

Original Article

Maternal and neonatal plasma n-3 and n-6 fatty acids of pregnant women and neonates from three regions of China with contrasting dietary patterns

Jian Zhang MSc¹, Yiqun Wang MPhil², Liping Meng MSc¹, Chunrong Wang¹,
Wenhua Zhao PhD³, Junshi Chen MD¹, Kebreab Ghebremeskel FIBiol²,
Michael A Crawford PhD²

¹Institute of Nutrition and Food Safety, Chinese Centre for Disease Control and Prevention, Beijing, China

²Institute of Brain Chemistry & Human Nutrition, London Metropolitan University, London, UK

³Center of Chronic Disease Control, Chinese Centre for Disease Control and Prevention, Beijing, China

The aim of this study was to investigate n-3 and n-6 fatty acid status of pregnant women and neonates from River/Lake, Coastal and Inland regions of China. Demographic, nutritional and anthropometric data, as well as blood samples (maternal and cord) were obtained. Plasma choline phosphoglyceride (CPG) fatty acids were analysed. Median daily fatty acid intakes of the women from River/Lake, Coastal and Inland women were linoleic acid (LA), 20.2, 22.1, 31.7 g; arachidonic acid (AA), 157.2, 95.6, 141.3 mg; α -linolenic acid (ALA), 4.2, 1.0, 1.8 g; eicosapentenoic acid (EPA), 22.4, 28.6, 3.1 mg; docosahexaenoic acid (DHA), 51.7, 54.7, 33.3 mg and the n-6 to n-3 fatty acid ratio, 4.7, 20.9, 17.2. The median maternal and cord plasma CPG AA levels of River/Lake, Coastal and Inland groups were 7.3% and 15.7%, 6.7% and 16.1%, and 7.2% and 16.9%. The median maternal and neonatal DHA levels in the three regions were 3.2% and 4.7%, 3.0% and 4.3%, and 2.0% and 3.6%. There appears to be a close association between dietary intake of AA, EPA, DHA and the corresponding levels of the fatty acids in maternal plasma CPG ($p < 0.05$). The results of the study indicate low intake of the n-3 long chain polyunsaturated fatty acids, EPA and DHA, and n-6 to n-3 fatty acid imbalance are the main problems of dietary fat intake of Chinese pregnant women. Measures such as increased supply of oily fish and ALA-rich edible oils should be taken to help enhance n-3 fatty acid intake of pregnant Chinese women.

Key Words: docosahexaenoic acid, arachidonic acid, eicosapentenoic acid, Chinese pregnant women, neonate

INTRODUCTION

The long chain n-3 and n-6 polyunsaturated fatty acids, arachidonic (AA, 20:4 n6) and docosahexaenoic (DHA, 22:6 n3) play a crucial role in fetal growth and development. DHA accumulates in fetal tissues, particularly the central nervous system during the latter part of pregnancy.^{1,2} Adequate maternal intake of dietary DHA enhances visual acuity and neurodevelopment in infants,^{3,4} while reduced retinal and brain DHA are associated with decreased visual function, and impaired learning and behavioural alteration, respectively.⁵ Because of the accumulated published evidence and acceptance of the beneficial role of DHA in growth, development and health, interest on n-3 long chain polyunsaturated fatty acids, particularly DHA, has gained a wide ground in China.

It has been shown, maternal dietary intake of DHA mirrors closely with maternal plasma DHA and the status of the fatty acid in developing fetus.^{6,7} During the past twenty years, as the result of rapid economic development, conspicuous changes in dietary habits, primarily manifested by a significant increase of vegetable oil consumption, has occurred in China. Data from the National Nutrition Survey in China shows, between 1992 and 2002, vegetable oil intake of urban and rural dwellers increased by 24.1% and 74.8%, respectively. Although the situation

is not as serious as in the USA, this rapid dietary transition has precipitated n-6/n-3 imbalance because the vegetable oils consumed are high in the n-6 fatty acid, linoleic. The estimated n-6/n-3 fatty acid ratio of American and Chinese diets are 10:1⁹ and 5-7:1,¹⁰ respectively.

Although there has been marked improvement in health and wellbeing of pregnant women in recent years, concern still remains about the sufficiency of intake of certain vital nutrients. A lot of research has been done on nutrition problems associated with trace elements and vitamins, such as folate, in this population. However, there is a scarcity of fatty acid nutritional status data in Chinese pregnant women. The available published reports were restricted to a single local population and/or the sample sizes were small.¹¹⁻¹³

Corresponding Author: Dr Jian Zhang, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing 100050, China

Tel: +86-010-83132560; Fax: +86-010-83132021

Email: zhjian6708@yahoo.com.cn

Manuscript received 2 March 2009. Initial review completed 14 September 2009. Revision accepted 15 September 2009.

The aim of present study is to investigate fatty acid status of Chinese pregnant women from three regions with contrasting dietary patterns, and to obtain additional robust evidence for dietary guidelines for pregnant women.

MATERIAL AND METHODS

Subjects and recruitment

Healthy women (n=150) with uncomplicated singleton pregnancy were recruited from local hospitals of Jurong county, Jiangsu province (River/Lake, n=50), Jimo county, Shandong province (Coastal, n=50) and Hui county, Henan province (Inland, n=50) between 24 and 28 weeks of gestation (Fig 1). The exclusion criteria were: residency of less than 6 years, regular consumption of fatty acid supplements, hypertension, diabetes, and metabolic, renal, psychiatric and neurological disease.

Fasting maternal blood (5 ml) and demographic, clinical, nutritional and obstetric data from the mothers at gestation week 35, as well as cord blood (5 ml) and anthropometric information from the babies at delivery were collected. The study was approved by the Ethics Committee of the Institute of Nutrition & Food Safety, China Disease Control and Prevention Center, and written consent was obtained from the expectant mothers.

Dietary assessment

A semi-quantitative food frequency questionnaire (FFQ) which included 156 foods developed for our previous studies^{14,15} was used to estimate macro nutrients, fat and fatty acid intake at 28 and 35 week of gestation. The food questionnaires were completed by trained local hospital dietitians or nurses face-to-face with explanations and aid of some food models, bowls, plates and cups. Macro nutrients and fatty acids intakes were calculated using the Chinese Food Composition Table (version 2002).

Blood processing and fatty acid analysis

Maternal and cord blood specimens were collected in

heparin tubes and centrifuged at 1000g for 10 minutes at -4°C to separate plasma and red cells. The plasma was carefully transferred to another tube and stored at -20°C for less than one month and subsequently transported in dry ice to the Institute of Nutrition and Food Safety for storage at -70°C until analysis.

Plasma total lipids were extracted with the method of Folch et al¹⁶ by homogenising the samples in chloroform and methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) under nitrogen. Phosphoglyceride classes were separated by thin-layer chromatography on silica gel plates using the developing solvents chloroform, methanol, water (60:30:4 v/v) containing 0.01% BHT. The bands were detected by spraying the developed plate with a methanolic solution of 2,7 dichloro-fluorescein (0.01% w/v), visualized under ultraviolet light and identified by comparison with phospholipid standards (Beijing Chemical Reagent Ltd) and run on the same plate. The choline phosphoglyceride (CPG) band was scraped from the silica plate and transferred to a tube, sealed and methylated with 15% acetyl chloride in methanol by heating at 70°C for 3 hours under nitrogen. The resulting fatty acid methyl esters (FAMES) were separated by gas chromatography (Shimadzu GC 14B, Japan) fitted with a capillary column (50 m × 0.25 mm ID, 0.2 µm film, CP-SIL 88). Helium was used as a carrier gas at a flow rate of 2 ml/min. The split ratio, and injector and detector temperatures were 40:1, 260°C and 260°C, respectively. The oven programme had an initial temperature of 140°C which was held for 5 minutes and subsequently increased to 240°C at 4°C per minute. FAMES were identified by comparison with retention times of authentic standards (Sigma-Aldrich Ltd. UK) and peak areas were quantified with the use of computer data system (CBM-101 workstation).

