Geographic variations and determinants of EPA plus DHA and EPA alone in pregnant and lactating women from China

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Abstract

EPA and DHA are essential for maternal and fetal health, but epidemiological data are sparse in China. We examined the trends of EPA alone and a combination of EPA plus DHA in pregnant and lactating women in three distinct geographic regions in China and explored their potential influencing factors. A total of 1015 healthy women during mid-pregnancy, late pregnancy or lactation were recruited from Weihai (coastland), Yueyang (lakeland) and Baotou (inland) cities of China between May and July of 2014. Maternal EPA and DHA concentrations (percentage of total fatty acids) in plasma and erythrocytes were measured by capillary GC. Adjusted EPA plus DHA concentrations in both plasma and erythrocytes significantly declined from mid-pregnancy (2·92 %, 6·95 %) to late pregnancy (2·20 %, 6·42 %) and lactation (2·40 %, 6·29 %) ($P_{trend} < 0\cdot001$); and both concentrations were highest in coastland, followed by lakeland, and lowest in inland ($P < 0\cdot001$). Regarding EPA alone, the concentrations were higher in women during lactation or late pregnancy and in women in coastland and inland areas. Moreover, concentrations of EPA or EPA plus DHA were higher in women with older age, higher education, higher annual family income per capita and higher dietary intake of marine aquatic product and mutton. In lactating women, erythrocyte EPA concentration was higher in those having breastfeeding partially *v.* exclusively. In conclusion, maternal plasma and erythrocyte concentrations of EPA plus DHA or EPA alone differed with geographic regions, physiological periods and maternal characteristics, indicating a need of population-specific health strategies to improve fatty acids status in pregnant and lactating women.

Keywords: EPA: DHA: Pregnant women: Lactating women: Plasma: Erythrocyte

Adequate EPA (20:5n-3) and DHA (22:6n-3), exerting important effects on neurological and immunological systems, have been linked to longer gestation, improved perinatal outcomes and better neuro-/visual development in offspring[1–4]. EPA and DHA depletion likely happen to pregnant or lactating women, due to increased demands for maternal deposition and fetal or infant development[5,6]. EPA and DHA predominantly depend on dietary intake, since they are not sufficiently converted *in vivo*. For example, the conversion rate from α-linolenic acid (ALA, 18:3n-3) to EPA is approximately 21 % and 9 % to DHA[7]. EPA and DHA are abundant in aquatic products, especially in marine fish oils and flesh of oily fish, with per 100 g containing approximately 133 mg of EPA plus DHA[8]. WHO and FAO of the United Nations recommended a minimum intake of 300 mg EPA plus DHA per day for pregnant and lactating women[9,10].

However, the maternal EPA plus DHA intakes in most countries were lower than the recommendation and differed significantly across countries[11–14]. The intakes in American and Canadian pregnant women were 98 mg/d and 160 mg/d, respectively[13,14] and were 220 mg/d and 135 mg/d for Dutch women in early pregnancy and lactation, respectively[11,12]. Maternal intakes were even less in developing countries[15,16]. Insufficient dietary intake can lead to poor DHA and EPA status. The concentrations of a combination of DHA plus EPA in erythrocytes, also called n-3 index, were 5·5 % for early/mid-pregnant women in Belgium[17], 7·1 % for late pregnant women in Italy[18] and 6·4 % and 5·5 % for late pregnant and lactating women in Germany, respectively[19], all lower than the optimal range 8–11 %[20].

The concentration varied with physiological stages. Studies from Germany, Japan and the USA consistently showed a

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decreasing trend of DHA plus EPA concentration from pregnancy to postpartum[19,21,22], but not for EPA alone. The EPA concentration decreased in British, Spanish and Japanese women[21,23,24], but increased in African-American women or Dutch women, from mid- or late pregnancy to postpartum[12,22], suggesting disparities of EPA status in different population and geographic regions. For Chinese women, especially for lactating women, little is known about the EPA or EPA plus DHA status. To our knowledge, besides our previous study on DHA status in pregnant and lactating women[25], only one Chinese study has examined the DHA and EPA status in 138 pregnant women[26].

Therefore, the purposes of the present study were to (1) investigate the concentrations and trends of EPA plus DHA and EPA alone in pregnant and lactating women living distinct regions of China and (2) evaluate potential factors that could influence the concentrations.

Subjects and methods

Subjects

In this cross-sectional study, women during mid-pregnancy (17 ± 2 gestational weeks), late pregnancy (39 ± 2 gestational weeks) or lactation (42 ± 7 d postpartum) were recruited while attending a prenatal or postnatal care visit between May and July of 2014 in hospitals in three cities of China: Weihai (coastland), Yueyang (lakeland) and Baotou (inland), which has been described in detail elsewhere[25,27,28]. The eligibility criteria of study participants were (1) generally healthy women aged 18–35 years who have not been diagnosed with cardiovascular, metabolic, renal diseases or mental disorder; (2) having a singleton pregnancy or delivering a healthy singleton who was currently breastfed and (3) local residents of one of the three cities. Women were excluded if they were (1) suffering from severe vomiting after 16 weeks of gestation; (2) being allergic to fish, shrimp, shellfish or other foods enriched with DHA; (3) having severe heart, liver, kidney or lung disorders or serious mental health problems or (4) participating in another research study within previous 30 d. A total of 1254 women during pregnancy or lactation were initially enrolled, forty-three women were excluded due to failure to meet inclusion criteria. For this present analysis, women with EPA below the limit of detection (0·01 % for EPA weight percentage of total fatty acids, *n* 196)[29,30] were further excluded and 1015 were finally included in the analysis (online Supplementary Fig. 1).

The study protocol was approved by the Institutional Review Board/Human Subjects Committee at Peking University Health Science Center (IRB00001052–14 012). All women were informed about the study procedures and gave signed informed consent.

Baseline data and blood sample collection

During enrollment, each woman completed a self-administered questionnaire containing information on characteristics related to birthdate, ethnicity, education, height, pre-pregnancy weight and parity. For pregnant women, gestational age at enrolment was calculated by subtracting the date of last menstrual period from date of enrolment. For lactating women, self-reported information on gestational age at delivery, delivery mode and breast-feeding mode was collected and checked against medical records by trained obstetricians or nurses.

Dietary information over the past month was collected using a newly designed, electronic, DHA-specific, semi-quantitative FFQ, as described previously[27,28,31]. The FFQ was developed originally to capture all DHA-containing foods mentioned in China Food Composition Table (Version 2009)[32], including marine aquatic products (seventy-five items such as mackerel, lobster and crab), freshwater aquatic products (thirty-eight items such as carp, shrimp and river crab) and mutton. For each food item, the standard portion size with nine options ranging from 25 g to 250 g and the frequency of consumption with twelve options ranging from 'once per month' to 'three times per day' were categorised. The average daily dietary intake was calculated as converting the reported frequency of consumption to per day, and then multiplying by the average amount of that food per time. Additional dietary information on the formula containing DHA was also collected.

A fasting venous blood sample (about 5 ml) was taken from each woman and collected into EDTA-containing tubes. Detailed methods for blood processing, storage and transportation have been described in our previous studies[25,27,28]. In brief, all blood samples were kept in a refrigerator at 5°C for approximately 30 min before plasma, erythrocyte were separated by centrifugation at 3000 *g* for 10 min and the erythrocytes were further washed out with 0·9 % (*w/v*) saline. Then the plasma and erythrocyte specimens were immediately stored at –20°C in the local hospitals for about 10 d before being transferred on dry ice to the central laboratory and stored at –80°C until analysis.

