

# Dietary Intake of Chinese Lactating Women Is Associated with the Fatty Acid Profile of Their Milk

Íris R. Montez de Sousa<sup>a</sup> Zhixu Wang<sup>b</sup> Rui Hu<sup>c</sup> Bernd Stahl<sup>d,e</sup> Yi Jin<sup>f</sup>  
Simone R.B.M. Eussen<sup>d</sup> Jing Li<sup>g</sup>

<sup>a</sup>Division of Human Nutrition and Health, Wageningen University, Wageningen, The Netherlands; <sup>b</sup>Department of Maternal, Child and Adolescent Health, School of Public Health, Nanjing Medical University, Nanjing, China; <sup>c</sup>Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China; <sup>d</sup>Danone Nutricia Research, Utrecht, The Netherlands; <sup>e</sup>Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands; <sup>f</sup>Danone Nutricia Research, Shanghai, China; <sup>g</sup>Department of Neonatology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China

## Keywords

Diet · Fat · Lactation · Human milk composition · China · Postpartum

## Abstract

**Introduction:** The present study aimed to explore the relationship between the dietary intake and the human milk (HM) fatty acid (FA) profile of Chinese lactating women. **Methods:** HM samples and food records were obtained from 122 Chinese women over 5 visits between 0 and 51 days postpartum. Adjusted multiple regression was performed to explore associations between maternal dietary intakes of energy, macronutrients, FAs and foods, and the HM FA profile. Analyses were performed separately for colostrum and mature milk. **Results:** Dietary intakes of total polyunsaturated FAs (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid were positively associated with the HM contents of PUFAs, omega-6 (n-6) PUFAs, and linoleic acid (LA), and the intakes of n-3 PUFAs and α-linolenic acid (ALA) were negatively associated with saturated FA levels in HM. Associations

were stronger for mature milk. Intakes of milk/dairy, meat/poultry, and eggs were negatively associated with n-6 PUFAs, LA, and EPA in mature milk, whereas the opposite was seen for fish/shrimp. Positive associations were also found between fish/shrimp and total and n-3 PUFAs in mature milk.

**Conclusion:** The HM FA profile of Chinese women is associated with their diet, and in particular with their FA intake. Tailored nutritional advice based on HM FA composition may optimize HM FA profile and thereby contribute to healthy infant development.

© 2021 S. Karger AG, Basel

## Introduction

Breastfeeding is the best way to supply infants with healthy nutrition to achieve optimal growth and development [1]. Human milk (HM) composition is very variable; among other factors, it changes according to the mother's health status and diet, during its course (fore- vs. hindmilk), throughout the day, and also over

the stages of lactation [2]. Colostrum, the milk produced during the first few days postpartum, has a high content of protein and immunomodulatory components [3]. Moving from colostrum to transitional and later mature milk, there is an increase in lactose and total fat content [3–5].

HM fat provides about 50% of the infant's caloric intake [6, 7]. Besides providing energy, fat also delivers essential fatty acids (FAs), including the long-chain polyunsaturated FAs (PUFAs). PUFAs are critical for the growth and maturation of the infant nervous and neurological system, contribute to the development of the immune system and affect gene expression through various mechanisms, including changes in membrane composition, intracellular calcium levels, and eicosanoid production [2, 7–9]. Arachidonic acid (AA), an omega-6 (n-6) PUFA, and docosahexaenoic acid (DHA), an n-3 PUFA, are of particular physiological importance in early life as neonates' ability to convert linoleic acid (LA) and  $\alpha$ -linolenic (ALA) into AA and DHA, respectively, is very low [10]. Therefore, exclusively breastfed infants rely on the supply of these PUFAs from HM [11], and hence, it is very important that they receive adequate amounts from HM [12]. One of the factors suggested to influence HM FA composition is maternal dietary intake. Various studies have reported relationships between maternal intake of specific nutrients and foods/food groups with the FA profile of HM [5]. According to literature, the FAs with stronger demonstrated associations between dietary intake and HM content are total n-6 and n-3 PUFAs, LA, DHA, eicosapentaenoic acid (EPA), and oleic acid [6].

So far, only a limited number of studies focused on associations between maternal dietary intake and HM FA profile in Chinese lactating women [13–19]. Most studies focused solely on mature milk [13, 16–19] and were performed in rural areas of China [13, 19]. It is important to further investigate this topic specifically for China as the diets of Chinese women, mainly from urban regions, are shifting from traditional diets to more Westernized ones. This may affect the availability of total and specific PUFAs in HM [15, 20, 21].

Therefore, the present study aims to explore the relationship between the dietary intake and the HM FA profile of a group of women ( $n = 122$ ) dwelling in Shanghai during the puerperium period, that is, between 0 and 51 days postpartum. We hypothesize that the HM FA profile is associated with maternal diet, and in particular with maternal FA intake.

## Materials and Methods

### *Study Subjects and Design*

This research was part of the MuRu study, an exploratory, observational, single-center study conducted at the Shanghai Children's Medical Center (SCMC). The subjects included in the study were Chinese lactating women aged 20–40 years with a healthy term (37–42 weeks) newborn whose weight was between the 10th and 90th percentiles according to the Chinese birth weight charts. A total of 179 potentially eligible subjects were screened of whom 33 (18%) were excluded because of deviation from in- or exclusion criteria [21]. During the observational period, 24 (13%) mothers discontinued the study before the last visit due to: lost to follow-up ( $n = 20$ ), an adverse event, that is, mastitis ( $n = 1$ ), and early withdrawal ( $n = 3$ ), ultimately leading to a final sample size of 122 women.

Most mothers were recruited during the 3rd trimester of pregnancy from the obstetrics departments of the Ren-ji hospital, a level III general hospital or from the Shanghai First Maternity and Infant Health Hospital. Women recruited from the Ren-ji hospital were taken care of by the family after discharge from the hospital (Community group,  $n = 92$ ), whereas women recruited from the Shanghai First Maternity and Infant Health Hospital stayed at the clinics for 4 weeks postpartum (Maternal Care Center [MCC] group,  $n = 30$ ).

Study visit dates (windows) were logged as follows [21]: Visit (V) 1 at postpartum day 4 (0–7), V2 at postpartum day 10 (8–13), V3 at postpartum day 17 (14–21), V4 at postpartum day 28 (22–35), and V5 at postpartum day 42 (36–51). The women in the MCC group attended all the visits, whereas the community subjects only attended V1, V4, and V5.

At V1, infant's date of birth, sex, birth weight, length, and head circumference, gestational age, mode of delivery, and occurrence of problems during pregnancy and/or delivery were registered. Mothers were also asked for information about current medical treatments or medication use and to record any infant illness symptoms in a study diary that was provided. At every visit, maternal dietary intake was assessed, maternal weight and infant's weight, length, and head circumference were measured, and HM was sampled. Further details on the study design, subjects recruitment, and inclusion and exclusion criteria are published elsewhere [21].

### *Maternal Dietary Intake*

Maternal dietary intake information was collected via traditional food records along with photographic diaries. The Community group kept a 1-day food record during the day before each visit, while the MCC group kept a 3-day food record starting 3 days before each visit. The staff of the MCC provided the recipes and portion size details for the MCC group at V1–V4, that is, when the mothers stayed at the clinics. All food records were checked by trained investigators. Food intakes were calculated from the records using the “Food Graph Reference for Retrospective Dietary Survey” designed by Professor Wang of Nanjing Medical University [21], and macronutrient and FA intakes were calculated using the China Food Composition Book [22].