Statistical analysis

Depending on distribution, the data are expressed as mean

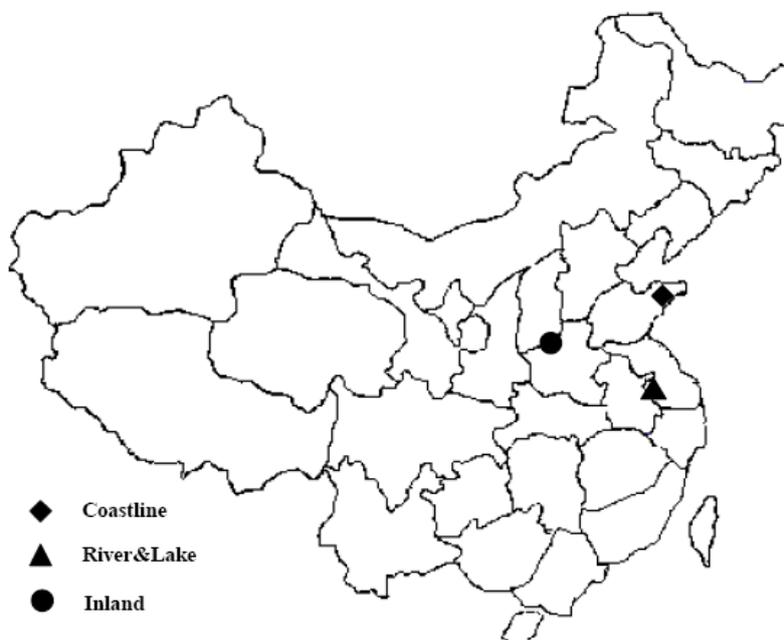


Figure 1. Schematic map of China and the three regions

and standard deviation (mean±SD), proportion and median and 25th (p25) 75th (p75) percentiles. Similarly, one-way ANOVA plus LSD, Kruskal-Wallis or Mann-Whitney *U* test were used to test inter-group differences. Spearman correlation coefficients were calculated to assess the relationship between dietary arachidonic acid (AA, 20:4 n-6), eicosapentenoic acid (EPA, 20:5 n-3) and DHA and the corresponding levels in maternal and cord plasma CPG. A *p*-value of less than 0.05 was considered statistically significant. All data were analysed with the use of the statistical software SPSS (version 11.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics

Demographic characteristics of the three groups of mothers and anthropometric measurements of their new-born babies are shown in Table 1. A total number of 47, 48, 43 pregnant women from River/Lake, Coastal and Inland regions, respectively, completed the study. The women from river/lake region were younger than their counterparts from coastal and inland regions (*p*<0.05). Similarly, they had lower body weight compared with the Coastal group at 28 and 35 weeks of gestation (*p*<0.05) and

inland women at 35 weeks of gestation (*p*<0.05). Moreover, in contrast to the Coastal and Inland groups, the women from River/Lake region delivered after a shorter gestational period (*p*<0.05).

The neonates of the Coastal women were longer (*p*<0.05) and heavier (*p*<0.05) than those of River/Lake and comparable to those of the inland. In contrast, the neonates of the Coastal group had shorter head circumference than those from the Inland region, and lower ratio of head circumference to length and head circumference to weight than their counterparts from the River/Lake and Inland regions (*p*<0.05). In addition, there was a higher proportion of male babies born to the Inland group in compared with that of Coastal group (*p*<0.05).

Animal foods, Macro nutrient and fatty acid intake

Major animal foods - As shown in Table 2, the aquatic foods intake among pregnant women from both River/Lake and Coastal region were higher than that of women from the Inland region (*p*<0.05) and the Coastal group had the highest seafood intake while the River/Lake group had the highest freshwater fish intake (*p*<0.05). Comparing to those from River/Lake and Inland regions, Coastal women had lower intake of eggs (*p*<0.05).

Table 1. Maternal demographic and neonatal anthropometric characteristics, Mean±SD

	River/Lake region (N=47)	Coastal region (N=48)	Inland region (N=43)
Pregnant women			
Age (y) †‡§	23.8±2.4	27.5±3.7	25.3±3.9
Height (cm)	160±4.0	161±4.3	160±4.3
BW (kg, 28 week) †	61.9±7.0	66.5±8.3	64.7±7.0
BW (kg, 35 week) †‡	64.9±7.2	69.7±8.8	69.6±7.2
Gestational age at delivery (wks) †‡	39.2±1.0	39.7±1.2	39.6±1.1
Supplements (%)	34.0	47.9	34.9
Neonates			
Birth length (cm) †	49.3±1.8	50.3±1.4	50.1±2.8
Birth weight (kg) †	3.33±0.40	3.49±0.38	3.44±0.39
Head circumference (cm) ‡§	33.7±1.0	33.2±1.2	34.5±1.86
Head circumference/birth weight (cm/kg) †§	10.2±1.1	9.6±1.0	10.1±1.1
Head circumference/birth length †§	0.68±0.02	0.66±0.03	0.69±0.05
Gender, male (%) §	57.4	54.2	67.4

† Significant difference between River/Lake and Coastal regions at *p*<0.05;

‡ Significant difference between River /Lake and Inland regions at *p*<0.05;

§ Significant difference between Coastal and Inland regions at *p*<0.05.

Table 2. Major animal foods intake of pregnant women in three regions (g/d, median, p25, p75)

Animal foods	River/Lake region (N=47)		Coastal region (N=48)		Inland region (N=43)	
	Median	(P 25, P 75)	Median	(P 25, P 75)	Median	(P 25, P 75)
Meat	70.4	(50.0,111)	57.8	(24.3,110)	67.6	(33.7,126)
Aquatic foods † ‡§	100	(60.7,159)	116.2	(81.6,208)	9.2	(0.8,21.8)
Seafoods †‡§	10.7	(0.0,35.7)	112.3	(76.7,200)	0.8	(0.0,4.3)
Freshwater fish †‡	81.2	(42.9,116)	3.3	(0.0,14.3)	7.5	(0.0,17.1)
Egg †§	60.0	(41.3,82.5)	34.3	(13.6,60.0)	72.9	(38.6,111)
Milk †	125	(30.0,220)	187.2	(125,250)	150	(102,286)

† Significant difference between River/Lake and Coastal regions at *p*<0.05;

‡ Significant difference between River /Lake and Inland regions at *p*<0.05;

§ Significant difference between Coastal and Inland regions at *p*<0.05.