Fatty acid analysis

The fatty acid analysis has been described in detail previously[25]. The total lipids were extracted from plasma and erythrocyte specimens according to a modified method described by Folch *et al.*[33] and were analysed to determine fatty acids concentrations using Agilent 6890N capillary gas chromatography (Agilent Technologies). The specimens were mixed with internal standard solution containing methyl undecanoate (C11:0), then with boron trifluoride and methanol. The mixture was heated at 115°C for 20 min, subsequently cooled to room temperature and extracted with n-hexane. Finally, the n-hexane-containing methyl esters of total lipids were analysed by capillary GC equipped with a capillary column (CP-Sil 88, 50 m, 0·25 mm i.d., 0·20 μm film) and a flame ionisation detector at 280°C. The injector temperature was held at 250°C (split ratio of 1:5). The oven temperature was programmed 2°C /min from 120°C to 166°C, held there for 10 min, then temperature-programmed 2°C/min to 200°C and held there for 10 min. Fatty acids were individually identified by comparing with reference standards, and related data were collected by the Agilent Open LAB software (Agilent Technologies). The relative concentrations of EPA and DHA were expressed as their percentages of total fatty acids.

Statistical analysis

Categorical variables were expressed as frequency (percentage) and continuous variables as mean (standard deviation). χ^2 test or ANOVA was used to compare maternal characteristics among physiological period and geographic region. Logarithmic transformations were applied to normalise skewed distributions in concentrations of EPA plus DHA and EPA alone. Values were back-transformed by exponentiating and presented as geometric mean (GM) with 95 % CI. Multivariable linear regression models were used to test the differences in adjusted GM of EPA plus DHA and EPA concentrations across categories of physiological period, geographic region and other socio-demographic characteristics, and post hoc Bonferroni tests were further used for multiple comparisons.

Multivariable linear regression models were also used to explore potential influencing factors for EPA plus DHA and EPA concentrations. The variables in multivariable models included maternal age (18·0–24·9, 25·0–29·9 and 30·0–34·9 years), education (middle school or less, high school and college or above), ethnicity (Han and others), annual family income per capita (< 30 000, 30 000–50 000 and ≥ 50 000 Yuan), parity (nulliparous and multiparous), pre-pregnancy BMI (< 18·5, 18·5–23·9 and ≥ 24·0 kg/m^2), physiological period (mid-pregnancy, late pregnancy and lactation), geographic region (coastland, lakeland and inland) and dietary intake (low and high) of aquatic products, mutton and formula (yes and no); delivery mode (vaginal delivery and cesarean delivery) and breast-feeding mode (exclusive breast-feeding and partial breast-feeding) were additionally included in the models for lactating women. A sensitivity analysis where EPA concentrations below the limit of detection were imputed using the value of the half limit of detection (0·005 %) was performed[29,30], and the results were similar to those in the available data analysis (online Supplementary Table 1). To explore the coherence of the results, stratified analyses were performed according to physiological period and geographic region, respectively.

To reveal the relationships of logarithmic plasma with erythrocyte EPA plus DHA and EPA alone, multivariable fractional polynomial models with generalised linear regression were performed[34,35]. Because the nonlinear relationships and multivariable fractional polynomial-algorithm-based inflection points were observed, separate linear regression with knot values of 1·3 % for logarithmic EPA plus DHA and 0·3 % for EPA in erythrocytes that were then conducted.

All analyses were performed using SPSS version 24.0 (IBM; Corp), and a two-side $P < 0.05$ was considered as statistically significant.

Results

Maternal characteristics

The mean age of the 1015 pregnant and lactating women was 27·7 ± 3·0 (range, 18·8 to 35·0) years. Women residing in lakeland v. those in coastland and inland areas were more likely to be younger, to have lower education, higher annual family income per capita and lower pre-pregnancy BMI ($P < 0.01$).

Women in inland v. those in coastland and lakeland areas were more likely to be ethnic minority and to have had a cesarean section ($P < 0.01$). Details of maternal characteristics according to geographic region and physiological period are presented in Table 1.

Concentrations of EPA plus DHA and EPA alone

Overall, the GM (95 % CI) of EPA plus DHA and EPA concentrations were 2·56 % (1·39, 4·72 %) and 0·31 % (0·08, 1·29 %) in plasma and 6·63 % (4·23, 10·39 %) and 0·40 % (0·18, 0·90 %) in erythrocytes, respectively. The concentrations significantly varied by geographic regions (Table 2). The adjusted GM of EPA plus DHA concentrations in both plasma and erythrocytes were highest in coastland area (2·87 %, 2·70, 3·05 %; 7·40 %, 7·07, 7·75 %), followed by lakeland (2·39 %, 2·25, 2·54 %; 6·62 %, 6·34, 6·92 %) and lowest in inland (2·25 %, 2·13, 2·38 %; 5·72 %, 5·49, 5·96 %) ($P_{trend} < 0.001$), whereas the adjusted GM for EPA concentrations in plasma and erythrocytes were higher in inland (0·33 %, 0·29, 0·38 %; 0·43 %, 0·40, 0·47 %) and coastland areas (0·30 %, 0·26, 0·35 %; 0·42 %, 0·39, 0·46 %), as compared with those in lakeland (0·23 %, 0·20, 0·27 %; 0·36 %, 0·33, 0·39 %) ($P < 0.001$).

The concentrations also differed by physiological periods (Table 2). The adjusted GM (95 % CI) of EPA plus DHA concentrations in plasma and erythrocytes decreased from mid-pregnancy (2·92 %, 2·76, 3·07 %; 6·95 %, 6·68, 7·23 %) to late pregnancy (2·20 %, 2·09, 2·33 %; 6·42 %, 6·16, 6·68 %) and lactation (2·40 %, 2·27, 2·54 %; 6·29 %, 6·03, 6·56 %) ($P_{trend} < 0.001$). In contrast, the EPA concentrations did not show significantly linear trends from pregnancy through lactation, with a higher plasma concentration in lactating women and a higher erythrocyte concentration in late-pregnant women. The patterns of EPA plus DHA and EPA concentrations by physiological periods were consistently observed in strata of geographic regions (Fig. 1). The multivariable linear regression models on the associations of EPA plus DHA and EPA with geographic region and physiological period showed similar results (Table 3).

The adjusted GM of EPA plus DHA and EPA concentrations were higher in women with older age, higher educational status, higher annual family income per capita, higher dietary intakes of marine aquatic product and higher dietary intakes of mutton (Table 2). Consistently, the multivariable linear regression models showed positive associations of the concentrations with maternal age, educational status, annual family income per capita, dietary intakes of marine aquatic product and mutton (Table 3). Most of the associations persisted across pregnancy and lactation (online Supplemental Table 2). During lactation, maternal EPA concentration in erythrocytes appeared lower in women who exclusively breastfed their infants as compared with those who partially breastfed (online Supplemental Table 2).