### *Collection and Analysis of HM Samples*

HM samples were collected at each study visit. Mothers were requested to express a minimum of 10 mL of foremilk, ideally collected in the morning before breakfast between 6:00 a.m. and 10:00

**Table 1.** Baseline characteristics of infants from the total group and divided per subgroup (MCC and Community)

	Total (n = 122)	MCC group (n = 30)	Community group (n = 92)
Gestational age, weeks, median (IQR)	39 (38–40)	39 (39–40)	39 (38–40)
Length at birth, cm, median (IQR)	50 (50–51)	50 (50–51)	50 (50–51)
Weight at birth, g, median (IQR)	3,425 (3,169–3,611)	3,378 (3,193–3,543)	3,448 (3,158–3,700)
Head circumference at birth, cm, median (IQR)	34 (34–35)	35 (34–35)	34 (34–35)
Delivery mode, n (%)			
Vaginal	71 (58)	19 (63)	52 (57)
Caesarean section	51 (42)	11 (37)	40 (44)
Sex, n (%)			
Male	71 (58)	17 (57)	54 (59)
Female	51 (42)	13 (43)	38 (41)

MCC, Maternal Care Center.

a.m., by breast pump or by hand, into sterile plastic containers that were stored at 0°C at home (Community group) or at the MCC group and sent to the hospital in ice boxes. At the hospital laboratory, the milk was carefully stirred and aliquoted into smaller sterile plastic containers and stored at –80°C. Macronutrient content was analyzed using a mid-infrared MIRIS Human Milk Analyzer (MIRIS AB, Uppsala, Sweden) by mixing a 6 mL aliquot with a suitable homogenizer and subjecting it to mid-infrared analysis according to the manufacturer's user manual. FA composition was analyzed using high-resolution quartz capillary gas chromatography GC 2010 (Shimadzu Co., Kyoto, Japan). Each sample was analyzed in duplicate.

#### Statistical Analyses

Infants' baseline sociodemographic and anthropometric data are presented as mean ± SD, median (IQR) or n (%) as appropriate. Since mothers from the MCC group had 3-day food records and mothers from the Community group only had 1 day, the intakes reported by the MCC group were averaged before statistical analysis. The distribution of all variables related to maternal intake and HM composition was checked for normality using the Shapiro-Wilk test and visual inspection of histograms. As data for nearly all variables were non-normally distributed, results are expressed as median (IQR). The stability of maternal dietary intake and HM composition over time was assessed using ANOVA with Tukey HSD test for multiple comparisons adjustment. Dietary intake (i.e., intakes of total energy in kilocalorie [kcal]/day, and of macronutrients, total and specific FAs, and food groups in g/1,000 kcal) at V1 and V2–5, and the composition of colostrum and mature milk (i.e., total energy in kcal/100 mL, total fat in g/100 mL, and specific FAs in percentage of total FAs) are presented for the total study group, as well as separately for the MCC and Community groups, and were analyzed using descriptive statistics and nonparametric tests for differences between visits/groups. Multiple linear regression models were used to analyze the associations between maternal dietary intake of macronutrients (in percent of energy, en %), and total and specific FAs and food groups (in grams per day), and the FA profile of HM. Given their abundance and/or important roles in the healthy development of infants' brain, eyes, and nerves [7, 12], we focused on the following FAs in diet and HM: saturated FAs (SFAs), monounsaturated FAs (MU-

FAs), PUFAs, n-6 PUFAs, LA, AA, n-3 PUFAs, ALA, EPA, and DHA. Moreover, dietary intakes of the ratios n-6/n-3 PUFAs, LA/ALA, and DHA/EPA were considered. Several HM FAs were logarithm-transformed to ensure model assumptions were met. Estimated effect sizes obtained on logarithm scales were back-transformed into estimates on the original scales for presentation. Outliers were identified and removed based on visual inspection of the residuals distribution from the individual regression models. The following covariates were defined prior to analysis and considered in the adjusted models because of their potential role as confounders: sex of the infant, infant length and weight at birth, mother's ethnicity, delivery mode, subgroup (Community or MCC), maternal total energy intake, maternal BMI, maternal age, maternal education, size of the house/apartment, and total yearly income in the family [23, 24]. Covariates were added one-by-one and then selected for inclusion in the model if they changed the regression coefficient of the independent variable by >10%. Subsequently, all selected variables were included simultaneously in the regression model and the final model was constructed through backward selection. No multiplicity adjustment was performed on the regression models. Analyses were conducted using IBM® SPSS® Statistics version 26 (IBM Corp. Int) and R version 3.1, Package Effectsize (RStudio, Inc.), and test findings associated with a *p* value of <0.05 were considered as statistically significant.

## Results

Maternal dietary intake was found to be reasonably stable over time, whereas HM composition significantly changed over time. In particular, statistically significant differences in macronutrient concentrations were observed between V1 and the subsequent visits. Therefore, analyses were performed separately for V1 (colostrum) and for V2–5 (mature milk) (online suppl. Tables 1, 2; see [www.karger.com/doi/10.1159/000520515](http://www.karger.com/doi/10.1159/000520515) for all online suppl. material). Dietary intake was reported at V1 by 19 (63%) and 90 (98%) mothers in the MCC and Commu-

**Table 2.** Energy (kcal/d) and macronutrient (g/1,000 kcal) intake (median [IQR]) at V1 and V2–5 of the total population and separately for the subgroups (MCC and Community)

	Total		MCC group		Community group		MCC versus community				
	V1 (n = 109) <sup>1</sup>	V2-5 (n = 122)	p value <sup>2</sup>	V1 (n = 19) <sup>1</sup>	V2-5 (n = 30)	p value <sup>2</sup>	V1 (n = 90) <sup>1</sup>	V2-5 (n = 92)	p value <sup>2</sup>	p value V1 <sup>3</sup>	p value V2-5 <sup>3</sup>
Total energy, kcal/day	1,854 (1,434–2,193)	2,148 (1,867–2,479)	<b>&lt;0.001</b>	2,122 (1,843–2,296)	2,234 (2,097–2,507)	<b>0.024</b>	1,786 (1,369–2,184)	2,060 (1,813–2,471)	<b>&lt;0.001</b>	<b>0.023</b>	<b>0.048</b>
Fat (g/1,000 kcal)	35.0 (29.2–39.3)	39.3 (35.0–42.8)	<b>&lt;0.001</b>	32.8 (29.0–37.8)	39.0 (36.9–40.0)	<b>&lt;0.001</b>	35.3 (29.6–39.9)	39.4 (34.0–45.2)	<b>0.001</b>	0.25	0.62
Carbohydrates (g/1,000 kcal)	120 (108–134)	112 (101–122)	<b>&lt;0.001</b>	119 (110–126)	108 (104–114)	<b>0.003</b>	120 (107–137)	115 (100–125)	<b>0.003</b>	0.30	0.25
Protein (g/1,000 kcal)	48.1 (40.4–54.1)	48.7 (43.4–53.3)	0.48	57.6 (52.4–63.7)	52.9 (50.5–58.0)	0.051	46.1 (39.2–51.8)	46.3 (42.5–51.2)	0.37	<b>&lt;0.001</b>	<b>&lt;0.001</b>

Results are obtained from nonparametric Mann-Whitney U test. MCC, Maternal Care Center; V, visit. p values <0.05 are bolded. <sup>1</sup> There are fewer subjects included at V1 because 11 subjects from the MCC group and 2 subjects from the Community group did not report their dietary intake. <sup>2</sup> p value for difference between V1 and V2–5. <sup>3</sup> p value for difference between MCC and Community groups at V1 and V2–5.