Table 3. Macro-nutrient and fatty acid intake of pregnant women (median, p25, p75)

	River/Lake region (N=47)		Coastal region (N=48)		Inland region (N=43)	
	Median	(P 25, P 75)	Median	(P 25, P 75)	Median	(P 25, P 75)
Energy	11.9	(10.4,13.9)	12.3	(10.9,14.2)	12.4	(11.3,14.7)
Protein [§]	98.5	(78.4,111)	101	(88.7,119)	88.5	(78.7,112)
% of energy	13.4		13.8		12.4	
Fat ^{†§}	94.1	(73.0,121)	79.8	(64.2,94.7)	98.5	(82.7,129)
% of energy	29.9		24.1		31.2	
Carbohydrate [†]	402	(339,498)	443.4	(406,519)	421	(371,514)
% of energy	56.5		62.4		56.8	
12:0 (g)	0.1	(0.1,0.2)	0.1	(0.1,0.2)	0.1	(0.1,0.2)
14:0 (g)	0.9	(0.5,1.2)	0.9	(0.6,1.1)	0.9	(0.6,1.4)
16:0 (g) ^{‡§}	12.2	(8.8,15.8)	12.8	(10.5,15.0)	15.8	(12.8,21.1)
18:0 (g) ^{†§}	5.2	(3.6,6.1)	4.2	(3.1,5.3)	5.8	(4.1,7.7)
18:1 n-9 (g) ^{‡§}	25.5	(18.8,32.4)	29.0	(23.6,34.1)	36.4	(30.6,45.9)
22:1 n-9 (g) ^{†‡§}	11.6	(8.1,13.9)	0.05	(0.03,0.08)	0.08	(0.04,0.13)
18:2 n-6 (g) ^{‡§}	20.2	(14.5,31.3)	22.1	(18.8,26.4)	31.7	(24.3,37.2)
20:4 n-6 (mg) ^{†§}	157	(129,209)	95.6	(47.8,149)	141	(102,210)
18:3 n-3 (g) ^{†‡§}	4.2	(3.3,5.2)	1.0	(0.8,1.4)	1.8	(1.3,2.4)
20:5 n-3 (mg) ^{†‡§}	22.4	(8.1,44.9)	28.6	(17.1,58.9)	3.1	(0.4,8.4)
22:5 n-3 (mg) ^{†‡§}	5.5	(2.1,9.6)	8.8	(3.4,16.9)	0.7	(0.1,1.7)
22:6 n-3 (mg) ^{‡§}	51.7	(33.8,79.1)	54.7	(40.7,84.5)	33.3	(21.2,50.6)
SFA (g) ^{‡§}	21.5	(15.7, 25.3)	20.2	(16.2,23.3)	24.5	(19.0,33.6)
MUFA (g) ^{†§}	34.9	(29.2,44.4)	29.0	(23.7,34.1)	36.5	(30.7,46.0)
PUFA (g) ^{‡§}	24.6	(18.4,36.4)	23.2	(20.1,28.6)	33.6	(26.1,39.0)
Total n6 (g) ^{‡§}	20.3	(14.6,31.5)	22.2	(18.9,26.5)	31.9	(24.5,37.3)
Total n3 (g) ^{†‡§}	4.3	(3.4,5.3)	1.1	(0.9,1.5)	1.9	(1.3,2.4)
n6_n3 ratio ^{†‡§}	4.7	(3.6,6.4)	20.9	(15.9,25.3)	17.2	(13.0,19.1)

[†] Significant difference between River/Lake and Coastal regions at $p < 0.05$;

[‡] Significant difference between River/Lake and Inland regions at $p < 0.05$;

[§] Significant difference between Coastal and Inland regions at $p < 0.05$.

Micro-nutrients – As shown in Table 3, the Coastal group had higher protein intake than those of Inland and higher carbohydrate intake than those of River/Lake region ($p < 0.05$), as well as lower fat ($p < 0.05$) intake than their counterparts in the other two regions ($p < 0.05$).

Fatty acids – Dietary fatty acid intakes of pregnant women from the three regions are shown in Table 3. Of the three groups of women, those from the Inland region had the highest intake of palmitic acid (16:0), stearic acid (18:0), total saturated acids (SFA), oleic acid (18:1 n-9), linoleic acid (LA, 18:2 n-6) and total n-6 polyunsaturated fatty acids ($p < 0.05$). Whereas, those from River/Lake region had the highest intake of euricic acid (22:1 n-9), AA, α -linolenic (ALA, 18:3 n-3) and total n-3 polyunsaturated fatty acids ($p < 0.05$). In contrast, women from the Coastal region consumed more long chain n-3 polyunsaturated fatty acids, EPA and docosapentaenoic acid (DPA, 22:5 n-3) ($p < 0.05$). The intake of DHA in River/Lake and coastal groups was comparable ($p > 0.05$), and both higher than that of the Inland group ($p < 0.05$). Pregnant women from the River/Lake region had the lowest while those from the Coastal region had highest dietary n-6 to n-3 fatty acid ratio ($p < 0.05$).

Maternal and cord plasma choline phosphoglyceride fatty acids

Plasma CPG fatty acids of the pregnant women from the three regions are shown in Table 4. Compared with that in

the Coastal group, the percentage level of 18:0, eicosanoic acid (20:0), LA, n-6 PUFA, PUFA and essential fatty acid index (EFA index) were lower while 16:0, 18:1, AA, ALA, SFA and n-3 PUFA were higher in the River/Lake group ($p < 0.05$). When compared with that in the Inland group, the levels of LA, dihomogamma linolenic acid (DHGLA, 20:3 n-6), docosapentaenoic acid (22:5 n-6), n-6 PUFA, PUFA, EFA index and cervonic acid deficiency index (CADI) were lower while 18:1, EPA, DPA, DHA, MUFA, and cervonic acid sufficiency index (CASI) were higher in the River/Lake group ($p < 0.05$). The Inland group had higher levels of 16:0, DHGLA, AA, 22:5 n-6, SFA, n-6 PUFA, PUFA, EFA index, CADI while lower level of EPA, DHA, MUFA, n-3 PUFA, CASI than that found in the Coastal group.

Percent fatty acid composition of plasma CPG of neonates born to the women from the Inland, River/Lake and Coastal regions is presented in Table 5. Compared with the newborn babies from the River/Lake, and Coastal regions, the neonates of the inland group had elevated levels of AA, docosatetraenoic acid (22:4 n-6), 22:5 n-6 ($p < 0.05$), and reduced level of EPA, DPA, DHA and CASI ($p < 0.05$). The neonates of the River/Lake group had higher level of oleic acid, ALA, EPA, MUFA and the CASI while lower levels of 22:4 n-6, n-6 PUFA, and EFA index than that of the coastal group ($p < 0.05$).

Table 4. Fatty acids Profile in maternal plasma choline phosphoglycerides (w/w %)