Relationship of plasma with erythrocyte EPA plus DHA and EPA alone

The relationship between logarithmic plasma and erythrocyte concentrations of EPA plus DHA was J-shaped ($P < 0.01$ for nonlinearity, power p = 1 and q = 2), with a nadir at 1·3 % of

Table 1. Maternal characteristics by geographic region and physiological period*
(Numbers and percentages; mean values and standard deviations)

Characteristics	Coastland						Lakeland						Inland					
	Mid-pregnancy		Late pregnancy		Lactation		Mid-pregnancy		Late pregnancy		Lactation		Mid-pregnancy		Late pregnancy		Lactation	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Number of participants	110		108		95		113		131		135		95		102		126	
GA (week)/PP (day) at enrolment	17·6	1·4	37·9	0·9	42·7	2·4	17·4	1·3	38·4	1·0	41·7	4·1	17·3	2·1	38·9	1·2	42·2	3·9
Age (year)	27·9	2·5	28·3	2·8	28·4	2·6	26·8	3·1	27·1	3·2	27·1	3·0	27·9	2·9	28·5	3·3	28	3·0
Ethnicity																		
Han	107	97·3	107	99·1	91	95·8	111	98·2	128	97·1	132	97·8	78	82·1	96	94·1	115	91·3
Others	3	2·7	1	0·9	4	4·2	2	1·8	3	2·3	3	2·2	17	17·9	6	5·9	11	8·7
Education																		
College or above	81	73·6	72	66·7	68	71·6	53	46·9	80	61·1	84	62·2	76	80·0	67	65·7	90	71·4
High school	17	15·5	27	25·0	18	18·9	42	37·2	32	24·4	32	23·7	11	11·6	17	16·7	26	20·6
Middle school or less	12	10·9	9	8·3	9	9·4	18	15·9	19	14·5	19	14·1	8	8·4	18	17·7	10	7·9
Annual family income per capita (Yuan)																		
< 30 000	30	27·3	26	24·1	69	72·6	67	59·3	67	51·2	89	65·9	43	45·3	49	48·0	74	58·7
30 000–50 000	46	41·8	43	39·8	24	25·3	28	24·8	25	19·1	25	18·5	25	26·3	25	24·5	36	28·6
≥ 50 000	34	30·9	39	36·1	2	2·1	18	15·9	39	19·8	21	15·6	27	28·4	28	27·5	16	12·7
Parity																		
Nulliparous	99	90·0	102	94·4	84	88·4	85	75·2	104	79·4	110	81·5	82	86·3	69	67·6	110	87·3
Multiparous	11	10·0	6	5·6	11	11·6	28	24·8	27	20·6	25	18·5	13	13·7	33	32·4	16	12·7
Pre-pregnancy BMI (kg/m²)	20·9	2·8	21·3	2·6	21·9	3·3	20·2	2·4	19·8	1·9	20·1	2·9	21·1	2·8	21·4	2·8	21·1	3·1
Delivery mode																		
Vaginal delivery	–	–	–	–	77	81·1	–	–	–	–	79	58·5	–	–	–	–	46	36·5
Cesarean delivery	–	–	–	–	18	18·9	–	–	–	–	56	41·5	–	–	–	–	80	63·5
Feeding mode																		
Exclusive breastfeeding	–	–	–	–	48	50·5	–	–	–	–	87	64·4	–	–	–	–	75	59·5
Partial breastfeeding	–	–	–	–	47	49·5	–	–	–	–	48	35·6	–	–	–	–	51	40·5

* The en dash in subgroups of delivery mode and feeding mode represents unavailable information.

erythrocyte EPA plus DHA, whereas the relationship for EPA concentrations was inversely J-shaped ($P < 0.01$ for nonlinearity, power p = 1 and q = 3), with an apex at 0·3 % of erythrocyte EPA (Fig. 2). In the segmented analysis based on inflection point, a significant linear relationship was observed in women with erythrocyte EPA plus DHA concentration ≥ 1·3 % (n 999; $\beta = 1.01$, $P < 0.001$) but not in those with the concentration < 1·3 % (n 16; adjusted $\beta = -0.14$, $P = 0.71$), and observed in women with erythrocyte EPA concentration < 0·3 % (n 1002; $\beta = 1.08$, $P < 0.001$), but not in those with the concentration ≥ 0·3 % (n 13; adjusted $\beta = -1.84$, $P = 0.62$). Similar linear pattern persisted in most subgroups stratified by physiological periods and geographic regions (online Supplementary Table S3).

Discussion

In this large cross-sectional study in China, maternal EPA plus DHA concentrations in both plasma and erythrocytes were higher in the coastland/lakeland than in the inland and decreasing from mid-pregnancy to late pregnancy and lactation. Maternal EPA concentrations also varied by physiological periods but with different trends in plasma and erythrocytes, as concentration in plasma was higher in lactating women, while concentration in erythrocytes was higher in late-pregnant women. Regarding geographic variations, the EPA concentrations in both plasma and erythrocytes were higher in the inland and coastland *v.* in the lakeland. In addition, maternal age, education, annual family income per capita and breast-feeding mode were also related to the concentrations of EPA plus DHA or EPA alone.

The decreasing trends in EPA plus DHA concentrations from mid-pregnancy to lactation in our Chinese population were consistent to the findings from German, Japanese and the American women[19,21,22], which was probably attributed to the change in DHA status[23,25], a dominant contributor to the sum of EPA plus DHA. This decline in maternal DHA concentrations has been suggested to be due to the transfer from the mother to fetus via placenta during pregnancy or to infants via breast milk during lactation, to meet offspring demands for development[12,36,37]. An alternate possibility is that the decline might be related to lipolysis of hepatic *de novo* lipogenesis and adipose tissue that accompanies the diminished insulin sensitivity during pregnancy, which might cause a lower concentration of EPA plus DHA[38]. Unlike the sum of EPA plus DHA, the concentration of EPA alone in plasma was higher in lactation while the concentration in erythrocytes was higher in late pregnancy in the study. The inconsistent pattern in plasma and erythrocytes was possibly because EPA in erythrocytes reflects intakes aggregated over the lifespan of ∼120 d[39]. Several studies reported a decreasing trend of plasma EPA concentration throughout pregnancy in British and Japanese women[21,23] and for erythrocyte EPA concentrations in Spanish women[24]. Nevertheless, some previous studies reported that the maternal EPA concentrations both in plasma

Table 2. Geometric mean (95 % CI) of EPA plus DHA and EPA concentrations (%) by maternal characteristics*
(Mean values and 95 % confidence intervals)