**Table 3.** Total and specific FA (g/1,000 kcal) intake (median [IQR]) at V1 and V2–5 of the total population and separately for the subgroups (MCC and Community)

Intake in g/1,000 kcal	Total		MCC group		Community group		MCC versus community				
	V1 (n = 109) <sup>1</sup>	V2–5 (n = 122)	p value <sup>3</sup>	V1 (n = 19) <sup>1</sup>	V2–5 (n = 30)	p value <sup>3</sup>	V1 (n = 90) <sup>1</sup>	V2–5 (n = 92)	p value <sup>3</sup>	p value V1 <sup>4</sup>	p value V2–5 <sup>4</sup>
Total FAS	26.0 (22.2–31.6)	29.9 (25.2–32.8)	<b>0.002</b>	25.8 (23.4–30.9)	30.7 (28.3–32.1)	<b>0.006</b>	26.2 (22.1–32.5)	27.8 (24.4–33.9)	<b>0.030</b>	0.99	0.18
Total SFAs	9.03 (7.08–10.1)	9.55 (8.25–11.2)	<b>0.008</b>	7.66 (6.59–9.31)	9.82 (9.20–10.4)	<b>&lt;0.001</b>	9.18 (7.20–10.4)	9.33 (7.86–11.7)	0.17	0.062	0.50
Total MUFAs	9.29 (6.89–11.4)	10.7 (8.98–12.5)	<b>&lt;0.001</b>	8.94 (7.44–12.9)	11.2 (10.6–12.0)	<b>0.027</b>	9.34 (6.73–11.2)	9.91 (8.01–12.9)	<b>0.009</b>	0.54	0.071
Total PUFAs	8.61 (7.30–10.1)	9.22 (7.70–10.3)	0.094	9.22 (8.45–10.1)	9.39 (8.61–9.98)	0.97	8.48 (7.02–9.67)	8.87 (7.41–10.6)	0.13	0.080	0.23
Total n-6 PUFAs	7.97 (6.38–9.58)	8.39 (7.15–9.73)	0.12	8.70 (7.51–11.8)	8.67 (7.94–9.66)	0.76	7.58 (6.28–9.44)	8.19 (6.53–9.75)	0.13	<b>0.019</b>	0.11
LA (C18:2 n-6)	7.59 (6.15–9.33)	8.09 (6.91–9.41)	0.11	8.52 (7.39–11.5)	8.43 (7.71–9.45)	0.74	7.23 (6.00–9.18)	7.99 (6.33–9.38)	0.12	<b>0.016</b>	0.12
AA (C20:4 n-6)	0.05 (0.03–0.12)	0.10 (0.05–0.16)	<b>0.004</b>	0.09 (0.02–0.17)	0.11 (0.09–0.16)	0.23	0.05 (0.03–0.11)	0.07 (0.04–0.19)	<b>0.043</b>	0.49	0.091
Total n-3 PUFAs	1.04 (0.71–1.32)	1.11 (0.89–1.39)	0.069	1.40 (1.21–2.60)	1.40 (1.26–1.72)	0.89	0.94 (0.66–1.20)	1.02 (0.83–1.22)	0.11	<b>&lt;0.001</b>	<b>&lt;0.001</b>
ALA (C18:3 n-3)	0.99 (0.69–1.23)	1.05 (0.85–1.28)	0.084	1.25 (1.14–2.39)	1.27 (1.14–1.62)	0.82	0.93 (0.66–1.12)	0.94 (0.79–1.17)	0.13	<b>&lt;0.001</b>	<b>&lt;0.001</b>
EPA (C20:5 n-3)	0.01 (0.00–0.04)	0.02 (0.01–0.05)	<b>0.001</b>	0.04 (0.00–0.08)	0.05 (0.04–0.06)	0.18	0.00 (0.00–0.03)	0.01 (0.00–0.03)	<b>0.008</b>	<b>0.004</b>	<b>&lt;0.001</b>
DHA (C22:6 n-3)	0.01 (0.00–0.04)	0.02 (0.01–0.05)	<b>0.002</b>	0.04 (0.00–0.12)	0.05 (0.04–0.07)	0.59	0.00 (0.00–0.02)	0.01 (0.00–0.03)	<b>0.019</b>	<b>0.013</b>	<b>&lt;0.001</b>
n-6/n-3	7.73 (6.25–9.25)	7.58 (6.15–8.40)	0.30	5.70 (4.02–6.78)	6.14 (5.16–7.28)	0.35	8.03 (7.07–9.42)	8.06 (7.11–8.94)	0.50	<b>&lt;0.001</b>	<b>&lt;0.001</b>
LA/ALA	7.87 (6.66–9.13)	7.95 (6.62–8.75)	0.43	6.29 (4.20–7.11)	6.66 (5.54–7.50)	0.33	8.13 (7.37–9.38)	8.08 (7.39–9.07)	0.60	<b>&lt;0.001</b>	<b>&lt;0.001</b>
DHA/EPA <sup>2</sup>	0.92 (0.67–1.61)	0.83 (0.67–1.27)	0.42	1.14 (0.63–1.74)	1.03 (0.85–1.37)	0.45	0.83 (0.67–1.33)	0.73 (0.67–1.19)	0.27	0.31	<b>0.023</b>

Results are obtained from nonparametric Mann-Whitney U test. FA, fatty acid; MCC, Maternal Care Center; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n-6 PUFAs, omega-6 PUFAs; LA, linoleic acid; AA, arachidonic acid; n-3 PUFAs, omega-3 PUFAs; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; p values <0.05 are bolded. <sup>1</sup> There are fewer subjects included at V1 because 11 subjects from the MCC group and 2 subjects from the Community group did not report their dietary intake. <sup>2</sup> The ratio DHA/EPA is only reported for 58 subjects (15 in MCC and 43 in Community group) because the remaining subjects had an EPA intake of zero. <sup>3</sup> p value for difference between V1 and V2–5. <sup>4</sup> p value for difference between MCC and Community groups at V1 and V2–5.

**Table 4.** Food group (g/1,000 kcal) intake (median [IQR]) at V1 and V2–5 of the total population and separately for the subgroups (MCC and community)

Intake in g/1,000 kcal	Total		MCC group		Community group		MCC versus community				
	V1 (n = 109) <sup>1</sup>	V2–5 (n = 122)	p value <sup>2</sup>	V1 (n = 19) <sup>1</sup>	V2–5 (n = 30)	p value <sup>2</sup>	V1 (n = 90) <sup>1</sup>	V2–5 (n = 92)	p value <sup>2</sup>	p value V1 <sup>3</sup>	p value V2–5 <sup>3</sup>
Beans	0.00 (0.00–6.13)	3.59 (0.00–7.29)	<b>0.027</b>	4.57 (2.40–5.72)	6.66 (4.31–8.84)	<b>0.010</b>	0.00 (0.00–7.59)	2.53 (0.00–6.41)	0.14	<b>0.029</b>	<b>&lt;0.001</b>
Eggs	37.3 (23.1–68.7)	35.2 (22.2–49.4)	0.22	26.4 (18.7–37.2)	28.1 (25.8–34.5)	0.26	43.7 (24.8–74.7)	41.2 (21.5–55.8)	0.13	<b>0.002</b>	<b>0.022</b>
Fish/shrimp	33.5 (0.00–90.3)	63.2 (29.1–89.5)	<b>0.007</b>	93.4 (45.6–121)	76.5 (62.2–95.3)	0.67	13.6 (0.00–74.4)	51.8 (19.7–89.0)	<b>0.004</b>	<b>&lt;0.001</b>	<b>0.003</b>
Fruits	0.00 (0.00–68.7)	71.0 (23.9–124)	<b>&lt;0.001</b>	5.13 (0.00–29.2)	42.5 (17.0–78.0)	<b>&lt;0.001</b>	0.00 (0.00–79.9)	92.2 (27.7–159)	<b>&lt;0.001</b>	0.37	<b>0.008</b>
Grains	123 (110–157)	111 (100–123)	<b>&lt;0.001</b>	130 (117–141)	111 (103–118)	<b>&lt;0.001</b>	121 (104–158)	111 (100–125)	<b>&lt;0.001</b>	0.65	0.79
Meat/poultry	77.3 (40.9–114)	86.1 (68.5–107)	0.18	92.6 (71.4–130)	92.0 (77.5–112)	0.70	76.8 (37.9–107)	80.8 (62.8–106)	0.17	<b>0.041</b>	<b>0.049</b>
Milk/dairy	0.00 (0.00–96.4)	53.4 (0.00–83.4)	0.25	70.7 (0.00–95.2)	72.1 (61.7–86.1)	0.85	0.00 (0.00–100)	2.46 (0.00–80.3)	0.50	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Oils	10.3 (7.32–12.8)	9.82 (7.95–12.0)	0.53	11.1 (10.3–12.8)	10.4 (9.58–11.8)	0.059	9.52 (6.74–12.9)	9.11 (7.28–12.0)	0.79	<b>0.025</b>	<b>0.031</b>
Tubers	0.00 (0.00–0.00)	0.00 (0.00–11.4)	<b>0.001</b>	0.00 (0.00–21.7)	5.53 (3.31–10.2)	0.10	0.00 (0.00–0.00)	0.00 (0.00–13.2)	<b>0.049</b>	<b>0.044</b>	<b>&lt;0.001</b>
Vegetables	133 (77.8–178)	126 (84.8–161)	0.49	171 (149–197)	139 (123–156)	<b>0.002</b>	115 (67.5–175)	107 (78.6–165)	0.96	<b>0.001</b>	<b>0.034</b>

