Q1 (< 5·44)	324/223	1·00 (ref.)		1·00 (ref.)		1·00 (ref.)	
Q2 (5·44–< 6·26)	245/223	0·76	0·59, 0·97	0·76	0·58, 0·99	0·72	0·55, 0·94
Q3 (6·26–< 7·03)	155/223	0·48	0·37, 0·62	0·49	0·37, 0·64	0·45	0·34, 0·60
Q4 (≥ 7·03)	129/223	0·40	0·30, 0·52	0·39	0·29, 0·52	0·36	0·27, 0·49
P_{trend}		< 0·001		< 0·001		< 0·001	

ALA, α-linolenic acid; DPA, docosapentaenoic acid.
* Adjusted for age, educational level, BMI, passive smoking, alcohol drinking, physical activity, age at menarche, history of breast cancer in first-degree relatives, history of benign breast disease and total energy intake.
† Additionally adjusted for erythrocyte membrane total *n*-6 PUFA.

inverse relationship between erythrocyte membrane DHA and odds of breast cancer[12]. To our best knowledge, no study has reported that the erythrocyte membrane ALA is significantly associated with odds of breast cancer[10–14]. Our observation of an inverse association between the erythrocyte membrane ALA and odds of having breast cancer must be confirmed in further studies.

The conflicting results from different studies may be due to the variation in the contents of erythrocyte membrane *n*-3 PUFA (total and subclasses). Of the four studies mentioned above[11–14], the levels of erythrocyte membrane total *n*-3 PUFA were higher in the Shanghai[11], Italian[12] and Swedish[13] studies than in ours but were lower in the US study[14]. Regarding the individual levels of erythrocyte membrane *n*-3 PUFA, the EPA level was lower in three studies[11,12,14] than in ours except for the Swedish study[13]. The content of erythrocyte membrane DHA was higher in three studies[11–13] than in ours and except the US study[14]. Concerning the erythrocyte membrane DPA level, all the studies had higher levels than ours[11–14]. However, the Japanese study[10] revealed a similar median proportion of erythrocyte membrane EPA, DPA, DHA and total *n*-3 PUFA to ours, likely explaining the consistent results. Additionally, the relatively higher level of erythrocyte membrane ALA in our study compared with that in other studies may help explain our observed reduction of breast cancer with erythrocyte membrane ALA.

Our study demonstrated non-linear associations of the erythrocyte membrane ALA, DPA and total *n*-3 PUFA and odds of breast cancer. The findings revealed that the erythrocyte membrane ALA level of 0·48–0·91 % may provide the maximum benefit for odds of breast cancer. We also showed a similar pattern of erythrocyte membrane DPA at the level from 0·86 % to 4·85 %. Our observation of non-linear associations of erythrocyte membrane ALA, DPA and DHA with odds of breast cancer might also help to explain the inconsistent results across studies. Further investigation is warranted regarding the optimal level of erythrocyte membrane *n*-3 PUFA in different populations and the validation of cut-off points.

Possible mechanisms explaining the apparent benefit of *n*-3 PUFA on breast cancer risk are as follows. *n*-3 PUFA is precursor to a range of biologically active metabolites, such as resolvins[34] and *n*-3 PUFA-derived eicosanoids[35] that act as anti-inflammatory agents to alter cell signalling[36]. Animal studies have documented that *n*-3 PUFA modulates the tumour microenvironment, resulting in a decrease of neutrophils and macrophages infiltration and an increase of CD3+ lymphocytes infiltration, tumour cell apoptosis and IL-10 expression[37,38]. Additionally, *n*-3 PUFA reduce the production of pro-inflammatory PGE2 metabolites by inhibiting arachidonic acid synthesis, reducing breast cancer risk[39]. Overall, laboratory evidence demonstrates that the role of *n*-3 PUFA in inhibiting breast cancer occurs at all stages of cancer[5].