	River/Lake region (N=47)		Coastal region (N=48)		Inland region (N=43)	
	Median	(P 25, P 75)	Median	(P 25, P 75)	Median	(P 25, P 75)
14:0	0.40	(0.29,0.53)	0.37	(0.26,0.48)	0.38	(0.31,0.51)
16:0 ^{†§}	34.6	(33.1,36.4)	33.7	(31.7,35.1)	35.0	(33.3,36.8)
18:0 [†]	8.9	(8.1,9.4)	9.2	(8.6,9.8)	9.2	(8.5,10.1)
20:0 ^{†§}	0.06	(0.04,0.08)	0.10	(0.07,0.26)	0.06	(0.04,0.17)
16:1 n-7	0.92	(0.66,1.24)	0.85	(0.45,1.16)	0.89	(0.72,1.30)
18:1 n-9 ^{†‡§}	13.5	(11.2,16.0)	11.3	(8.8,13.5)	9.8	(8.0,10.4)
18:2 n-6 ^{†‡}	19.1	(17.6, 24.2)	24.6	(22.0,27.5)	25.0	(23.4,27.9)
20:3 n-6 ^{‡§}	3.0	(2.4,3.4)	2.8	(2.4,3.6)	3.5	(3.1,4.1)
20:4 n-6 ^{†§}	7.3	(6.0,8.1)	6.7	(5.4,7.6)	7.2	(6.5,7.9)
22:4 n-6	0.33	(0.20,0.49)	0.29	(0.21,0.47)	0.40	(0.23,0.55)
22:5 n-6 ^{‡§}	0.42	(0.34,0.55)	0.38	(0.30,0.56)	0.72	(0.64,0.96)
18:3 n-3 ^{†‡}	0.55	(0.19,0.79)	0.18	(0.12,0.26)	0.20	(0.14,0.28)
20:5 n-3 ^{†‡§}	0.32	(0.20,0.50)	0.20	(0.12,0.34)	0.10	(0.07,0.16)
22:5 n-3 [‡]	0.48	(0.23,0.60)	0.29	(0.18,0.51)	0.24	(0.19,0.36)
22:6 n-3 ^{‡§}	3.2	(2.6,3.8)	3.0	(2.5,3.7)	2.0	(1.6,2.5)
SFA ^{†§}	44.8	(42.8,46.8)	43.7	(42.1,45.3)	45.3	(43.5,46.7)
MUFA ^{†‡§}	14.8	(11.9,17.5)	12.6	(9.5,15.0)	10.9	(8.8,11.6)
Total n-6 ^{†‡§}	31.6	(28.6,34.5)	35.6	(34.0,37.7)	38.5	(36.6,39.9)
Total n-3 ^{†‡§}	4.6	(3.8,5.5)	3.9	(3.2, 4.7)	2.7	(2.3,3.2)
PUFA ^{†‡§}	36.9	(34. 3,38.8)	39.6	(38.5,40.9)	41.4	(39.5,42.2)
EFA index ^{†‡§}	2.5	(2.1,3.1)	3.2	(2.6,4.1)	3.8	(3.5,4.4)
CADI ^{‡§}	1.4	(1.1,1.8)	1.3	(0.9,1.9)	1.6	(1.4,2.1)
CASI ^{‡§}	7.7	(6.0,9.4)	8.1	(4.9,11.0)	2.7	(1.9,3.0)

EFA index: total (n-3 + n-6)/total (n-7+n-9); CADI: cervonic acid deficiency index, 22:5 n-6/22:4 n-6;

CASI: cervonic acid sufficiency index, 22:6n-3/22:5n-6.

[†] Significant difference between River/Lake and Coastal region at $p < 0.05$;

[‡] Significant difference between River/Lake and Inland region at $p < 0.05$;

[§] Significant difference between Coastal and Inland region at $p < 0.05$.

Table 5. Fatty acids profile in neonatal plasma choline phosphoglycerides (w/w %)

	River/Lake region (N=47)		Coastal region (N=48)		Inland region (N=43)	
	Median	(P 25, P 75)	Median	(P 25, P 75)	Median	(P 25, P 75)
14:0	0.34	(0.26, 0.42)	0.38	(0.28, 0.50)	0.36	(0.30, 0.43)
16:0	31.7	(30.1, 34.4)	31.3	(28.2, 33.6)	31.5	(30.2, 34.6)
18:0 [‡]	13.6	(12.7, 14.3)	14.3	(12.9, 15.1)	14.7	(13.8, 15.4)
20:0	0.11	(0.03, 0.20)	0.12	(0.02, 0.25)	0.10	(0.03, 0.13)
16:1n-7	0.82	(0.63,1.05)	0.79	(0.56, 1.15)	0.95	(0.70, 1.16)
18:1n-9 ^{†‡}	10.2	(8.8, 11.0)	8.9	(7.7, 10.3)	8.8	(7.5, 9.9)
18:2n-6	8.0	(7.2, 9.0)	8.3	(7.5,9.6)	7.9	(7.0, 9.2)
20:3n-6	4.6	(3.9, 5.4)	4.9	(4.3, 5.5)	5.0	(4.3, 5.3)
20:4n-6 ^{‡§}	15.7	(13.6, 17.1)	16.1	(14.7, 17.3)	16.9	(15.5, 18.4)
22:4n-6 ^{†‡§}	0.41	(0.21, 0.48)	0.45	(0.39, 0.55)	0.61	(0.49, 0.78)
22:5n-6 ^{‡§}	0.60	(0.49, 0.77)	0.72	(0.51, 0.88)	1.27	(1.00, 1.48)
18:3n-3 ^{†‡§}	0.08	(0.05, 0.27)	0.02	(0.00, 0.04)	0.05	(0.00, 0.08)
20:5n-3 ^{†‡§}	0.37	(0.29, 0.45)	0.18	(0.09, 0.29)	0.09	(0.06, 0.12)
22:5n-3 [‡]	0.42	(0.26, 0.52)	0.22	(0.14, 1.08)	0.19	(0.13, 0.37)
22:6n-3 ^{‡§}	4.7	(4.0, 5.3)	4.3	(3.6, 5.4)	3.6	(3.0, 4.5)
SFA	46.5	(44.4, 49.3)	46.7	(43.3, 49.1)	47.5	(45.6, 50.0)
MUFA ^{†‡}	11.1	(9.5, 12.3)	10.0	(8.3, 11.2)	9.9	(8.4, 11.2)
Total n-6 ^{†‡§}	30.1	(28.2, 32.5)	31.9	(30.3, 32.9)	32.4	(31.4, 34.5)
Totaln-3 ^{‡§}	5.8	(5.2, 6.7)	5.3	(4.2, 6.7)	4.0	(3.2, 4.9)
PUFA	35.6	(33.7, 37.9)	37.2	(35.6, 39.2)	37.0	(35.2, 39.1)
EFA index ^{†‡}	3.2	(2.9, 4.0)	3.7	(3.1, 4.8)	3.7	(3.3, 4.4)
CADI [§]	1.5	(1.2, 2.4)	1.5	(1.0, 1.8)	1.8	(1.5, 2.5)
CASI ^{†‡§}	7.6	(5.8, 9.5)	5.9	(4.4, 8.3)	2.8	(2.3, 3.6)

EFA index: total(n-3 + n-6)/total(n-7+n-9); CADI: cervonic acid deficiency index, 22:5n-6/22:4n-6;

CASI: cervonic acid sufficiency index, 22:6n-3/22:5n-6.

[†] Significant difference between River/Lake and Coastal region at $p < 0.05$;

[‡] Significant difference between River/Lake and Inland region at $p < 0.05$;

[§] Significant difference between Coastal and Inland region at $p < 0.05$.