Results are obtained from nonparametric Mann-Whitney U test. MCC, Maternal Care Center; V, visit. p values <0.05 are bolded. <sup>1</sup> There are fewer subjects included at V1 because 11 subjects from the MCC, group and 2 subjects from the Community group did not report their dietary intake. <sup>2</sup> p value for difference between V1 and V2–5. <sup>3</sup> p value for difference between MCC and Community groups at V1 and V2–5.

nity groups, respectively, whereas all participants ( $n = 122$ ) reported their dietary intake at least at one of the subsequent visits (V2–5 for mothers in the MCC group and V4–5 for mothers in the Community group). HM samples were collected at all timepoints from each woman, with the exception of one woman from the MCC group for which the V4 sample was missing.

### Maternal and Infants' Baseline Sociodemographic and Anthropometric Data

The mean age of the study population was  $29.3 \pm 3.4$  years (ranging from 20.4 to 39.4 years) with a mean baseline BMI of  $24.0 \pm 3.1 \text{ kg/m}^2$ . There were no apparent differences between the MCC and Community groups, except for a higher annual household income and more housewives in the MCC group [21]. Baseline sociodemographic and anthropometric data of the infants were similar for the MCC and the Community groups (Table 1).

### Maternal Dietary Intake

Maternal dietary intake at V1 and at V2–5 of energy, macronutrients, total and specific FAs, and food groups is presented in Tables 2–4 for the total group, as well as separately for the MCC and Community groups. Total energy intake and relative intakes of fat (Table 2), total SFAs, total MUFAs, AA, EPA, and DHA (Table 3), beans, fish/shrimp, fruits, and tubers (Table 4) were statistically significantly higher at V2–5 than V1, whereas relative carbohydrate (Table 2) and grains (Table 4) intakes were higher at V1 than V2–5. Total energy intake (Table 2), as well as relative intakes of protein (Table 2), total n-3 and n-6 PUFAs, LA, ALA, EPA, DHA (Table 3), and most food groups (Table 4) were higher in the MCC group than the Community group at both visits, whereas the n-6/n-3 and LA/ALA ratios (Table 3) and the relative intake of eggs (Table 4) were higher in the Community group.

### HM FA Profile

Table 5 shows the total energy and fat profile of colostrum and mature milk for the total group, and in addition separately for the MCC and Community groups. Total energy (in g/100 kcal) and total fat content (in g/100 mL), as well as relative total PUFA, total n-6 PUFA, LA, and ALA levels (as percentage of total FAs) were statistically significantly lower in colostrum compared to mature milk, whereas AA and EPA levels were significantly higher in colostrum compared to mature milk. Additionally, the ratios of n-6/n-3 FAs and DHA/EPA were significantly higher in mature milk than colostrum.

**Table 5.** Colostrum and mature milk energy content (kcal/100 mL), total FAs (g/100 mL) and specific FAs (% of total FAs) of the total group and divided per subgroup (MCC and Community)

	Total		MCC group		Community group		MCC versus community	
	colostrum (n = 122)	mature milk (n = 122)	p value <sup>1</sup>	colostrum (n = 30)	mature milk (n = 30)	p value <sup>1</sup>	colostrum <sup>2</sup>	p value mature milk <sup>2</sup>
Total energy (kcal/100 mL)	64.0 (58.0–72.0)	72.0 (66.3–82.0)	<b>&lt;0.001</b>	63.0 (57.0–64.3)	69.9 (66.3–72.9)	<b>&lt;0.001</b>	65.0 (58.3–74.0)	74.3 (65.8–84.9)
Total fat (g/100 mL)	3.05 (2.30–3.70)	4.18 (3.40–4.90)	<b>&lt;0.001</b>	3.05 (2.38–3.23)	3.98 (3.54–4.28)	<b>&lt;0.001</b>	3.05 (2.30–3.80)	4.25 (3.40–4.99)
Total SFAs (% of total FAs)	40.7 (38.2–44.6)	40.4 (37.0–43.5)	0.20	44.4 (41.5–46.3)	43.4 (41.8–46.3)	0.54	39.4 (37.5–43.1)	39.2 (36.2–42.9)
Total MUFAs (% of total FAs)	34.6 (31.6–37.1)	33.8 (31.3–35.6)	0.10	31.5 (30.4–33.0)	31.7 (30.0–33.2)	0.92	35.5 (32.8–37.6)	34.7 (31.9–36.1)
Total PUFAs (% of total FAs)	24.1 (22.7–26.2)	26.2 (22.7–28.8)	<b>&lt;0.001</b>	24.1 (22.2–26.6)	25.4 (22.7–26.5)	0.51	24.1 (22.8–26.1)	26.5 (23.3–29.0)
Total n-6 PUFAs (% of total FAs)	21.8 (19.7–23.6)	23.9 (20.4–25.8)	<b>&lt;0.001</b>	22.0 (19.6–23.7)	22.3 (20.3–23.9)	0.66	21.8 (19.6–23.6)	24.2 (20.6–26.5)
LA (C18:2 n-6) (% of total FAs)	20.5 (18.3–22.3)	22.9 (19.6–24.9)	<b>&lt;0.001</b>	20.3 (18.1–22.4)	21.5 (19.1–23.1)	0.20	20.5 (18.3–22.3)	23.3 (19.7–25.8)
AA (C20:4 n-6) (% of total FAs)	0.85 (0.63–1.18)	0.54 (0.50–0.58)	<b>&lt;0.001</b>	0.92 (0.85–1.14)	0.53 (0.49–0.58)	<b>&lt;0.001</b>	0.78 (0.61–1.32)	0.54 (0.50–0.58)
Total n-3 PUFAs (% of total FAs)	2.39 (1.99–2.82)	2.33 (1.83–2.84)	0.49	2.50 (2.29–2.76)	2.72 (2.47–3.00)	<b>0.039</b>	2.28 (1.90–2.87)	2.17 (1.74–2.64)
ALA (C18:3 n-3) (% of total FAs)	1.24 (1.03–1.50)	1.44 (1.09–1.83)	<b>0.003</b>	1.36 (1.16–1.54)	1.62 (1.48–1.92)	<b>0.002</b>	1.18 (0.95–1.49)	1.34 (1.03–1.75)
EPA (C20:5 n-3) (% of total FAs)	0.19 (0.13–0.27)	0.08 (0.06–0.10)	<b>&lt;0.001</b>	0.22 (0.18–0.24)	0.09 (0.06–0.10)	<b>&lt;0.001</b>	0.17 (0.11–0.28)	0.08 (0.05–0.10)
DHA (C22:6 n-3) (% of total FAs)	0.68 (0.55–0.90)	0.65 (0.48–0.89)	0.23	0.66 (0.57–0.89)	0.94 (0.80–1.07)	<b>0.001</b>	0.69 (0.52–0.90)	0.58 (0.45–0.78)
n-6/n-3	9.13 (7.52–10.7)	10.3 (8.21–13.1)	<b>0.001</b>	8.90 (8.00–9.64)	8.27 (7.53–9.15)	0.11	9.41 (7.28–11.4)	11.3 (9.12–14.1)
LA/ALA	16.3 (13.5–19.8)	15.4 (13.0–21.0)	0.97	15.0 (13.5–16.0)	13.4 (12.1–15.1)	<b>0.021</b>	17.0 (13.4–21.3)	18.3 (13.7–23.3)
DHA/EPA	3.61 (2.28–5.81)	7.65 (5.58–11.6)	<b>&lt;0.001</b>	3.37 (2.36–4.16)	10.5 (7.42–15.1)	<b>&lt;0.001</b>	3.87 (2.27–6.45)	6.92 (5.26–10.7)