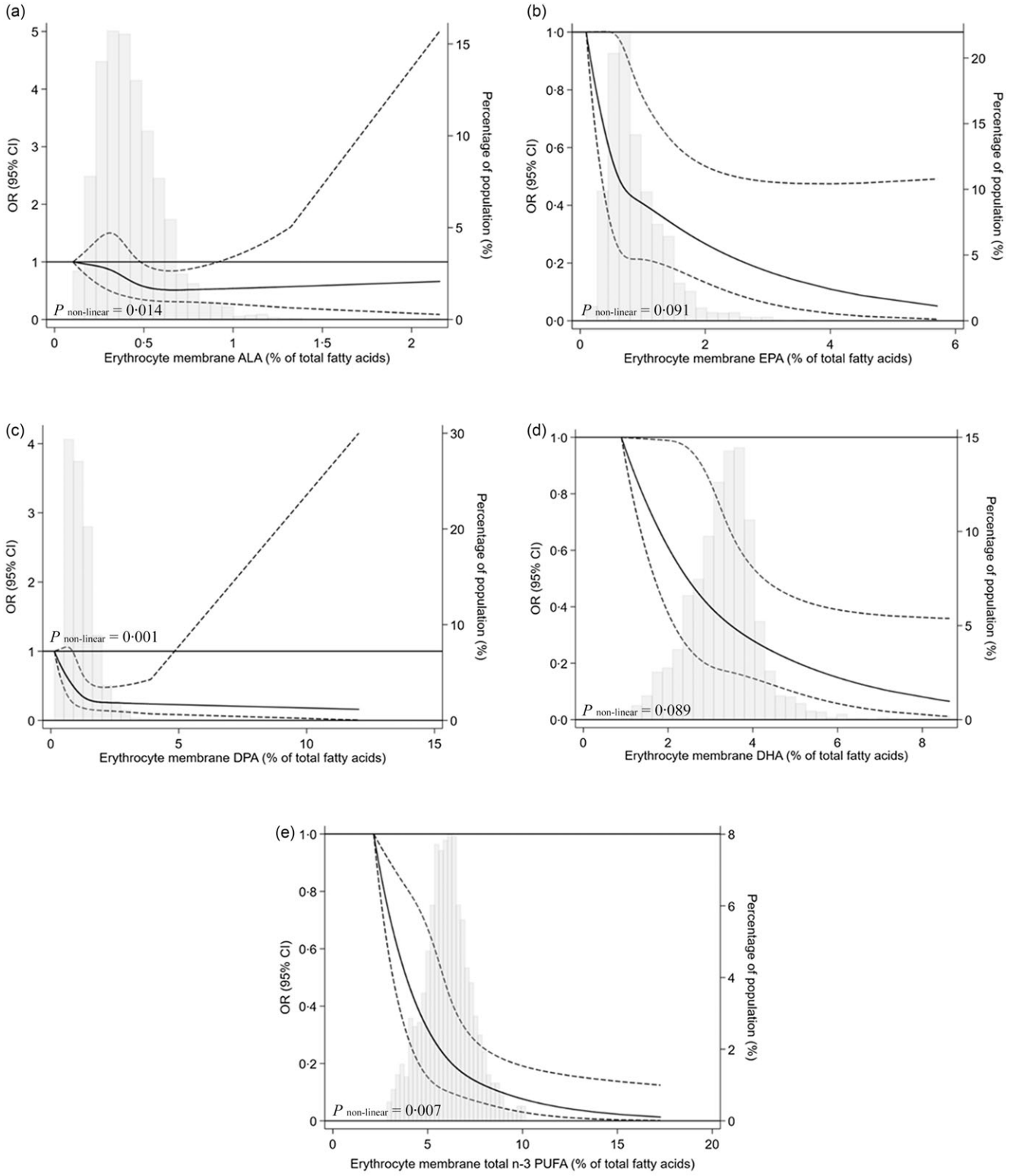

Fig. 1. Associations between the level of erythrocyte membrane *n*-3 PUFA (% of total fatty acids) and odds of breast cancer using restricted cubic splines. (a) ALA; (b) EPA; (c) DPA; (d) DHA and (e) total *n*-3 PUFA. Adjusted for age, educational level, BMI, passive smoking, alcohol drinking, physical activity, age at menarche, history of breast cancer in first-degree relatives, history of benign breast disease, total energy intake and erythrocyte membrane total *n*-6 PUFA. Solid and dashed lines represent OR and 95 % CI, respectively. The histograms represent the distributions of erythrocyte membrane *n*-3 PUFA levels among the study population. ALA, α-linolenic acid; DPA, docosapentaenoic acid.