Variables	Crude mean (95 % CI)								Adjusted mean (95 % CI)							
	Plasma EPA plus DHA		Erythrocyte EPA plus DHA		Plasma EPA		Erythrocyte EPA		Plasma EPA plus DHA		Erythrocyte EPA plus DHA		Plasma EPA		Erythrocyte EPA	
	Crude mean	95 % CI	Crude mean	95 % CI	Crude mean	95 % CI	Crude mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI
Geographic region																
Inland	2·27	2·20,2·35[a]	5·81	5·68,5·93[a]	0·36	0·33,0·39[b]	0·44	0·42,0·46[b]	2·25	2·13,2·38[a]	5·72	5·49,5·96[a]	0·33	0·29,0·38[b]	0·43	0·40,0·47[b]
Lakeland	2·45	2·38,2·53[b]	6·55	6·42,6·69[b]	0·26	0·24,0·27[a]	0·34	0·33,0·36[a]	2·39	2·25,2·54[b]	6·62	6·34,6·92[b]	0·23	0·20,0·27[a]	0·36	0·33,0·39[a]
Coastland	3·05	2·96,3·15[c]	7·69	7·52,7·87[c]	0·34	0·32,0·37[b]	0·44	0·42,0·46[b]	2·87	2·70,3·05[c]	7·40	7·07,7·75[c]	0·30	0·26,0·35[b]	0·42	0·39,0·46[b]
P	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
Physiological period																
Mid-pregnancy	3·04	2·94,3·13[c]	7·11	6·94,7·29[c]	0·31	0·28,0·33[b]	0·40	0·38,0·41[b]	2·92	2·76,3·07[c]	6·95	6·68,7·23[c]	0·28	0·24,0·32[a]	0·39	0·36,0·43[b]
Late pregnancy	2·30	2·23,2·38[a]	6·57	6·42,6·73[b]	0·25	0·23,0·27[a]	0·42	0·40,0·44[b]	2·20	2·09,2·33[a]	6·42	6·16,6·68[b]	0·23	0·20,0·26[a]	0·42	0·38,0·45[b]
Lactation	2·44	2·36,2·51[b]	6·27	6·12,6·41[a]	0·39	0·37,0·42[c]	0·39	0·37,0·40[a]	2·40	2·27,2·54[b]	6·29	6·03,6·56[a]	0·37	0·32,0·42[b]	0·40	0·36,0·43[b]
P	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
Age																
< 25	2·48	2·37,2·60	6·37	6·15,6·59[a]	0·29	0·26,0·33	0·38	0·36,0·41[a]	2·48	2·33,2·64	6·38	6·09,6·68[a]	0·30	0·25,0·35	0·41	0·37,0·45
25·0–29·9	2·57	2·51,2·63	6·65	6·53,6·77[b]	0·31	0·29,0·33	0·40	0·39,0·41[b]	2·46	2·33,2·59	6·48	6·24,6·73[a]	0·28	0·24,0·32	0·39	0·36,0·42
≥ 30	2·59	2·49,2·70	6·76	6·56,6·97[b]	0·33	0·30,0·36	0·42	0·40,0·45[b]	2·53	2·39,2·68	6·78	6·50,7·07[b]	0·28	0·24,0·32	0·40	0·37,0·44
P	0·350		0·031		0·308		0·045		0·361		0·008		0·535		0·399	
Education																
Middle school or less	2·43	2·30,2·57	6·30	6·05,6·56[a]	0·27	0·24,0·31[a]	0·38	0·36,0·41[a]	2·43	2·28,2·59	6·41	6·11,6·72	0·26	0·22,0·31[b]	0·40	0·36,0·44
High school	2·56	2·45,2·66	6·64	6·44,6·84[b]	0·29	0·27,0·32[a]	0·38	0·36,0·40[a]	2·51	2·37,2·66	6·62	6·34,6·91	0·29	0·25,0·33[a]	0·39	0·36,0·43
College or above	2·59	2·53,2·65	6·68	6·57,6·80[b]	0·33	0·31,0·35[b]	0·41	0·40,0·43[b]	2·53	2·40,2·66	6·61	6·37,6·87	0·31	0·27,0·35[a]	0·41	0·38,0·45
P	0·120		0·030		0·011		0·008		0·387		0·283		0·046		0·264	
Ethnicity																
Han	2·57	2·52,2·62[b]	6·65	6·55,6·75[b]	0·31	0·30,0·33	0·40	0·39,0·41	2·58	2·49,2·67	6·59	6·42,6·77	0·33	0·30,0·36	0·42	0·40,0·44
Others	2·35	2·15,2·56[a]	6·17	5·79,6·57[a]	0·27	0·22,0·33	0·39	0·35,0·44	2·40	2·22,2·61	6·50	6·12,6·90	0·29	0·20,0·31	0·39	0·34,0·43
P	0·041		0·024		0·151		0·627		0·072		0·618		0·054		0·141	
Annual family income per capita (Yuan)																
< 30 000	2·51	2·44,2·57	6·48	6·35,6·61[a]	0·31	0·30,0·33	0·38	0·37,0·40[a]	2·49	2·37,2·62	6·53	6·29,6·78	0·28	0·25,0·32	0·39	0·36,0·42[b]
30 000–50 000	2·63	2·54,2·73	6·76	6·58,6·94[b]	0·32	0·29,0·35	0·43	0·41,0·45[b]	2·48	2·34,2·62	6·52	6·26,6·79	0·28	0·24,0·33	0·41	0·38,0·45[a]
≥ 50 000	2·60	2·50,2·71	6·81	6·61,7·02[b]	0·30	0·27,0·33	0·42	0·39,0·44[b]	2·50	2·36,2·66	6·58	6·30,6·88	0·29	0·25,0·34	0·40	0·37,0·44[a]
P	0·070		0·005		0·639		0·001		0·908		0·849		0·914		0·045	
Parity																
Nulliparous	2·58	2·53,2·63	6·69	6·58,6·79[b]	0·31	0·30,0·33	0·40	0·39,0·41	2·49	2·37,2·61	6·66	6·42,6·91	0·27	0·24,0·31	0·40	0·37,0·43
Multiparous	2·47	2·36,2·59	6·33	6·11,6·55[a]	0·31	0·27,0·34	0·40	0·37,0·42	2·49	2·35,2·65	6·43	6·15,6·72	0·30	0·25,0·35	0·41	0·37,0·44

Table 2. (Continued)

Variables	Crude mean (95 % CI)								Adjusted mean (95 % CI)							
	Plasma EPA plus DHA		Erythrocyte EPA plus DHA		Plasma EPA		Erythrocyte EPA		Plasma EPA plus DHA		Erythrocyte EPA plus DHA		Plasma EPA		Erythrocyte EPA	
	Crude mean	95 % CI	Crude mean	95 % CI	Crude mean	95 % CI	Crude mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI
P	0·100		0·004		0·694		0·793		0·908		0·616		0·228		0·564	
Pre-pregnancy BMI (kg/m^2)																
< 18·5	2·55	2·44,2·66	6·45	6·25,6·66	0·29	0·27,0·32[a]	0·37	0·35,0·39[a]	2·55	2·41,2·70	6·52	6·25,6·80	0·27	0·23,0·31	0·38	0·35,0·42
18·5–23·9	2·56	2·51,2·62	6·67	6·57,6·79	0·31	0·30,0·33[a]	0·41	0·39,0·42[b]	2·47	2·36,2·59	6·56	6·33,6·79	0·27	0·24,0·31	0·41	0·38,0·44
≥ 24·0	2·57	2·40,2·76	6·62	6·29,6·96	0·38	0·32,0·44[b]	0·43	0·40,0·47[b]	2·45	2·28,2·63	6·56	6·22,6·92	0·31	0·26,0·37	0·42	0·37,0·46
P	0·954		0·175		0·026		0·005		0·299		0·933		0·279		0·120	
Marine aquatic product																
Low	2·34	2·29,2·40[a]	6·15	6·04,6·27[a]	0·30	0·28,0·32[a]	0·38	0·36,0·39[a]	2·46	2·34,2·59[a]	6·39	6·15,6·65[a]	0·27	0·23,0·30[a]	0·38	0·36,0·42[a]
High	2·79	2·72,2·87[b]	7·12	6·98,7·26[b]	0·33	0·31,0·35[b]	0·43	0·41,0·44[b]	2·52	2·39,2·66[b]	6·70	6·45,6·97[b]	0·31	0·27,0·35[b]	0·42	0·39,0·45[b]
P	< 0·001		< 0·001		0·048		< 0·001		< 0·001		0·001		0·005		0·002	
Fresh aquatic product																
Low	2·58	2·51,2·66	6·70	6·55,6·84	0·32	0·30,0·34	0·42	0·41,0·44[b]	2·46	2·34,2·59	6·57	6·32,6·83	0·28	0·24,0·32	0·40	0·37,0·44
High	2·54	2·47,2·60	6·56	6·44,6·68	0·31	0·29,0·33	0·38	0·37,0·39[a]	2·52	2·39,2·66	6·52	6·27,6·78	0·29	0·26,0·34	0·40	0·37,0·43
P	0·356		0·153		0·554		< 0·001		0·232		0·556		0·193		0·865	
Mutton																
Low	2·48	2·39,2·57[a]	6·34	6·18,6·51[a]	0·29	0·28,0·31[a]	0·38	0·36,0·39[a]	2·49	2·36,2·62	6·46	6·22,6·71	0·28	0·25,0·32	0·38	0·35,0·41[a]
High	2·60	2·55,2·66[b]	6·77	6·66,6·88[b]	0·36	0·33,0·39[b]	0·46	0·44,0·48[b]	2·49	2·35,2·64	6·64	6·36,6·93	0·29	0·25,0·33	0·42	0·39,0·46[b]
P	0·022		< 0·001		< 0·001		< 0·001		0·91		0·12		0·906		0·006	
Formula																
No	2·58	2·52,2·63	6·75	6·58,6·93	0·26	0·24,0·28[a]	0·38	0·36,0·4[a]	2·50	2·39,2·63	6·51	6·29,6·75	0·29	0·25,0·33	0·40	0·37,0·43
Yes	2·51	2·42,2·61	6·59	6·48,6·70	0·33	0·31,0·35[b]	0·41	0·4,0·42[b]	2·48	2·33,2·63	6·58	6·3,6·87	0·28	0·24,0·33	0·41	0·37,0·44
P	0·28		0·135		< 0·001		0·006		0·63		0·529		0·653		0·479	