Relation between dietary and plasma CPG AA and DHA

Data from three regions were pooled and Spearman correlation was tested for relation between diet, maternal and cord plasma CPG AA, EPA and DHA level. Maternal dietary AA, EPA, DHA intake showed positive correlations with the corresponding plasma CPG level (Figure 2-

1, AA $r=0.170$, $p<0.05$; EPA $r=0.301$, $p<0.05$; DHA $r=0.257$, $p<0.05$). Maternal plasma AA, EPA, DHA level also showed positive association with the corresponding cord plasma CPG level (Figure 2-2, AA $r=0.260$, $p<0.05$; EPA $r=0.403$, $p<0.05$; DHA $r=0.469$, $p<0.05$)

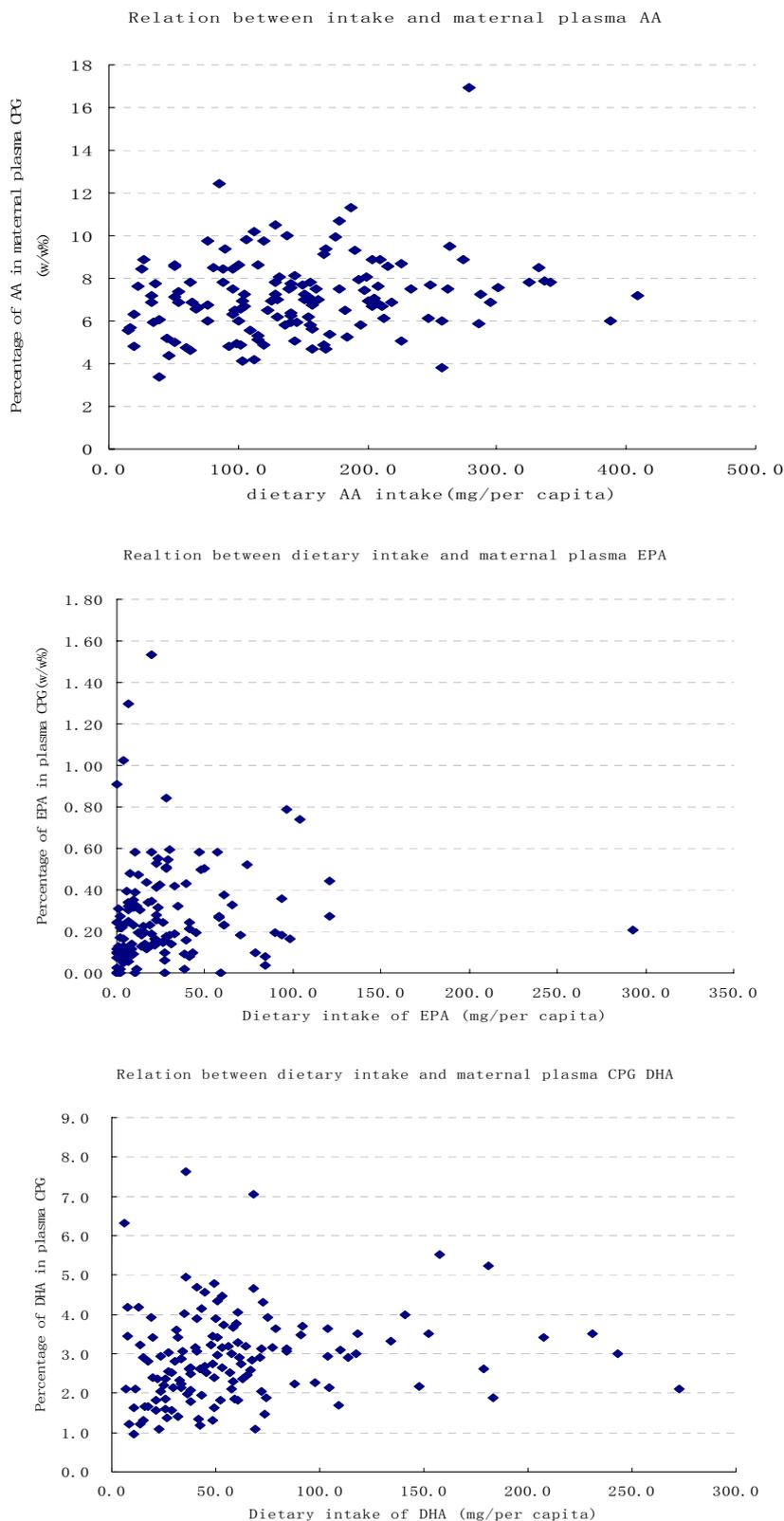


Figure 2-1. Relations between dietary intakes and maternal plasma CPG concentrations of arachidonic acid (AA; 20:4 n-6) $r=0.170$, $p<0.05$; eicosapentaenoic acid (EPA; 20:5 n-3) $r=0.301$, $p<0.05$; docosahexaenoic acid (DHA; 22:6 n-3) $r=0.257$, $p<0.05$, $n=138$.

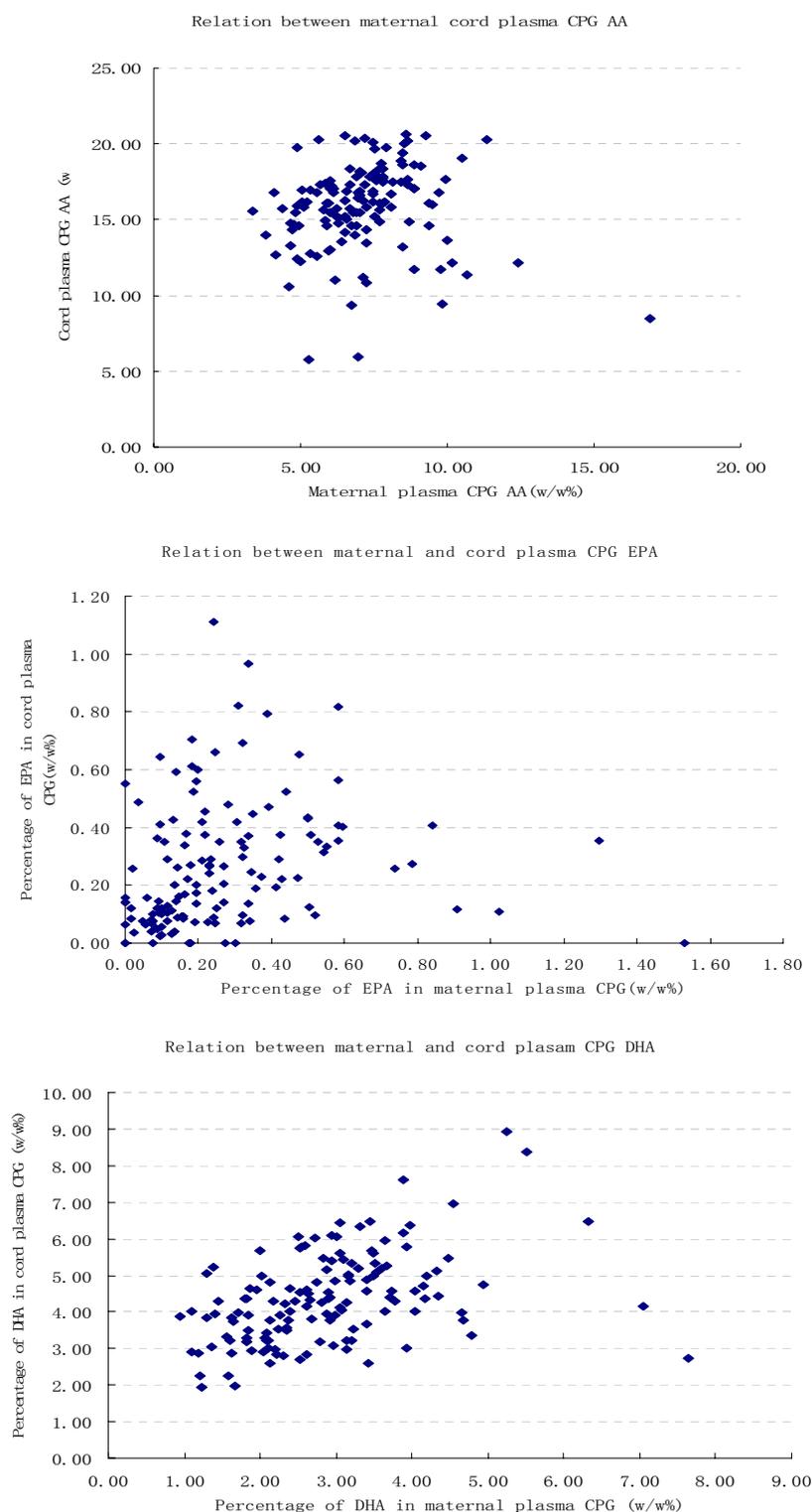


Figure 2-2. Relations between dietary maternal and cord plasma CPG concentrations of arachidonic acid (AA; 20:4 n-6) $r=0.260$, $p<0.05$; eicosapentaenoic acid (EPA; 20:5 n-3) $r=0.403$, $p<0.05$; docosahexaenoic acid (DHA; 22:6 n-3) $r=0.469$, $p<0.05$, $n=138$.