Table 3. Associations of the levels of erythrocyte membrane *n*-3 PUFA (% of total fatty acids) with odds of breast cancer stratified by menopausal status (95 % confidence intervals)

	Premenopausal women			Postmenopausal women			
Erythrocyte membrane *n*-3 PUFA	Cases/controls	Adjusted OR*	95 % CI	Cases/controls	Adjusted OR*	95 % CI	$P_{interaction}$
ALA							0·015
Q1	192/152	1·00 (ref.)		113/71	1·00 (ref.)		
Q2	126/135	0·81	0·57, 1·13	91/88	0·61	0·39, 0·96	
Q3	106/135	0·65	0·46, 0·92	69/88	0·42	0·26, 0·67	
Q4	115/134	0·80	0·56, 1·13	41/89	0·30	0·18, 0·50	
P_{trend}		0·094			< 0·001		
EPA							0·171
Q1	159/144	1·00 (ref.)		103/79	1·00 (ref.)		
Q2	164/134	1·15	0·82, 1·61	75/89	0·59	0·38, 0·94	
Q3	121/141	0·76	0·54, 1·08	71/82	0·65	0·40, 1·04	
Q4	95/137	0·62	0·43, 0·90	65/86	0·52	0·33, 0·84	
P_{trend}		0·002			0·013		
DPA							0·215
Q1	184/144	1·00 (ref.)		133/79	1·00 (ref.)		
Q2	134/128	0·71	0·50, 1·01	72/95	0·36	0·23, 0·57	
Q3	114/141	0·51	0·35, 0·74	56/82	0·30	0·18, 0·50	
Q4	107/143	0·50	0·34, 0·73	53/80	0·31	0·19, 0·52	
P_{trend}		< 0·001			< 0·001		
DHA							0·405
Q1	174/146	1·00 (ref.)		113/77	1·00 (ref.)		
Q2	151/134	0·86	0·61, 1·21	77/89	0·50	0·31, 0·80	
Q3	107/136	0·61	0·42, 0·88	70/87	0·45	0·28, 0·73	
Q4	107/140	0·59	0·41, 0·85	54/83	0·34	0·20, 0·56	
P_{trend}		0·001			< 0·001		
Total *n*-3 PUFA							0·026
Q1	196/155	1·00 (ref.)		128/68	1·00 (ref.)		
Q2	150/130	0·88	0·63, 1·23	95/93	0·42	0·27, 0·68	
Q3	102/130	0·61	0·42, 0·87	53/93	0·23	0·14, 0·38	
Q4	91/141	0·49	0·34, 0·70	38/82	0·18	0·10, 0·31	
P_{trend}		< 0·001			< 0·001		

ALA, α-linolenic acid; DPA, docosapentaenoic acid.
* Adjusted for age, educational level, BMI, alcohol drinking, passive smoking, physical activity, age at menarche, history of breast cancer in first-degree relatives, history of benign breast disease, total energy intake and erythrocyte membrane total *n*-6 PUFA.

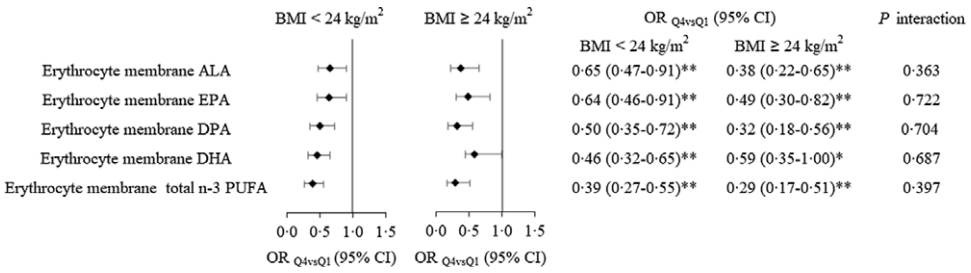

Fig. 2. Multivariable-adjusted associations of the level of erythrocyte membrane *n*-3 PUFA (% of total fatty acids) with odds of breast cancer stratified by BMI. Adjusted for age, educational level, passive smoking, alcohol drinking, physical activity, age at menarche, history of breast cancer in first-degree relatives, history of benign breast disease, total energy intake and erythrocyte membrane total *n*-6 PUFA. * $P < 0·05$; ** $P < 0·01$; ALA, α-linolenic acid; DPA, docosapentaenoic acid.

Breast cancer is a heterogeneous hormone-related disease with varying aetiologies depending on the menopausal status[40,41]. Our analysis showed an inverse association of erythrocyte membrane ALA with breast cancer in postmenopausal women but not in premenopausal women. Consistent with our results, a Canadian cohort showed a decreased risk for breast cancer in postmenopausal women with higher concentrations of plasma ALA and DHA[28]. This Canadian study and another meta-analysis[42] were concordant in observing a trend of a reduced risk of breast cancer associated with total *n*-3 PUFA in postmenopausal women, driven by a reduced risk associated with ALA. However, two prior studies conducted in postmenopausal women had inconsistent results when examining the relationships between erythrocyte membrane *n*-3 PUFA and odds of breast cancer[12,13]. One nested case–control study in Italy illustrated a borderline negative association between the erythrocyte membrane DHA and odds of breast cancer[12]. Another nested case–control study in Sweden found no associations[13]. Further studies are needed to explore the associations between erythrocyte membrane *n*-3 PUFA and odds of breast cancer by menopausal status.