Values are presented as median (IQR). Results are obtained from nonparametric Mann-Whitney U test. FAs, fatty acids; MCC, Maternal Care Center; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n-6 PUFAs, omega-6 PUFAs; LA, linoleic acid; AA, arachidonic acid; n-3 PUFAs, omega-3 PUFAs; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. <sup>1</sup> p value for difference between colostrum and mature milk. <sup>2</sup> p value for difference between MCC and Community groups for colostrum and mature milk.

**Table 6.** Adjusted multiple linear regression models for HM FA profile (% of total FAs) depending on the macronutrients intake (en %) – per lactation period

Lactation period	Predictor	Standardized β	95% CI	p value	Adjusted explained variance
HM n-3 PUFA content (% of total FAs) as outcome	Colostrum				
	Mature milk	No correlations with p value <0.10 were found			
	Protein intake (en %) <sup>2</sup>	1.35	[1.12; 1.62]	<b>0.002</b>	0.127
HM AA content (% of total FAs) as outcome	Colostrum				
	Mature milk	Protein intake (en %) <sup>1</sup>			
	No correlations with p value <0.10 were found	0.73	[0.61; 0.90]	<b>0.003</b>	0.159
HM EPA content (% of total FAs) as outcome	Colostrum				
	Mature milk	Protein intake (en %) <sup>1</sup>			
	No correlations with p value <0.10 were found	0.83	[0.66; 1.02]	0.079	0.073
HM DHA content (% of total FAs) as outcome	Colostrum				
	Mature milk	Protein intake (en %) <sup>1</sup>			
	No correlations with p value <0.10 were found	1.28	[1.03; 1.60]	<b>0.023</b>	0.024
	Carbohydrates intake (en %) <sup>1</sup>	0.82	[0.66; 1.00]	<b>0.048</b>	0.011
	No correlations with p value <0.10 were found				

HM, human milk; FA, fatty acid; n-3 PUFAs, omega-3 polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Only p values <0.10 are included in the table. p values <0.05 are bolded. <sup>1</sup> The regression models were adjusted for total energy intake and subgroup (Community or Maternal Care Center). <sup>2</sup> These regression models were also adjusted for maternal BMI and infant gender.

Mothers in the Community group had significantly higher relative levels of MUFAs and LA/ALA ratio, and lower levels of SFAs and ALA in both colostrum and mature milk than the MCC group. Moreover, AA levels in colostrum were significantly lower in the Community group, whereas levels of total energy, n-6 PUFAs, LA and n-6/n-3 ratio were significantly higher and levels of total n-3 PUFAs, DHA, and DHA/EPA ratio were significantly lower in mature milk of mothers in the Community group compared to the MCC group (Table 5).

#### *Associations of Maternal Dietary Intake of Macronutrients, FAs and Food Groups with HM FA Profile*

The associations between the HM FA profile of colostrum and mature milk, and either maternal dietary intake of macronutrients, specific FAs, and food groups is presented in Tables 6–8, respectively. The percentage of energy intake from protein was significantly positively associated with AA and DHA content in colostrum, and with total n-3 PUFA content in mature milk. Additionally, the percentage of energy intake from carbohydrates was positively associated with DHA content in colostrum (Table 6).

Relative (n-3) PUFA and ALA intakes were significantly negatively associated with SFA content and positively associated with MUFA (only for n-3 PUFA intakes), PUFA, n-6 PUFA and LA (not significantly for ALA intakes) content in colostrum. In mature milk, significant positive associations were observed between PUFA, EPA and DHA intakes and PUFA, n-6 PUFA, LA, n-3 PUFA (only for EPA and DHA intakes), and ALA (only for EPA intakes) HM content, whereas a negative association was observed between the relative intake of ALA and EPA HM content. In addition, the dietary intake ratio of n-6/n-3 FAs was significantly positively associated with EPA in colostrum and with SFAs and ALA in mature milk, and negatively associated with PUFAs, n-6 PUFAs, and LA in mature milk. Lastly, the dietary intake ratio of LA/ALA was positively associated with SFA and EPA contents in mature milk and colostrum, respectively (Table 7).

Table 8 shows the associations between maternal food intake and the FA profile of HM. The intake of eggs was significantly negatively associated with SFAs and positively associated with n-3 PUFAs in colostrum, whereas in mature milk negative associations between the intake of eggs and total and n-6 PUFAs, and LA were seen. In contrast, fish/shrimp intakes were positively associated with these specific PUFAs, as well as with n-3 PUFAs in

mature milk. The intake of milk/dairy was statistically significantly positively associated with SFAs and EPA in colostrum and with AA in mature milk, and negatively associated with MUFAs in colostrum and with n-6 PUFA, LA and EPA contents in mature milk. Moreover, the dietary intakes of both oils and vegetables were positively associated with colostrum contents of PUFAs, n-6 PUFAs, and LA and negatively associated with the colostrum and mature milk (only for vegetables) contents of SFAs. Lastly, intakes of meat/poultry and beans were positively correlated with n-3 PUFA and ALA content in colostrum (only meat/poultry) and mature milk, and additionally meat/poultry intakes were negatively correlated with total PUFA content in mature milk.

## **Discussion**

HM contains essential FA that are crucial for the healthy development of infants. The present study explored associations between maternal dietary intake and the FA composition of HM in lactating Chinese women during the puerperium. The first 30 days of this period, that is, the *Zuo yuezi*, are of particular importance for Chinese women to heal after childbirth. During this time, rest, avoidance of physical work and a nutritious diet are promoted along with other practices [21, 25]. This “confinement” diet typically includes foods with high amounts of proteins and fats and low amounts of fruits and fresh vegetables because it is believed that “cold foods” should be avoided in order to boost HM quantity and quality [16, 25]. Indeed, intakes of fruits and vegetables by women in both groups in this study [21] were below the dietary recommendations set by the Chinese Nutrition Society [26], as well as below intakes of non-pregnant, nonlactating Chinese women [27].

In line with earlier findings [5, 10, 13, 28–33], our study showed that the FA composition of HM is associated with maternal diet, and in particular with maternal FA intake. The percentage of energy intake from protein was positively associated with total and specific (i.e., EPA and DHA) n-3 PUFA contents in HM, a finding similar to that of a study from Iceland [10]. Fish and seafood are the main sources of EPA and DHA in the diet [34], and these foods are important sources of protein too [35]. Indeed, our data show that EPA and DHA were strongly correlated with protein intake (DHA:  $r = 0.648$ ,  $p < 0.001$ ; EPA:  $r = 0.660$ ,  $p < 0.001$ ). Surprisingly, the percentage of energy intake from carbohydrates was positively associated with DHA content in colostrum, a finding that has not been reported before to our knowledge.