DISCUSSION

The three investigated regions are small cities of middle class economic level with different dietary patterns, in particular, for aquatic foods intake. Pregnant women from these three regions had access to adequate foods supply but had a relatively low dairy intake and very low intake of aquatic foods intake among women residing in the Inland region. Thus the energy and macronutrients intake

meet the recommendation for Chinese pregnant women¹⁷ and association between low birth weight and inadequacy in terms of intake of some foods, as reported in other countries, were not observed in the present study.¹⁸

Similar to that in the general population, the saturated to monounsaturated to polyunsaturated (S:M:P) fatty acid ratio of pregnant women is comparable, 1:1.6:1.1, 1:1.4:1.1 and 1:1.5:1.4 for the River/Lake, Coastal and Inland

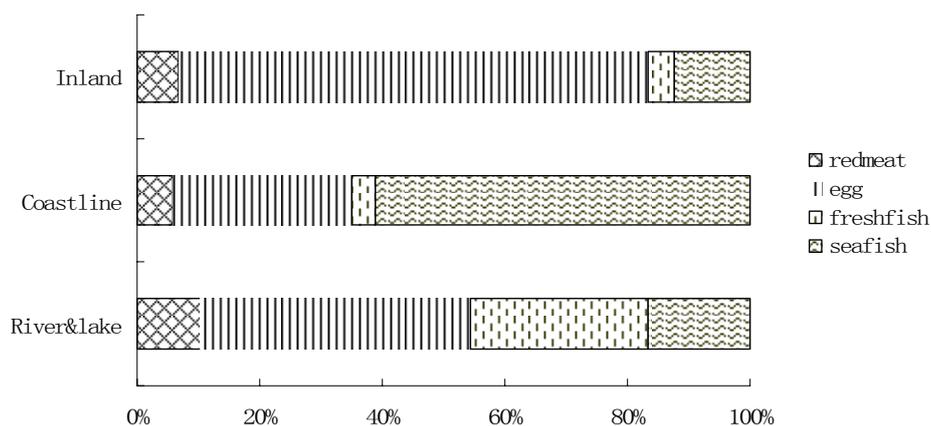


Figure 3-1. Food source of dietary docosahexaenoic acid (%)

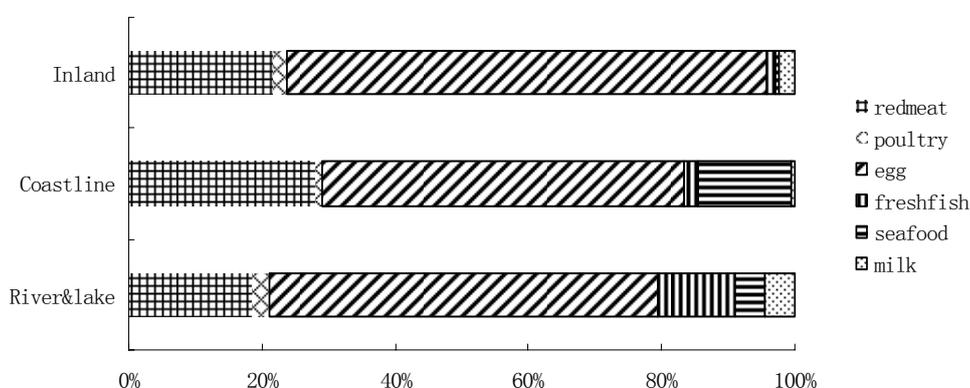


Figure 3-2. Food source of dietary arachidonic acid (%)

groups respectively. However, the median n-6:n-3 PUFA ratio reached 17.2 and 20.9 among the Inland and Coastal groups, which were much higher than the recommended ratio of 4-6:1 and the ratio of the general population in China.¹⁰ Different from that found in the other two regions, the median n-6:n-3 PUFA ratio of the River/Lake group is 4.7 and the 25th and 75th percentiles are 3.6, 6.4 respectively. This is largely due to the high intake of ALA. It had been reported that the mean intake of ALA is 1.6 g/d, 1.0 g/d, 1.0 g/d, 1.4 g/d in pregnant women residing in the United States, Canada, Holland and Belgium.^{9,19-21} In the present study, pregnant women residing in Inland and Coastal regions had 1.8 g/d and 1.0 g/d ALA intake, which is comparable to that in other populations but lower than the level recommended by the International Society for the Study of Fatty Acids and Lipids (ISSFAL) (0.7 energy%). River/Lake groups had a median intake of 4.2 g/d ALA, which was much higher than that in other populations and recommended level, and thus had a rational dietary n-6:n-3 PUFA ratio.

ALA had been shown to be further metabolized by D-6 desaturation, elongation, and D-5 desaturation to EPA, and then involving 2 sequential elongations of EPA to 6,9,12,15,18,21-tetracosenoic (24:6 n-3), followed by transport to the peroxisomes, and then a single cycle of β -oxidation to yield DHA.²² Animal studies indicated that

ALA supplementation resulted in the accretion of ALA-derived DHA in the brains of baboon fetuses.²³ But some supplemental studies in human indicated that increased dietary intakes of ALA by supplementation did not increase DHA in the blood of either pregnant women nor their newborn infants.²⁴ However, Otto *et al.* obtained some indications that LCP synthesis from EFA precursors may be enhanced during pregnancy²⁵ and Burdge GC, *et al.* showed that although endogenous DHA synthesis from dietary ALA is limited in men, this conversion capacity is intensified in young women.²⁶⁻²⁸ In the present study, in addition to higher ALA intake, the River/Lake group had a comparable DHA intake to the Coastal group and their maternal plasma CPG DHA level is similar to that of the Coastal group. It seems that a high intake of ALA do not promote increasing DHA levels in maternal plasma. However, the river/lake group had significant lower EPA, DPA intake but higher corresponding plasma CPG level in comparison with the coastal group. This suggests that high ALA intake may increase long chain n-3 fatty acids concentrations such as EPA and DPA in the plasma.

Rapeseed oil had been shown to promote myocardial lesions in male adult rats but the same effect was not observed in a piglet model later.^{29,30} In spite of the controversy, it is a commonly consumed edible oil in the

River/Lake region and is also the predominant source of ALA since it contains $\approx 8\%$ ALA. In contrast, the commonly consumed edible oil in the Inland and Coastal regions is peanut oil which contains less than 0.5% ALA while nearly 40% LA. In the present study, the median LA intake is 22.1 g/d and 31.7 g/d among Coastal and Inland groups, much higher than the recommended level of 2% of total energy by ISSFAL and that in some Western populations (11.2-22.5 g/d). The maximum conversion of ALA to EPA and DHA are critically dependent on the amount of LA in the diet since when dietary LA is increased to 30 g/d, conversion of ALA to n-3 LCPUFA is reduced by $\approx 40\%$.³¹ Therefore, in order to attain optimum conversion of ALA to DHA, it is necessary to suggest pregnant women to increase the consumption of vegetable oils with relatively high ALA content such as rapeseed oil at the expense of other LA rich oils. Blended edible oil products containing a mixture of a variety of edible oils with rational fatty acids ratio, particularly with the emphasis on n-3 fatty acids, are available in many big cities. In addition, several varieties of locally consumed edible oil such as flaxseed oil ($\approx 50\%$ ALA), perilla oil ($\approx 60\%$ ALA) have been developed as commercial edible oils and being introduced to consumers. Hopefully, with these efforts, the intake of n-3 PUFA, mainly ALA will significantly increase and the reduction of the n-6 to n-3 ratio will be witnessed in the future.