Likewise, breast cancer as a heterogeneous disease is classified into different subtypes according to the expression

Table 4. Associations of the levels of erythrocyte membrane *n*-3 PUFA (% of total fatty acids) with odds of breast cancer stratified by tumour oestrogen and PR status (95 % confidence intervals)

Erythrocyte membrane *n*-3 PUFA	ER+			ER–				PR+			PR–			
	Cases	Adjusted OR*	95 % CI	Cases	Adjusted OR*	95 % CI	$P_{heterogeneity}$†	Cases	Adjusted OR*	95 % CI	Cases	Adjusted OR*	95 % CI	$P_{heterogeneity}$‡
ALA							0·821							0·660
Q1	208	1·00 (ref)		76	1·00 (ref)			179	1·00 (ref)		104	1·00 (ref)		
Q2	150	0·74	0·55, 1·00	52	0·71	0·46, 1·08		124	0·70	0·51, 0·96	77	0·78	0·54, 1·13	
Q3	114	0·54	0·39, 0·73	54	0·68	0·44, 1·03		103	0·54	0·39, 0·75	65	0·62	0·42, 0·91	
Q4	109	0·57	0·42, 0·78	40	0·58	0·37, 0·91		88	0·53	0·38, 0·73	60	0·64	0·43, 0·94	
P_{trend}		< 0·001			0·016				< 0·001			0·010		
EPA							0·119							0·536
Q1	174	1·00 (ref)		71	1·00 (ref)			158	1·00 (ref)		87	1·00 (ref)		
Q2	155	0·89	0·66, 1·21	70	1·03	0·69, 1·54		123	0·79	0·57, 1·08	101	1·20	0·83, 1·72	
Q3	136	0·76	0·56, 1·04	49	0·71	0·46, 1·09		112	0·69	0·50, 0·95	73	0·87	0·59, 1·27	
Q4	116	0·64	0·46, 0·87	32	0·45	0·28, 0·72		101	0·60	0·43, 0·84	45	0·52	0·34, 0·80	
P_{trend}		0·003			< 0·001				0·003			0·001		
DPA							0·624							0·487
Q1	218	1·00 (ref)		82	1·00 (ref)			190	1·00 (ref)		110	1·00 (ref)		
Q2	142	0·52	0·39, 0·71	49	0·53	0·34, 0·81		118	0·49	0·35, 0·67	71	0·57	0·39, 0·84	
Q3	109	0·38	0·27, 0·52	50	0·49	0·32, 0·77		88	0·34	0·24, 0·49	70	0·52	0·35, 0·78	
Q4	112	0·41	0·30, 0·58	41	0·42	0·26, 0·67		98	0·41	0·29, 0·57	55	0·42	0·28, 0·64	
P_{trend}		< 0·001			< 0·001				< 0·001			< 0·001		
DHA							0·167							0·183
Q1	200	1·00 (ref)		71	1·00 (ref)			169	1·00 (ref)		102	1·00 (ref)		
Q2	156	0·68	0·51, 0·92	57	0·80	0·52, 1·22		134	0·70	0·51, 0·96	78	0·71	0·49, 1·04	
Q3	121	0·53	0·39, 0·73	46	0·67	0·43, 1·05		102	0·53	0·38, 0·74	64	0·61	0·41, 0·91	
Q4	104	0·46	0·33, 0·63	48	0·68	0·44, 1·06		89	0·46	0·32, 0·64	62	0·59	0·40, 0·88	
P_{trend}		< 0·001			0·060				< 0·001			0·005		
Total *n*-3 PUFA							0·861							0·972
Q1	219	1·00 (ref)		85	1·00 (ref)			188	1·00 (ref)		116	1·00 (ref)		
Q2	168	0·73	0·55, 0·98	64	0·78	0·52, 1·16		140	0·70	0·52, 0·96	91	0·81	0·57, 1·16	
Q3	103	0·44	0·32, 0·60	41	0·49	0·32, 0·77		86	0·43	0·31, 0·60	58	0·49	0·33, 0·72	
Q4	91	0·37	0·27, 0·51	32	0·35	0·22, 0·57		80	0·37	0·26, 0·52	41	0·34	0·22, 0·52	
P_{trend}		< 0·001			< 0·001				< 0·001			< 0·001		

ALA, α-linolenic acid; DPA, docosapentaenoic acid; ER, oestrogen receptor; PR, progesterone receptor.