* Values in a column that do not share a common superscript letter differed with the use of Bonferroni-correction procedures for multiple comparisons: a < b < c, $P < 0.05$. P values for crude and adjusted geometric means are from univariate and multivariable linear regression, respectively, adjusted for maternal age, education, ethnicity, annual family income per capita, parity, pre-pregnancy BMI, physiological period, geographic region and dietary intakes of aquatic product, mutton and formula.
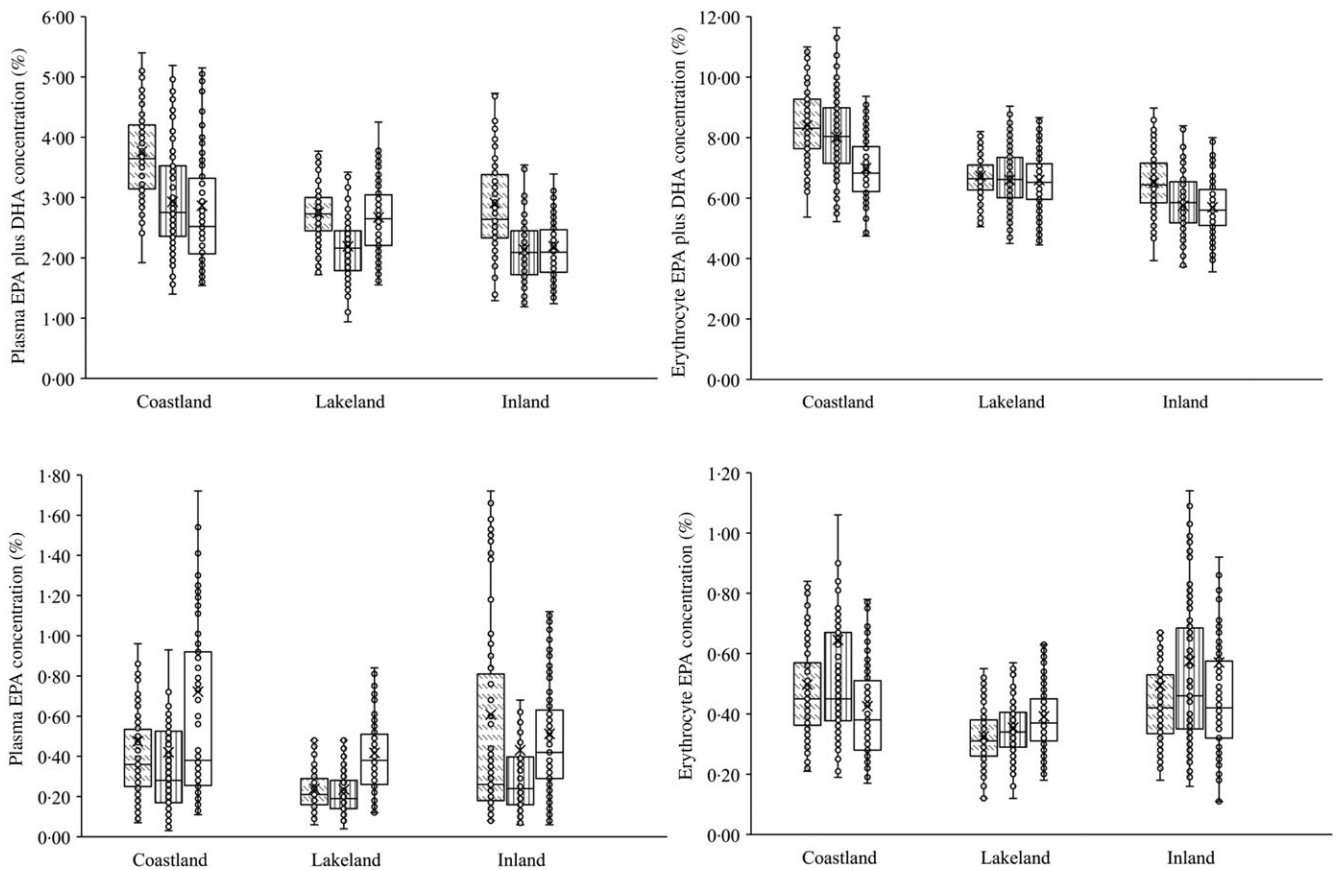
Fig. 1. Geometric mean (95 % CI) of EPA plus DHA and EPA concentrations (%) by geographic region across physiological periods. ▨, Mid-pregnancy; ▥, late pregnancy; ☐, lactation

and erythrocytes significantly increased from mid- or late pregnancy to postpartum in African-American women and Dutch women, similar to our findings[12,22]. These results suggested that the synthesis and metabolism of EPA and DHA *in vivo* might follow different mechanisms from pregnancy to lactation[12]. As pregnancy progresses, the efficiency at converting ALA to EPA was 12- to 29-fold higher than that at ALA to DHA[40,41]. Given that the metabolic pathway of ALA-to-DHA was mediated by EPA, the limited capacity for EPA conversion might be constrained severely for DHA conversion[7].

The erythrocyte EPA plus DHA concentrations in our population (6·63 %) was higher than African-American (< 4·23 %)[22], Chilean (< 6·0 %)[42] and German women (6·23 %)[19], whereas lower than Norwegian (> 6·8 %)[43] and Japanese women (> 7·53 %)[6]. The erythrocyte EPA concentration in our population (0·40 %) was substantially lower than those aforementioned women (0·55 % to 1·03 %) except for African-American women (< 0·35 %). The inconsistent results were likely due to different population or region.

A recent review included 298 original studies from fifty-four countries/regions found that the EPA plus DHA concentration in healthy adults was > 8 % in coastal countries surrounding Sea of Japan or adjoining Scandinavia, but < 4 % in countries like USA, Brazil or Africa[44]. Similarly, we found higher EPA plus DHA concentrations in coastland and lakeland areas *v.* inland. We also found that maternal EPA concentration in inland area was similar to coastland but higher than those in lakeland, inconsistent with a European study that reported higher maternal EPA concentration in coastal countries than in land countries[5]. The different patterns in EPA by geographic regions was possibly due to different dietary habits. In our study, higher intake of mutton was linked to higher erythrocyte EPA concentration, and the inland women was recruited from Inner Mongolia where mutton was commonly consumed[27], likely increasing the exogenous intake of EPA. The EPA content in mutton is approximately 100 mg per 100 g, higher than those in aquatic products even in some marine aquatic products (e.g. mantis shrimp, 66 mg/100 g, shrimp, 39·6 mg/100 g and clam, 22 mg/100 g). In addition, mutton is also rich in ALA (39·6 mg per 100 g of mutton), possibly enhancing the endogenous conversion of EPA[45]. In addition, our study was conducted during the fish close season (from 1 May to 1 September), which might limit acquisition of aquatic products and EPA intake for coastland/lakeland women.