It is well known that maternal intake of DHA during pregnancy modulates the DHA status of neonates and the development of their nervous system. The rapid transition in food supply urges people to do more research to address the current status of dietary nutrients intake including DHA. Based on current available scientific evidence, the EU workshop in the European research project Perilip proposed that pregnant women should aim to achieve an average dietary intake of at least 200 mg DHA/d.³² Results from other groups of pregnant women showed that the amount of dietary DHA is in the range of 80-300 mg/d and a study in pregnant women of three European cohorts showed that 90% of subjects reached the DHA recommended intake of 200 mg per day.³³⁻³⁵ The food source of dietary DHA in figure 3-1 showed that as the predominant food source of n-3 long chain polyunsaturated fatty acids, seafoods contributed more than 60% of DHA in the diet of pregnant women in coastal areas. However, as a large proportion of seafoods consumed are shrimp, scallop, and lean seafish, the median maternal DHA intake in this area was just about 55 mg/d, far below the recommended level. The DHA intake of the River/Lake group was comparable to that of the coastal group, but largely due to consumption of lean freshwater fish. The percentage of DHA from aquatic foods is less than 50%, 29.1% from fresh water fish and 16.6% from seafoods. Unlike that of other populations, egg was an important food source of DHA in these pregnant women's diets as it contributed 43.9%, 76.6% of dietary DHA for the River/Lake, Inland groups respectively. Similar to the results from other studies, a close link between total diet DHA intake and maternal plasma DHA levels was observed in this study and the association between maternal and umbilical plasma DHA concentration appears to be more significant. This suggest that increasing total dietary

DHA intake will benefit the improvement of n-3 long chain unsaturated fatty acids status. As an economical and high nutrient dense food, egg is largely consumed in China, particularly by pregnant women and infants. Result from the present study showed that, on average, approximately one egg per day was consumed among the River/Lake and Inland groups. It seems impractical to significantly increase DHA intake by continuing to emphasize on egg consumption.

Due to high content of n-3 long chain fatty acids, oily fish consumption are considered to be a more effective way to increase dietary DHA and have been proved to be closely associated with serum DHA concentrations.³⁶ Some oily fish such as salmon, halibut, mackerel, herring are rich sources of EPA and DHA. Data from this study indicated that the average intake of aquatic foods is about 100 g per day, but due to the lack of advanced nutrition knowledge, food habit, limited supply, most pregnant women did not consume oily fish as an important part of their diet. Therefore, it is necessary to let more pregnant women know about the health benefits of oily fish and enlarge supply of these kinds of foods so as to effectively increase dietary DHA and EPA intake.

Initial brain AA-accretion is also critical for cognitive outcome and balanced intake of DHA and AA is important.³⁷ In the present study, dietary AA intake also influenced the corresponding plasma level in both the mother and neonate, and a significant association between them was observed. The median AA intake was from 95.6 mg to 157 mg, which is comparable to the level reported from Australia and Canada.^{19,38} Different from that in other populations, egg was the predominant food source of AA, 58.5% for the River/Lake, 54.4% for the Coastal and 72.0% for the Inland groups (Figure 3-2).

It should be stated that the survey site for Coastal pregnant women in present study is in the central of China's coastline where a different dietary pattern and fish varieties from that in the coastal region of South China have been observed. Therefore, it is inappropriate to take this data as the representative of all coastal regions of China. In spite of this, the present study provides more evidence in terms of long chain unsaturated fatty acids among pregnant women in China. In 2005, according to the contract with the European Commission, recommendations of DHA and n-3 LCPUFA for pregnant women were developed from the European research project Perilip. In contrast, the recommendations of long chain polyunsaturated fatty acid intake was not included in the recently released Chinese Dietary Guidelines for pregnant, lactation women, as well as those for children aged 0-6 years. Thus, it is necessary, in the near future, to propose recommended intakes for n-6 and n-3 fatty acids on the basis of evidence from the Chinese population.

In conclusion, we showed that the ratio of saturated, monounsaturated and polyunsaturated fatty acids (S:M:F) in the diet of Chinese pregnant women is in a rational range, whereas the ratio of n-6 to n-3 fatty acid is high above the recommended value in the coastal and inland regions. DHA and EPA intakes are significantly lower than the recommended level among all pregnant women. The health importance of n-3 PUFA intake among pregnant women should be highlighted and more research

projects to address recommendations of fatty acids intake for the Chinese population should be undertaken. Practical approaches for improvement in consideration of the different dietary patterns should be formulated.

ACKNOWLEDGEMENTS

This research was funded by the Danone Nutrition Education Fund in China. We thank all the pregnant women who participated in this study, as well as the doctors and midwives in three local hospitals and related staff of the three local CDCs.

AUTHOR DISCLOSURES

Li Jiang, Rong Hua, Rong Yu, Jun Yang, Xueping Lu, no conflicts of interest.