**Table 7.** Adjusted linear regression models for HM FA profile (% of total FAs) depending on the intake of specific FAs (g/day) – per lactation period

Lactation period	Predictor	Standardized $\beta$	95% CI	p value	Adjusted explained variance
HM SFA content (% of total FAs) as outcome	Colostrum				
	n-3 PUFA intake, <sup>1</sup> g/day	-0.19	[-0.37, -0.01]	<b>0.036</b>	0.194
	n-6 PUFA intake, <sup>1</sup> g/day	-0.19	[-0.39, 0.01]	0.069	0.186
	LA intake, <sup>1</sup> g/day	-0.19	[-0.39, 0.01]	0.066	0.186
	ALA intake, <sup>1</sup> g/day	-0.19	[-0.36, -0.01]	<b>0.035</b>	0.195
	AA intake, <sup>1</sup> g/day	0.18	[0.00, 0.35]	0.053	0.189
	Mature milk				
	LA/ALA <sup>1</sup>	0.19	[0.02, 0.36]	<b>0.028</b>	0.191
	n-6/n-3 <sup>1</sup>	0.20	[0.02, 0.37]	<b>0.027</b>	0.191
	HM MUFA content (% of total FAs) as outcome				
Colostrum	n-3 PUFA intake, <sup>1</sup> g/day	0.24	[0.06, 0.42]	<b>0.008</b>	0.229
	LA/ALA <sup>1</sup>	-0.17	[-0.35, 0.01]	0.064	0.202
	DHA/EPA <sup>1</sup>	0.21	[-0.03, 0.46]	0.089	0.196
	Mature milk				
	No correlations with p value <0.10 were found				
	HM PUFA content (% of total FAs) as outcome				
	Colostrum				
	PUFA intake, <sup>1</sup> g/day	0.42	[0.15, 0.68]	<b>0.003</b>	0.112
	ALA intake, <sup>1</sup> g/day	0.24	[0.05, 0.42]	<b>0.013</b>	0.085
	Mature milk				
Colostrum	PUFA intake, <sup>1</sup> g/day	0.31	[0.07, 0.54]	<b>0.012</b>	0.123
	EPA intake, <sup>1</sup> g/day	0.36	[0.16, 0.55]	<b>&lt;0.001</b>	0.165
	DHA intake, <sup>1</sup> g/day	0.27	[0.07, 0.48]	<b>0.009</b>	0.127
	LA/ALA <sup>1</sup>	-0.17	[-0.35, 0.01]	0.065	0.101
	n-6/n-3 <sup>1</sup>	-0.21	[-0.39, -0.03]	<b>0.022</b>	0.115
	HM n-6 PUFA content (% of total FAs) as outcome				
	Colostrum				
	PUFA intake, <sup>1</sup> g/day	0.36	[0.10, 0.63]	<b>0.008</b>	0.082
	ALA intake, <sup>1</sup> g/day	0.22	[0.03, 0.41]	<b>0.022</b>	0.067
	Mature milk				
Colostrum	PUFA intake, <sup>1</sup> g/day	0.29	[0.06, 0.52]	<b>0.014</b>	0.169
	EPA intake, <sup>1</sup> g/day	0.26	[0.06, 0.45]	<b>0.011</b>	0.172
	DHA intake, <sup>1</sup> g/day	0.21	[0.01, 0.41]	<b>0.040</b>	0.156
	LA/ALA <sup>1</sup>	-0.17	[-0.34, 0.01]	0.062	0.151
	n-6/n-3 <sup>1</sup>	-0.20	[-0.37, -0.02]	<b>0.031</b>	0.159
	HM LA content (% of total FAs) as outcome				
	Colostrum				
	PUFA intake, <sup>1</sup> g/day	0.37	[0.11, 0.64]	<b>0.006</b>	0.073
	ALA intake, <sup>1</sup> g/day	0.18	[-0.01, 0.37]	0.057	0.042
	Mature milk				
Colostrum	PUFA intake, <sup>1</sup> g/day	0.30	[0.07, 0.54]	<b>0.012</b>	0.146
	EPA intake, <sup>1</sup> g/day	0.28	[0.08, 0.48]	<b>0.005</b>	0.156
	DHA intake, <sup>1</sup> g/day	0.24	[0.03, 0.44]	<b>0.022</b>	0.138
	LA/ALA <sup>1</sup>	-0.16	[-0.33, 0.02]	0.086	0.121
	n-6/n-3 <sup>1</sup>	-0.19	[-0.37, -0.01]	<b>0.040</b>	0.130
	HM AA content (% of total FAs) as outcome				
	Colostrum				
	EPA intake, <sup>1</sup> g/day	0.82	[0.67, 0.99]	0.051	0.123
	n-6/n-3 <sup>1</sup>	1.20	[0.99, 1.45]	0.057	0.114
	Mature milk				
	No correlations with p value <0.10 were found				

Table 7 (continued)

Lactation period	Predictor	Standardized $\beta$	95% CI	p value	Adjusted explained variance
HM n-3 PUFA content (% of total FAs) as outcome	Colostrum				
	MUFA intake, <sup>1</sup> g/day	0.80	[0.62, 1.03]	0.088	0.010
	AA intake, <sup>1</sup> g/day	0.83	[0.68, 1.01]	0.063	0.015
	Mature milk				
	MUFA intake, <sup>1</sup> g/day	0.78	[0.59, 1.01]	0.061	0.069
HM ALA content (% of total FAs) as outcome	Colostrum				
	EPA intake, <sup>1</sup> g/day	1.34	[1.08, 1.65]	<b>0.007</b>	0.062
	LA/ALA <sup>1</sup>	0.84	[0.70, 1.02]	0.081	0.027
	DHA/EPA <sup>2</sup>	0.83	[0.67, 1.01]	0.059	0.027
	n-6/n-3 <sup>1</sup>	0.83	[0.68, 1.00]	<b>0.045</b>	0.035
HM EPA content (% of total FAs) as outcome	Colostrum				
	n-3 PUFA intake, <sup>1</sup> g/day	0.83	[0.68, 1.01]	0.067	0.075
	LA/ALA <sup>1</sup>	1.23	[1.01, 1.51]	<b>0.036</b>	0.085
	n-6/n-3 <sup>1</sup>	1.23	[1.01, 1.51]	<b>0.043</b>	0.083
	ALA intake, <sup>1</sup> g/day	-0.25	[-0.43, -0.08]	<b>0.005</b>	0.049

HM, human milk; FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 PUFA; LA, linoleic acid; AA, arachidonic acid; n-3 PUFA, omega-3 PUFA; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Only p values < 0.10 are included in the table. p values < 0.05 are bolded. <sup>1</sup> The regression models were adjusted for total energy intake and subgroup (Community or Maternal Care Center). <sup>2</sup> These regression models were also adjusted for maternal BMI, and infant gender.