Our study also showed that EPA plus DHA and EPA concentrations were associated with maternal age and education, which was consistent with previous studies[46,47]. A recent review indicated that older maternal age and higher educational level were related to higher *n*-3 long-chain PUFA concentrations[46]. The associations of DHA and EPA with maternal socio-demographic characteristics could be partly explained by the difference in consumption profiles of foods enriched with fatty acids or supplements. A large birth cohort study on Chinese, Malay, and

Table 3. Regression coefficient (β) and 95 % confidence interval (95 % CI) of EPA plus DHA and EPA concentrations by maternal characteristics*
(Regression coefficient and 95 % confidence interval)

| | Plasma EPA plus DHA | | | | Erythrocyte EPA plus DHA | | | | Plasma EPA | | | | Erythrocyte EPA | | | |
| | Crude | | Adjusted | | Crude | | Adjusted | | Crude | | Adjusted | | Crude | | Adjusted | |
Variables	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI
Geographic region																
Inland	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Lakeland	0·08†	0·03, 0·12†	0·06†	0·00, 0·12†	0·12†	0·09, 0·1†	0·15†	0·10, 0·19†	−0·34†	−0·44, −0·23†	−0·36†	−0·51, −0·20†	−0·25†	−0·31, −0·19†	−0·18†	−0·27, −0·10†
Coastland	0·29†	0·25, 0·34†	0·24†	0·19, 0·30†	0·28†	0·25, 0·31†	0·26†	0·22, 0·30†	−0·04	−0·15, 0·07	−0·09	−0·22, 0·05	−0·01	−0·06, 0·06	−0·01	−0·09, 0·10
Physiological period																
Mid–pregnancy	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Late pregnancy	−0·28†	−0·32, −0·23†	−0·28†	−0·32, −0·24†	−0·08†	−0·11, −0·04†	−0·08†	−0·11, −0·05†	−0·21†	−0·31, −0·10†	−0·22†	−0·32, −0·11†	0·06†	0·00, 0·13†	0·06†	0·00, 0·12†
Lactation	−0·22†	−0·26, −0·18†	−0·19†	−0·23, −0·15†	−0·13†	−0·16, −0·09†	−0·10†	−0·13, −0·07†	0·25†	0·15, 0·36†	0·27†	0·16, 0·38†	−0·03	−0·09, 0·04	0·01	−0·05, 0·07
Age																
< 25	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
25·0–29·9	0·03	−0·02, 0·09	−0·01	−0·06, 0·04	0·04†	0·01, 0·08†	0·02	−0·02, 0·05	0·05	−0·07, 0·18	−0·07	−0·19, 0·05	0·04	−0·03, 0·11	−0·04	−0·11, 0·03
≥ 30	0·04	−0·02, 0·11	0·02	−0·04, 0·08	0·06†	0·01, 0·11†	0·06	0·02, 0·10†	0·11	−0·03, 0·26	−0·06	−0·22, 0·09	0·10†	0·01, 0·18†	−0·02	−0·11, 0·07
Education																
Middle school or less	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
High school	0·05	−0·02, 0·12	0·04	−0·02, 0·09	0·05†	0·01, 0·10†	0·04	0·00, 0·08	0·08	−0·07, 0·24	0·08	−0·07, 0·23	−0·01	−0·11, 0·07	−0·03	−0·11, 0·06
College or above	0·06†	0·00, 0·12†	0·03	−0·03, 0·09	0·06†	0·02, 0·10†	0·04†	0·00, 0·08†	0·19†	0·05, 0·33†	0·15†	0·01, 0·29†	0·07	−0·01, 0·15	0·03	−0·06, 0·11
Ethnicity																
Han	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Others	−0·09	−0·18, 0·01	−0·07	−0·15, 0·01	−0·07	−0·14, 0·01	−0·01	−0·07, 0·04	−0·15	−0·26, 0·06	−0·27	−0·47, 0·00	−0·03	−0·15, 0·09	−0·09	−0·20, 0·03
Annual family income per capita																
< 30 000	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
30 000–50 000	0·04	0·01, 0·07	0·00	−0·04, 0·03	0·01†	−0·10, 0·12†	0·00	−0·03, 0·03	0·11	0·05, 0·17	0·00	−0·10, 0·10	0·11	0·05, 0·17	0·06	0·00, 0·12
≥ 50 000	0·05†	0·02, 0·09†	0·00	−0·04, 0·05	−0·05†	−0·16, 0·07†	0·01†	0·00, 0·04†	0·08†	0·02, 0·15†	0·02	−0·09, 0·13	0·08	0·02, 0·15	0·03	−0·03, 0·10
Parity																
Nulliparous	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Multiparous	−0·04	−0·09, 0·01	0·00	−0·05, 0·05	−0·06†	−0·09, −0·02†	−0·03	−0·07, 0·00	−0·02	−0·14, 0·10	0·08	−0·05, 0·21	−0·01	−0·08, 0·06	0·02	−0·05, 0·10
Pre-pregnancy BMI																
< 18·5	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
18·5–23·9	0·01	−0·04, 0·06	−0·03	−0·07, 0·01	0·03	0·00, 0·07	0·01	−0·03, 0·04	0·06	−0·06, 0·17	0·02	−0·09, 0·12	0·09†	0·03, 0·16†	0·06	0·00, 0·12
≥ 24·0	0·01	−0·07, 0·09	−0·04	−0·11, 0·03	0·03	−0·03, 0·09	0·01	−0·05, 0·06	0·26†	0·07, 0·45†	0·14	−0·04, 0·32	0·15†	0·05, 0·26†	0·08	−0·02, 0·19
Marine aquatic product																
Low	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
High	0·17†	0·14, 0·21†	0·08†	0·04, 0·12†	0·15†	0·12, 0·17†	0·05†	0·02, 0·07†	0·09†	0·00, 0·18†	0·14†	0·04, 0·24†	0·13†	0·08, 0·18†	0·09†	0·03, 0·15†
Fresh aquatic product																
Low	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
High	−0·02	−0·06, 0·02	0·02	−0·01, 0·06	−0·02	−0·05, 0·01	−0·01	−0·04, 0·02	−0·03	−0·12, 0·06	0·06	−0·03, 0·16	−0·10†	−0·15, −0·05†	0·00	−0·06, 0·05
Mutton																
Low	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
High	−0·05†	−0·09, −0·01†	0·00	−0·04, 0·05	−0·07†	−0·09, −0·04†	0·03	−0·01, 0·06	0·21†	0·11, 0·30†	0·01	−0·11, 0·13	0·20†	0·15, 0·25†	0·10†	0·03, 0·17†
Formula																
No	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Yes	−0·03	−0·07, 0·02	−0·01	−0·05, 0·03	0·02	−0·01, 0·06	0·01	−0·02, 0·04	−0·23†	−0·33, −0·12†	−0·03	−0·14, 0·09	−0·07†	−0·14, −0·01†	0·02	−0·04, 0·09