* Adjusted for age, educational level, BMI, passive smoking, alcohol drinking, physical activity, age at menarche, history of breast cancer in first-degree relatives, history of benign breast disease, total energy intake and erythrocyte membrane total *n*-6 PUFA.

† $P_{heterogeneity}$ for ER + *v.* ER–.

‡ $P_{heterogeneity}$ for PR + *v.* PR–.

of ER and PR[43]. Genes and pathways differentially express on ER− and ER+ cells in response to EPA and arachidonic acid by microarrays[44]. However, no significant heterogeneity existed in the ER/PR status between erythrocyte membrane *n*-3 PUFA and odds of breast cancer, a finding that was consistent with other study findings concerning the relationships of plasma[25], serum[30] and erythrocyte membrane[14] *n*-3 PUFA with breast cancer risk. Given the small number of ER− and PR− patients in the available studies, suggestive results must be confirmed with larger case numbers.

We did not find that the obesity status significantly modified the inverse associations of erythrocyte membrane individual and total *n*-3 PUFA with odds of breast cancer. However, the inverse associations between erythrocyte membrane *n*-3 PUFA and odds of breast cancer, except for erythrocyte membrane DHA, were stronger in overweight/obese women than in women with BMI < 24 kg/m^2. Similarly, a nested case–control study[14] observed significant inverse associations of erythrocyte membrane total *n*-3 PUFA, ALA, EPA and DPA in overweight/obese women, while no associations were observed among women with BMI < 25 kg/m^2. Obesity is a critical risk factor for breast cancer[45]. Inflammation has been proposed as a potential link between obesity and breast cancer[46], and *n*-3 PUFA have anti-inflammatory effects. Thus, more investigations are warranted to carefully assess how the BMI may modulate the association of total *n*-3 PUFA and *n*-3 PUFA subclasses with breast cancer.

Our study has several strengths. The level of erythrocyte membrane *n*-3 PUFA is considered a good biomarker to reflect a relatively long-term intake of dietary *n*-3 PUFA[47]. Additionally, a relatively large number of study subjects allowed us to explore the relationship between erythrocyte membrane *n*-3 PUFA and odds of breast cancer stratified by BMI or menopausal status.

This study has some limitations that must be noted. First, the cases and control subjects were recruited from large tertiary hospitals; thus, selection bias may occur. To minimise this bias, we recruited cases from two major hospitals consecutively as well as control subjects with several conditions that were not related to either dietary causes or breast cancer. Additionally, the level of erythrocyte membrane total *n*-3 PUFA in the control subjects in our study was similar to that in a previous study in a community-dwelling Chinese population (participants without incident metabolic syndrome: 7·09 (SD 1·93) %)[48]. Second, the level of *n*-3 PUFA in the blood may change over time during blood banking/storage. However, one study showed that no statistically significant increase was observed in the level of *n*-3 PUFA over time in the Cardiovascular Health Study across 13 consecutive years of measures[49]. Furthermore, the half-life of erythrocytes is 4 months. Therefore, a single pre-operative blood collection can still represent the long-term status of *n*-3 PUFA.

In summary, our findings showed that total and individual erythrocyte membrane *n*-3 PUFA were inversely associated with odds of having breast cancer. Other factors, such as menopause and hormone receptor status, may warrant further investigation when examining the association between *n*-3 PUFA and breast cancer. However, due to the lack of measuring other biomarkers of dietary intake, increased *n*-3 PUFA levels could be related more to healthier dietary habits in general. Therefore, increased *n*-3 PUFA may suggest an overall healthier eating which might result in a reduced risk of breast cancer. It is needed to measure more biomarkers of food intake in future studies and to examine their relationship with breast cancer.

Acknowledgements

We gratefully acknowledge the contribution of the study participants; without them, the study would not have been finished.

This work was supported by the National Natural Science Foundation of China (No: 81973020, No. 81102188). The funders had no role in the design of the study, analysis of the data or writing of this manuscript.

Conceptualisation, C. X. Z. and Z. L. Z.; data curation, C. X. Z. and Z. L. Z.; formal analysis, Z. L. Z. and D. D. S.; funding acquisition, C. X. Z.; investigation, Z. L. Z., D. D. S., L. X. and Q. X. W.; methodology, C. X. Z. and Z. L. Z.; project administration, C. X. Z.; resources, X. X. Z. and C. X. Z.; supervision, C. X. Z. and S. C. H.; visualisation, C. X. Z. and Z. L. Z.; writing – original draft, Z. L. Z.; writing – review and editing, C. X. Z. and S. C. H.

The authors declare that they have no competing interests.

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