REFERENCES

- Crawford MA, Hassam AG, Stevens PA. Essential fatty acid requirements in pregnancy and lactation with special reference to brain development. *Prog Lipid Res.* 1981;20:31-40.
- Innis SM. Essential fatty acids in growth and development. *Prog Lipid Res.* 1991;30:39-103.
- O'Connor DL, Auestad N, Jacobs J. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective randomized controlled trial. *Pediatrics.* 2001;108:359-72.
- Jacobson JL, Jacobson SW, Muckle G, Kaplan-Estrin M, Ayotte P, Dewailly E. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the inuit of arctic Quebec. *J Pediatr* 2008;152:356-64.
- SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics.* 2000;105:1292-8.
- De Vriese SR, Matthys C, De Henauw S, De Backer G, Dhont M, Christophe AB. Maternal and umbilical fatty acid status in relation to maternal diet. *Prostaglandins Leukot Essent Fatty Acids.* 2002;67:389-96.
- Ghebremeskel K, Crawford MA, Lowy C, Min Y, Thomas B, Golfetto I, D Bitsanis. Arachidonic and docosahexaenoic acids are strongly associated in maternal and neonatal blood. *Euro J Clin Nutr.* 2000;54:50-6.
- Zhai FY, Yang XG. China Nutrition and Health Survey in 2002, Book II-Foods and Nutrients intake PMPH, 2006 ISBN 7-117-07565-1/R. 7566.(in Chinese)
- Kris-Etherton PM, Taylor DS, Yu-Poth SM, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao GX, Etherton TD. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr.* 2000;71(Suppl1):179S-188S.
- Zhang J, Wang CR, Gao JQ, Li XW, Chen JS. Study on dietary lipid intakes in Chinese Residents. *Acta Nutrimenta Sinica.* 2004;26:167-71. (in Chinese)
- Zhao ZZ, Xue FQ, Xu H, Sun XL, Li LJ. Effect of maternal docosahexaenoic acid on the intelligence development of normal term babies. *Chin J Pract Gynecol Obstet.* 2000;7:413-5. (in Chinese)
- Zhang Y, Liu Z, Tian LY, Zhang ST, Zhang BH. Factors relating to the long chain polyunsaturated fatty acid levels in preterm infants. *Chin J Pediatrics.* 1999;37:73-5. (in Chinese)
- Li HJ, Liu DH. Analysis of long-chain fatty acids composition of maternal and neonatal plasma. *China Public Health.* 2001;17:6-7. (in Chinese)
- Zhao W, Hasegawa K, Chen J. The use of food-frequency questionnaires for various purposes in China. *Public Health Nutr.* 2002;5:829-33.
- Li YP, Song J, Pan H, Yao MJ, Hu XQ, Ma GS. Validity of food frequency questionnaire to investigate the dietary energy and nutrients intake. *Acta Nutrimenta Sinica.* 2006;28:143-7.
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem.* 1957;226:497-509.
- Dietary Guideline for Chinese pregnant, lactating women and children aged 0-6y. China Nutrition Society PMPH, 2008 ISBN 978-7-117-09920-2/R. 9921. (in Chinese)
- Muthayya S, Dwarkanath P, Thomas T, Ramprakash S, Mehra R, Mhaskar A. The effect of fish and omega-3 LCPUFA intake on low birth weight in Indian pregnant women. *Eur J Clin Nutr.* 2009;63:340-6.
- Innis SM, Elias SL. Intake of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women. *Am J Clin Nutr.* 2003;77:473-8.
- Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Changes in the maternal essential fatty acid profile during early pregnancy and the relation to diet. *Am J Clin Nutr.* 2001;73:302-7.
- De Vriese SR, De Henauw S, De Backer G, Dhont M, Christophe AB. Estimation of dietary fat intake of Belgian pregnant women. Comparison of two methods. *Ann Nutr Metab.* 2001;45:273-8.
- Sprecher H, Chen Q, Yin FQ. Regulation of the biosynthesis of 22:5n-6 and 22:6n-3: complex intracellular process. *Lipids.* 1999;34:S153-6.
- Greiner RC, Winter J, Nathanielsz PW, Brenna JT. Brain docosahexaenoate accretion in fetal baboons: bioequivalence of dietary alpha-linolenic and docosa-hexaenoic acids. *Pediatr Res.* 1997;42:826-34.
- de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of alpha linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. *Am J Clin Nutr.* 2004;79:251-60.
- Otto SJ, Houwelingen AC, Antal M, Manninen A, Godfrey K, Lopez-Jaramillo P, Hornstra G. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. *Eur J Clin Nutr.* 1997;51:232-42.
- Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of α -linolenic acid metabolism in young men. *Br J Nutr.* 2002;88:355-63.
- Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr.* 2003; 90:993-4.
- Williams CM, Burdge GC. Long-chain n-3 PUFA: plant v marine sources. *Proc Nutr Soc.* 2006;65:42-50.
- Clandinin MT, Yamashiro S. Dietary factors affecting the incidence of dietary fat-induced myocardial lesions. *J Nutr.* 1982;112:825-8.
- Kramer JK, Farnworth ER, Johnston KM, Wolynetz MS, Modler HW, Sauer FD. Myocardial changes in newborn piglets fed sow milk or milk replacer diets containing different levels of erucic acid. *Lipids.* 1990;25:729-37.
- Emken EA, Adolf RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and ALAs in young adult males. *Biochim Biophys Acta.* 1994;1213:277-88.
- Koletzko B, Cetin I, Brenna JT, Perinatal Lipid Intake Working Group, Child Health Foundation, Diabetic Pregnancy Study Group et al. Dietary fat intakes for pregnant and lactating women. *Br J Nutr.* 2007;98:873-7.
- Denomme J, Stark KD, Holub BJ. Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women

- are lower than current dietary recommendations. *J Nutr.* 2005;135:206-11.
34. Kris-Etherton PM, Grieger JA, Etherton TD. Dietary reference intakes for DHA and EPA. *Prostaglandins Leukot Essent Fatty Acids.* 2009;81:99-104.
 35. Franke C, Verwied-Jorky S, Campoy C, Trak-Fellermeier M, Decsi T, Dolz V, Koletzko B. Dietary intake of natural sources of docosahexaenoic acid and folate in pregnant women of three European cohorts. *Ann Nutr Metab.* 2008; 53:167-74.
 36. Philibert A, Vanier C, Abdelouahab N, Chan HM, Mergler D. Fish intake and serum fatty acid profiles from freshwater fish. *Am J Clin Nutr.* 2006;84:1299-307.
 37. Hadders-Algra M. Prenatal long-chain polyunsaturated fatty acid status: the importance of a balanced intake of docosahexaenoic acid and arachidonic acid. *J Perinat Med.* 2008; 36:101-9.
 38. Mann NJ, Johnson LG, Warrick GE, Sinclair AJ. The arachidonic acid content of the Australian diet is lower than previously estimated. *J Nutr.* 1995;125:2528-35.

Original Article

Maternal and neonatal plasma n-3 and n-6 fatty acids of pregnant women and neonates from three regions of China with contrasting dietary patterns

Jian Zhang MSc¹, Yiqun Wang MPhil², Liping Meng MSc¹, Chunrong Wang¹,
Wenhua Zhao PhD³, Junshi Chen MD¹, Kebeab Ghebremeskel FIBiol²,
Michael A Crawford PhD²

¹*Institute of Nutrition and Food Safety, Chinese Centre for Disease Control and Prevention, Beijing, China*

²*Institute of Brain Chemistry & Human Nutrition, London Metropolitan University, London, UK*

³*Center of Chronic Disease Control, Chinese Centre for Disease Control and Prevention, Beijing, China*

中国三个不同膳食模式地区孕妇及新生儿血浆 n-3 和 n-6 系列脂肪酸水平

本研究目的是探讨中国河湖、沿海、内陆地区孕妇和新生儿 n-3 和 n-6 脂肪酸的营养状况。收集了研究对象的人口统计学、营养和体格测量资料，并收集了血液样品（包括孕妇静脉血和脐带血）。分析了血浆胆磷脂(CPG)脂肪酸含量。河湖、沿海、内陆孕妇每日膳食脂肪酸摄入量的中位数分别为：亚油酸(LA)20.2、22.1、31.7 克，花生四烯酸(AA)157.2、95.6、141.3 毫克； α -亚麻酸(ALA)4.2、1.0、1.8 克，二十碳五烯酸(EPA)22.4、28.6、3.1 毫克，二十二碳六烯酸(DHA)51.7、54.7、33.3 毫克；n-6/n-3 脂肪酸比值分别为 4.7、20.9、17.2。河湖、沿海、内陆地区孕妇静脉血和新生儿脐带血血浆胆磷脂的 AA 水平分别是 7.3%和 15.7%、6.7%和 16.1%、7.2%和 16.9%；DHA 水平分别是 3.2%和 4.7%、3.0%和 4.3%、2.0%和 3.6%。孕妇膳食 AA、EPA、DHA 摄入量与血浆 CPG 中相应脂肪酸水平呈现显著相关($p < 0.05$)。基于这些结果，可以认为：在中国，孕妇人群膳食脂肪摄入状况中的主要问题是 n-3 长链多不饱和脂肪酸，特别是 EPA、DHA 摄入量低和 n-6/n-3 脂肪酸比例不平衡。建议采取增加食用肥鱼或富含 ALA 植物油等方法增加中国孕妇膳食 n-3 脂肪酸的摄入量。

关键字：二十二碳六烯酸，花生四烯酸，二十碳五烯酸、中国孕妇、新生儿