* P values for crude and adjusted β coefficients are from univariate and multivariable linear regression, respectively, adjusted for maternal age, education, ethnicity, annual family income per capita, parity, pre-pregnancy BMI, physiological period, geographic region and dietary intakes of aquatic product, mutton and formula.
† The values represents $P < 0.05$.
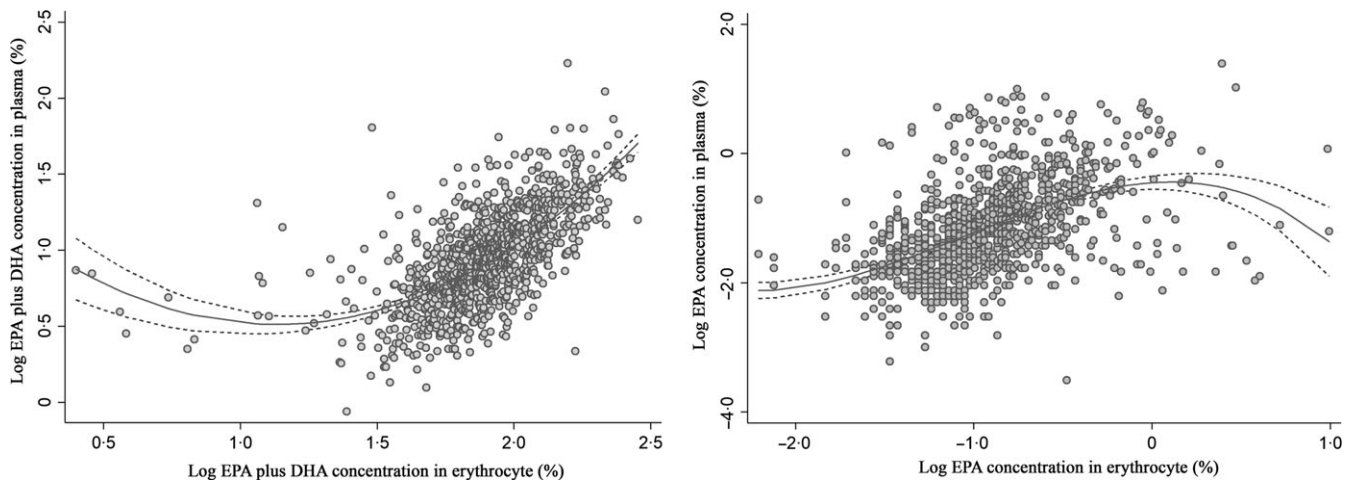
Fig. 2. The relationships between logarithmic plasma and erythrocyte concentrations of EPA plus DHA and EPA alone. The relationships were assessed using multi-variable fractional polynomial models with generalised linear regression, adjusted for maternal age, education, ethnicity, annual family income per capita, parity, pre-pregnancy BMI, physiological period and geographic region.

Indian pregnant women showed that the n-3 long-chain PUFA concentrations were positively correlated to maternal age[48], presumably because the women who chose to deliver children later v. earlier in life were more likely to have a higher socioeconomic status and have healthier dietary habits. A nationwide study comprising 15 407 American adults also indicated that higher education level was associated with better overall dietary profiles and higher consumption of sea foods enriched with fatty acids[49]. Our study also found that maternal EPA concentration in erythrocytes appeared lower in lactating women having breastfeeding exclusively v. partially, possibly due to more secretion of EPA into breast milk. Although no study has examined the association of breastfeeding mode with maternal n-3 long-chain PUFA status, a cross-sectional study in Hungary showed that erythrocyte EPA concentration was lower in infants breastfed exclusively v. partially[47].

Non-linear relationships were observed between plasma and erythrocyte EPA plus DHA and EPA concentrations, with inflection points of erythrocyte concentrations at 1·3 % for EPA plus DHA and 0·3 % for EPA alone. When erythrocyte EPA plus DHA ≥ 1·3 % (adjusted $\beta = 1·01$) or erythrocyte EPA < 0·3 % (adjusted $\beta = 1·08$), the relations between plasma and erythrocyte concentrations were linear; whereas no significant relation observed when erythrocyte EPA plus DHA < 1·3 % (n 16) or EPA ≥ 0·3 % (n 13), possibly due to the small samples in these strata. One interpretation of inflection points was that erythrocytes could serve as a reservoir for fatty acids, likely transporting them through dynamic interaction with plasma when maternal fatty acids levels were lower or higher[50]. On the other hand, the sample storage at −20°C might cause degradation of fatty acids, in particular in those with lower concentrations (such as EPA), thus leading to a flawed estimate deviated from the true linear relation[51,52]. Caution is needed when interpreting these results.

The present cross-sectional study in China, to the best of our knowledge, firstly investigated the concentrations of EPA alone and in combination with DHA and explored their potential determinants including geographic regions, periods of pregnancy and lactation, and maternal socio-demographic characteristics. The data collection and fatty acids measurements were performed by trained research personnel, following a standard protocol. The relatively large sample size provided accurate estimates for discrepancy and allowed for subgroup analyses. Several limitations of this study should be considered. First, potential selection bias may have occurred because the women were recruited in hospitals that were responsible for antenatal visit in urban areas. Second, because of the cross-sectional nature of the study design, casual inferences could not be made. Third, the mean EPA and DHA concentrations might be somewhat underestimated due to temporary storage of blood samples at −20°C. Finally, data on other potential confounders such as duration of living in the current residence were not collected.

Conclusion

In summary, maternal plasma and erythrocyte concentrations of EPA plus DHA and EPA alone differed with geographic regions, physiological periods and maternal characteristics, indicating a need of population-specific health strategies to improve fatty acids status in pregnant and lactating women. Further studies are warranted to elucidate the mechanisms underlying the aforementioned variations in EPA and DHA.

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There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114521003731

References

1. Oken E & Belfort MB (2010) Fish, fish oil, and pregnancy. *JAMA* **304**, 1717–1718.
2. Middleton P, Gomersall JC, Gould JF, *et al.* (2018) *n*-3 fatty acid addition during pregnancy. *Cochrane Database Syst Rev* **11**, CD003402.
3. Jasani B, Simmer K, Patole SK, *et al.* (2017) Long chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev* **3**, CD000376.
4. Bernard JY, Agostini MD, Forhan A, *et al.* (2013) The dietary *n*-6:*n*-3 fatty acid ratio during pregnancy is inversely associated with child neurodevelopment in the EDEN mother-child cohort. *J Nutr* **143**, 1481.
5. Otto SJ, Houwelingen AC, Antal M, *et al.* (1997) Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. *Eur J Clin Nutr* **51**, 232–242.
6. Kawabata T, Kagawa Y, Kimura F, *et al.* (2017) Polyunsaturated fatty acid levels in maternal erythrocytes of Japanese women during pregnancy and after childbirth. *Nutrients* **9**, 245–254.
7. Burdge GC & Wootton SA (2002) Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* **88**, 411–420.
8. Tvrzicka E, Kremmyda LS, Stankova B, *et al.* (2011) Fatty acids as biocompounds: their role in human metabolism, health and disease – a review. Part 1: classification, dietary sources and biological functions. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **155**, 117–130.
9. Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (2010) *Fat and Fatty Acids in Human Nutrition: Report of an Expert Consultation*. Rome, Italy: Food and Agriculture Organization.
10. European Food Safety Authority (EFSA) Panel on Dietetic Products NaAN (2010) Scientific opinion on dietary reference values for dat, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J* **8**, 1461–1519.
11. Otto SJ, van Houwelingen AC, Badart-Smook A, *et al.* (2001) Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. *Am J Clin Nutr* **73**, 302–307.
12. Otto SJ, Houwelingen AC, van Badart-Smook A, *et al.* (2001) Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. *Am J Clin Nutr* **73**, 1074–1079.
13. Zhang ZY, Fulgoni VL, Kris-Etherton PM, *et al.* (2018) Dietary intakes of EPA and DHA *n*-3 fatty acids among US childbearing-age and pregnant women: an analysis of NHANES 2001–2014. *Nutrients* **10**, 416–433.
14. Friesen RW & Innis SM (2010) Linoleic acid is associated with lower long-chain *n*-6 and *n*-3 fatty acids in red blood cell lipids of Canadian pregnant women. *Am J Clin Nutr* **91**, 23–31.
15. Mani I, Dwarkanath P, Thomas T, *et al.* (2016) Maternal fat and fatty acid intake and birth outcomes in a South Indian population. *Int J Epidemiol* **45**, 523–531.
16. Nishimura RY, Barbieiri P, de Castro GSF, *et al.* (2014) Dietary polyunsaturated fatty acid intake during late pregnancy affects fatty acid composition of mature breast milk. *Nutrition* **30**, 685–689.
17. Hoge A, Bernardy F, Donneau AF, *et al.* (2019) Importance of *n*-3 PUFA consumption during pregnancy: perception discrepancies between pregnant women and gynaecologists-obstetricians in Belgium. *Public Health Nutr* **22**, 1259–1268.
18. Massari M, Novielli C, Mando C, *et al.* (2020) Multiple micronutrients and docosahexaenoic acid supplementation during pregnancy: a randomized controlled study. *Nutrients* **12**, 2432.
19. Gellert S, Schuchardt JP & Hahn A (2016) Higher *n*-3 index and DHA status in pregnant women compared to lactating women: results from a German nation-wide cross-sectional study. *Prostaglandins Leukotrienes Essent Fatty Acids* **109**, 22–28.
20. Von Schacky C (2020) *n*-3 fatty acids in pregnancy-the case for a target *n*-3 index. *Nutrients* **12**, 898.
21. Kitamura Y, Kogomori C, Hamano H, *et al.* (2017) Relationship between changes in fatty acid composition of the erythrocyte membranes and fatty acid intake during pregnancy in pregnant Japanese women. *Ann Nutr Metab* **70**, 268–276.
22. Stark KD, Beblo S, Murthy M, *et al.* (2005) Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum. *J Lipid Res* **46**, 516–525.
23. Miles EA, Noakes PS, Lefkothea-Stella K, *et al.* (2011) The Salmon in pregnancy study: study design, subject characteristics, maternal fish and marine *n*-3 fatty acid intake, and marine *n*-3 fatty acid status in maternal and umbilical cord blood. *Am J Clin Nutr* **94**, 1986S–1992S.
24. Matorras R, Ruiz JI, Perteagudo L, *et al.* (2001) Longitudinal study of fatty acids in plasma and erythrocyte phospholipids during pregnancy. *J Perinat Med* **29**, 293–297.
25. You L, Hong-Tian L, Leonardo T, *et al.* (2015) DHA in pregnant and lactating women from Coastland, Lakeland, and Inland Areas of China: results of a DHA Evaluation in Women (DEW) Study. *Nutrients* **7**, 8723–8732.
26. Zhang J, Wang Y, Meng L, *et al.* (2009) Maternal and neonatal plasma *n*-3 and *n*-6 fatty acids of pregnant women and neonates in three regions in China with contrasting dietary patterns. *Asia Pac J Clin Nutr* **18**, 377–388.
27. Liu MJ, Li HT, Yu LX, *et al.* (2016) A correlation study of DHA dietary intake and plasma, erythrocyte and breast milk dha concentrations in lactating women from Coastland, Lakeland, and Inland Areas of China. *Nutrients* **8**, 312–325.
28. Zhou YB, Li HT, Leonardo T, *et al.* (2017) A correlation study of DHA intake estimated by a FFQ and concentrations in plasma and erythrocytes in mid- and late pregnancy. *Nutrients* **9**, 1256–1270.