**Table 8.** Adjusted linear regression models for HM FA profile (% of total FAs) depending on food intake (g/day) – per lactation period

Lactation period	Predictor	Standardized $\beta$	95% CI	<i>p</i> value	Adjusted explained variance
HM SFA content (% of total FAs) as outcome					
Colostrum	Eggs, <sup>1</sup> g/day	−0.20	[−0.39; −0.02]	<b>0.031</b>	0.196
	Fruits, <sup>1</sup> g/day	0.17	[−0.02; 0.36]	0.072	0.185
	Milk/dairy, <sup>2</sup> g/day	0.32	[0.14; 0.51]	<b>0.001</b>	0.287
	Oils, <sup>1</sup> g/day	−0.23	[−0.42; −0.03]	<b>0.027</b>	0.198
	Vegetables, <sup>1</sup> g/day	−0.20	[−0.40; 0.01]	0.060	0.187
Mature milk	Vegetables, <sup>1</sup> g/day	−0.22	[−0.39; −0.06]	<b>0.010</b>	0.204
HM MUFA content (% of total FAs) as outcome					
Colostrum	Grains, <sup>1</sup> g/day	0.21	[−0.03; 0.45]	0.083	0.198
	Milk/dairy, <sup>2</sup> g/day	−0.26	[−0.44; −0.08]	<b>0.005</b>	0.304
Mature milk	No correlations with <i>p</i> value <0.10 were found				
HM PUFA content (% of total FAs) as outcome					
Colostrum	Eggs, <sup>1</sup> g/day	0.18	[−0.02; 0.38]	0.082	0.056
	Fish/shrimp, <sup>1</sup> g/day	0.19	[−0.02; 0.41]	0.076	0.057
	Oils, <sup>1</sup> g/day	0.40	[0.19; 0.61]	<b>&lt;0.001</b>	0.151
	Vegetables, <sup>1</sup> g/day	0.41	[0.20; 0.62]	<b>&lt;0.001</b>	0.154
Mature milk	Eggs, <sup>1</sup> g/day	−0.18	[−0.36; 0.00]	<b>0.046</b>	0.093
	Fish/shrimp, <sup>1</sup> g/day	0.27	[0.09; 0.44]	<b>0.004</b>	0.127
	Meat/poultry, <sup>1</sup> g/day	−0.26	[−0.49; −0.03]	<b>0.029</b>	0.099
HM n-6 PUFA content (% of total FAs) as outcome					
Colostrum	Oils, <sup>1</sup> g/day	0.36	[0.15; 0.57]	<b>0.001</b>	0.116
	Vegetables, <sup>1</sup> g/day	0.30	[0.09; 0.52]	<b>0.006</b>	0.087
Mature milk	Eggs, <sup>1</sup> g/day	−0.20	[−0.37; −0.02]	<b>0.029</b>	0.160
	Fish/Shrimp, <sup>1</sup> g/day	0.21	[0.04; 0.38]	<b>0.017</b>	0.167
	Meat/Poultry, <sup>1</sup> g/day	−0.20	[−0.43; 0.03]	0.093	0.146
	Milk/dairy, <sup>1</sup> g/day	−0.22	[−0.39; −0.05]	<b>0.013</b>	0.170
HM LA content (% of total FAs) as outcome					
Colostrum	Oils, <sup>1</sup> g/day	0.34	[0.13; 0.56]	<b>0.002</b>	0.097
	Vegetables, <sup>1</sup> g/day	0.34	[0.12; 0.56]	<b>0.002</b>	0.089
Mature milk	Eggs, <sup>1</sup> g/day	−0.19	[−0.37; −0.01]	<b>0.037</b>	0.131
	Fish/shrimp, <sup>1</sup> g/day	0.22	[0.04; 0.39]	<b>0.015</b>	0.143
	Milk/dairy, <sup>1</sup> g/day	−0.21	[−0.38; −0.03]	<b>0.019</b>	0.140
HM AA content (% of total FAs) as outcome					
Colostrum	No correlations with <i>p</i> value <0.10 were found				
Mature milk	Milk/dairy, <sup>1</sup> g/day	0.81	[0.68; 0.98]	<b>0.029</b>	0.017
HM n-3 PUFA content (% of total FAs) as outcome					
Colostrum	Eggs, <sup>1</sup> g/day	1.23	[1.01; 1.52]	<b>0.039</b>	0.023
	Grains, <sup>1</sup> g/day	1.26	[0.96; 1.63]	0.092	0.009
	Meat/poultry, <sup>1</sup> g/day	0.76	[0.61; 0.95]	<b>0.017</b>	0.037
	Beans, <sup>1</sup> g/day	1.23	[1.03; 1.48]	<b>0.026</b>	0.081
Mature milk	Fish/shrimp, <sup>1</sup> g/day	1.34	[1.12; 1.58]	<b>0.002</b>	0.119
	Meat/poultry, <sup>1</sup> g/day	0.73	[0.57; 0.91]	<b>0.007</b>	0.098
HM ALA content (% of total FAs) as outcome					
Colostrum	Meat/poultry, <sup>1</sup> g/day	0.79	[0.63; 0.98]	<b>0.035</b>	0.078
	Vegetables, <sup>1</sup> g/day	1.22	[0.79; 1.20]	0.077	0.066
Mature milk	Beans, <sup>1</sup> g/day	1.27	[1.06; 1.54]	<b>0.010</b>	0.057
	Fish/shrimp, <sup>1</sup> g/day	1.20	[0.99; 1.43]	0.063	0.031
	Meat/poultry, <sup>1</sup> g/day	0.78	[0.61; 0.99]	<b>0.041</b>	0.037
HM EPA content (% of total FAs) as outcome					
Colostrum	Milk/dairy, <sup>1</sup> g/day	0.73	[0.59; 0.89]	<b>0.002</b>	0.153
Mature milk	Grains, <sup>1</sup> g/day	0.23	[−0.02; 0.49]	0.076	0.011
	Meat/poultry, <sup>1</sup> g/day	−0.22	[−0.47; 0.02]	0.073	0.012
	Milk/dairy, <sup>1</sup> g/day	−0.20	[−0.38; −0.01]	<b>0.037</b>	0.022
	Tubers, <sup>1</sup> g/day	−0.17	[−0.34; 0.01]	0.069	0.013

HM, human milk; FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6 PUFA, omega-6 PUFA; LA, linoleic acid; AA, arachidonic acid; n-3 PUFA, omega-3 PUFA; ALA, alpha-linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Only *p* values <0.10 are included in the table. *p* values <0.05 are bolded. <sup>1</sup> The regression models were adjusted for total energy intake and subgroup (Community or Maternal Care Center). <sup>2</sup> The regression models were also adjusted for maternal education and infant birth weight. <sup>3</sup> These regression models were also adjusted for maternal BMI and infant gender.

In contrast to protein and carbohydrates, the percentage of energy from total fat was not associated with HM FA profile. Yet, maternal intakes of *specific* FAs showed many associations with HM FA composition. This may suggest that the type of fat consumed is a more important predictor for the HM FA profile than the amount of fat [36]. As shown by others (e.g., [10, 29–33]), we found that the dietary intakes of total PUFAs, EPA, and DHA were positively associated with the HM contents of PUFAs, n-6 PUFAs, and LA, and the intakes of n-3 PUFAs and ALA were negatively associated with SFA levels in HM. The associations between maternal FA intakes and HM FA composition in Chinese women were strongest for mature milk, which is in agreement with previous findings [37]. It is known that during pregnancy, maternal fat stores build up to ensure adequate provision of FAs to the infant. PUFA reserves go down throughout lactation, therefore it is reasonable to assume that dietary intake starts having a bigger contribution to HM FA composition at a later stage when reserves are lower [7, 37]. For protein and carbohydrates intakes, the associations were strongest for colostrum suggesting that maternal storage and usage of these macronutrients follows a different mechanism.