29. Government of Canada, Health Canada, Pest Management Regulatory Agency (2003) Assigning Values to Nondetected/Nonquantified Pesticide Residues in Food (Science Policy Notice SPN2003–02) (Health Canada). https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/science-policy-notes/2003/assigning-values-nondetected-nonquantified-pesticide-residues-food-spn2003-02.html (accessed July 2003).

30. The Environmental Protection Agency QA (2000) Guidance for Data Quality Assessment: Practical Methods for Data Analysis. https://www.epa.gov/sites/default/files/2015-06/documents/g9-final.pdf (accessed July 2000).

31. Zhou YB, Yan LL, Li HT, et al. (2021) Patterns and determinants of essential and toxic elements in chinese women at mid-pregnancy, late pregnancy, and lactation. Nutrients 13, 668.

32. Yang YX, Wang GY & Pan XC (2009) China Food Compotion Table, 2nd ed. Beijing, China: Peking University Medical Press.

33. Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Bid Chem 226, 497–509.

34. Shewhart WA & Wilks SS (2008) Multivariable Model-Building: A pragmatic approach to regression analysis based on fractional polynomials for modelling continuous variables. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9780470770771 (accessed April 2008).

35. Royston P, Ambler G & Sauerbrei W (1999) The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 28, 964–974.

36. Larque E, Demmelmair H, Gil-Sanchez A, et al. (2011) Placental transfer of fatty acids and fetal implications. Am J Clin Nutr 94, 1908s–1913s.

37. Hornstra G (2000) Essential fatty acids in mothers and their neonates. Am J Clin Nutr 71, 1262s–1269s.

38. Kuipers RS, Luxwolda MF, Sang WS, et al. (2011) Postpartum changes in maternal and infant erythrocyte fatty acids are likely to be driven by restoring insulin sensitivity and DHA status. Med Hypotheses 76, 794–801.

39. Arab L (2003) Biomarkers of fat and fatty acid intake. J Nutr 133, 925S–932S.

40. Vermunt SHF, Mensink RP & Hornstra SG (2000) Effects of dietary α-linolenic acid on the conversion and oxidation of13C-α-linolenic acid. Lipids 35, 137–142.

41. Salem N, Pawlosky R, Wegher B, et al. (1999) In vivo conversion of linoleic acid to arachidonic acid in human adults.
Prostaglandins Leukotrienes Essent Fatty Acids 60, 407–410.

42. Barrera C, Valenzuela R, Chamorro R, et al. (2018) The impact of maternal diet during pregnancy and lactation on the fatty acid composition of erythrocytes and breast milk of chilean women. Nutrients 10, 839.

43. Markhus MW, Rasinger JD, Malde MK, et al. (2015) Docosahexaenoic acid status in pregnancy determines the maternal docosahexaenoic acid status 3-, 6- and 12 months postpartum. results from a longitudinal observational study. PLoS One 10, e0136409.

44. Stark KD, Van Elswyk ME, Higgins MR, et al. (2016) Global survey of the n-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. Prog Lipid Res 63, 132–152.

45. Urwin HJ, Zhang J, Gao YX, et al. (2013) Immune factors and fatty acid composition in human milk from river/lake, coastal and inland regions of China. Br J Nutr 109, 1949–1961.

46. Wilson NA, Mantzioris E, Middleton PF, et al. (2020) Influence of sociodemographic, lifestyle and genetic characteristics on maternal DHA and other polyunsaturated fatty acid status in pregnancy: a systematic review. Prostaglandins Leukotrienes Essent Fatty Acids 152, 1–11.

47. Minda H, Molnar S, Burus I, et al. (2002) Effect of different types of feeding on fatty acid composition of erythrocyte membrane lipids in full-term infants. Acta Paediatr 91, 874–881.

48. Lim WY, Chong M, Calder PC, et al. (2015) Relations of plasma polyunsaturated fatty acids with blood pressures during the 26th and 28th week of gestation in women of Chinese, Malay, and Indian Ethnicity. Medicine 94, e571–e577.

49. Lisa J, Susan R, Luann J, et al. (2014) Intake of seafood in the US varies by age, income, and education level but not by race-ethnicity. Nutrients 6, 6060–6075.

50. Ghebremeskel K, Min Y, Crawford MA, et al. (2000) Blood fatty acid composition of pregnant and nonpregnant Korean women: red cells may act as a reservoir of arachidonic acid and docosahexaenoic acid for utilization by the developing fetus. Lipids 35, 567–574.

51. Pottala JV, Espeland MA, Polreis J, et al. (2012) Correcting the effects of –20°C storage and aliquot size on erythrocyte fatty acid content in the Women's Health Initiative. Lipids 47, 835–846.

52. Metherel AH, Henao JJA & Stark KD (2013) EPA and DHA Levels in Whole Blood Decrease More Rapidly when Stored at –20°C as Compared with Room Temperature, 4 and –75°C. Lipids 48, 1079–1091.