During the confinement diet, “cold foods” such as fresh vegetables are traditionally avoided. Therefore, it is likely that mothers choose to consume vegetables stir-fried in oil. That may explain why maternal intake of vegetables and oils, particularly during the colostrum phase when the confinement diet is most strictly followed [25], often showed similar associations with FAs in HM, that is, positive associations with total and n-6 PUFAs, and LA, and negative correlation with SFAs. Soybean and peanut oil, both high in n-6 PUFAs and LA, are types of oils that are frequently used in China [16]. Indeed, when vegetables and oils were included in the same regression model, the associations between the intake of vegetables and the HM FA levels were lost. Another food that is commonly avoided during the traditional Chinese confinement diet is milk [16]. In our study population, the intake of milk and calcium [21] was far below the recommendations [26]. We showed that the intake of milk and dairy products was positively associated with SFAs in HM and negatively associated with HM MUFAs, n-6 PUFAs, LA and EPA, that is, mostly associated with changes toward a less healthy HM FA composition [38]. Meat/poultry and eggs were other foods that were negatively associated with n-6 PUFAs, LA, and/or EPA in HM. In contrast, maternal intake of fish/shrimp was positively associated with these FAs, as well as with total and n-3 PUFAs, suggesting

a favorable effect of fish/shrimp intake on HM FA profile. Although the *combined* intake of meat/poultry, eggs and fish/shrimp was in line with Chinese recommendations [21, 26], intakes of EPA + DHA, whose main sources are fish and seafood [39], were far below the adequate intake of these FAs (i.e., 350–450 mg/day) [40], particularly in the Community group.

There were also some unexpected results from this study. The findings that the dietary intake ratios of n-6/n-3 FAs and LA/ALA correlated positively with colostrum EPA and negatively with n-6 PUFAs in mature milk, and the observed negative correlation between ALA intake and EPA content in mature milk were unexpected. To date, there are no other studies (to our knowledge) that have reported these correlations. A potential explanation could be that not only the recent dietary intake as assessed in the 1- and 3-day dietary records, but also the habitual intake of FAs has an influence on the HM FA profile. The usual diet consumed by the mothers may have been different to the confinement diet they were following while breastfeeding, which is still reflected in their HM FA profile [41]. Yet, we cannot exclude the possibility of false positive findings due to multiple testing. Because of the exploratory character of this study, we decided not to correct for multiple testing [42–44]. Other limitations of this study include the small sample size and the predefined V1 window (between 0 and 7 days postpartum) which is relatively long considering the dramatic changes in milk composition during the first days after infant's birth [5]. Moreover, to reduce the burden of the participants, the dietary intakes of the mothers from the Community and MCC groups were assessed by a 1- and 3-day food record, respectively. It is known that the dietary intake of individuals is not stable from day to day; hence, it may be debatable whether 1 or even 3-day food records are enough to estimate maternal dietary intake [45]. Yet, during the confinement period many Chinese women adhere to strict dietary guidelines, which likely reduce the variability in intake levels [25]. Lastly, the observational nature of our study design limits the ability to assign causality and may have led to residual confounding from unmeasured factors.

Strengths of the study are the repeated dietary intake assessment and HM analyses over the first 6 weeks postpartum, including the colostrum phase as well as the full period in which women typically follow the confinement diet [25]. Additionally, the selected study population of lactating women from an urban coastal area in China and residing either in the community or in a MCC during the first 4 weeks after infant's birth, allows for a comparison

with other Chinese regions or subsets of the populations. For example, our results show that women belonging to the Community group (generally having a lower socioeconomic status) had lower intakes of n-3 and n-6 PUFAs and higher dietary intake ratios of n-6/n-3 and LA/ALA, which was reflected in the composition of their milk. The Community group had higher mature milk contents of MUFAs, total n-6 PUFAs, LA and of the ratio LA/ALA and lower contents of total n-3 PUFAs, ALA, and DHA, all directing toward a potentially less healthy HM FA profile.

In conclusion, this study adds to our understanding of the diet of lactating women living in an urban coastal region of China, which is influenced by both traditional beliefs typical of the Chinese confinement diet as well as a shift in dietary patterns toward a more Westernized diet and was shown to be associated with the HM FA profile. It is important to further investigate how to optimize the diets of breastfeeding women in China and their HM composition, to assess differences between various Chinese regions and characteristics of the mother and infant, and to better understand the relationship between diet and HM FA composition in order to create tailored nutritional advices that optimize the FA profile of HM and, consequently, improve infant's health.

## Acknowledgments

The research has received scientific contribution from Danone Nutricia Research, the Netherlands and Singapore. The dietary photos and records were analyzed by nutritionists from the Department of Clinical Nutrition of SCMC and a professional maternal nutrition team of Nanjing Medical University School of Public Health. The FAs in HM were analyzed by Shanghai TCM University. The authors would like to thank J. Pfeil and S. Amodio for giving statistical advice and suggestions during the preparation of this paper.

## References

- Horta BL, Victora CG. [Long-term effects of breastfeeding: a systematic review](#). Geneva, Switzerland: World Health Organization; 2013.
- Stam J, Sauer PJ, Boehm G. Can we define an infant's need from the composition of human milk? *Am J Clin Nutr*. 2013 Aug;98(2):521s–8s.
- Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am*. 2013 Feb;60(1):49–74.
- Chung MY. Factors affecting human milk composition. *Pediatr Neonatol*. 2014 Dec;55(6):421–2.
- Bravi F, Wiens F, Decarli A, Dal Pont A, Agostoni C, Ferraroni M. Impact of maternal nutrition on breast-milk composition: a systematic review. *Am J Clin Nutr*. 2016 Sep;104(3):646–62.
- Keikha M, Bahreynian M, Saleki M, Kelishadi R. Macro- and micronutrients of human milk composition: are they related to maternal diet? A comprehensive systematic review. *Breastfeed Med*. 2017 Nov;12(9):517–27.
- Demmelmair H, Koletzko B. Lipids in human milk. *Best Pract Res Clin Endocrinol Metab*. 2018 Jan;32(1):57–68.
- Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu Rev Nutr*. 2005;25:317–40.
- Molto-Puigmartí C, Castellote AI, Carbonell-Estrany X, Lopez-Sabater MC. Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. *Clin Nutr*. 2011 Feb;30(1):116–23.
- Olafsdottir AS, Thorsdottir I, Wagner KH, Elmadfa I. Polyunsaturated fatty acids in the diet and breast milk of lactating Icelandic women with traditional fish and cod liver oil consumption. *Ann Nutr Metab*. 2006 May;50(3):270–6.

## Statement of Ethics

Ethical approval was obtained from the Institutional Review Board of the SCMC (SCMCIRBK2013026), and all women provided written informed consent.

## Conflict of Interest Statement

B.S., Y.J., and S.R.B.M.E. are employees of Danone Nutricia Research.

## Funding Sources

The research has received financial support and scientific contribution from Danone Nutricia Early Life Nutrition, China. This work was also supported by the MCC, Renji Hospital, and Shanghai First Maternity and Infant Health Hospital.

## Author Contributions

Z.W., R.H., B.S., Y.J., and J.L. are responsible for the conception and design of the study. Z.W., R.H., and J.L. are responsible for the acquisition of the data and I.R.M.S., Y.J., and S.R.B.M.E. are responsible for the initial analysis and interpretation of the data. I.R.M.S. and S.R.B.M.E. drafted the manuscript, and all authors are responsible for revising the work critically for important intellectual content and gave their final approval of this version. All the authors are accountable for all aspects of the work.

## Data Availability Statement

All data generated or analysed during this study are included in this article and its online supplementary Files. Further enquiries can be directed to the corresponding author.

- 11 Martin MA, Lassek WD, Gaulin SJ, Evans RW, Woo JG, Geraghty SR, et al. Fatty acid composition in the mature milk of Bolivian forager-horticulturalists: controlled comparisons with a US sample. *Matern Child Nutr.* 2012 Jul;8(3):404–18.
- 12 Gao C, Liu G, Whitfield KC, Kroehn H, Green TJ, Gibson RA, et al. Comparison of human milk fatty acid composition of women from Cambodia and Australia. *J Hum Lact.* 2018 Aug;34(3):585–91.
- 13 Xiang M, Harbige LS, Zetterström R. Long-chain polyunsaturated fatty acids in Chinese and Swedish mothers: diet, breast milk and infant growth. *Acta Paediatr.* 2005 Nov;94(11):1543–9.
- 14 Urwin HJ, Zhang J, Gao Y, Wang C, Li L, Song P, et al. Immune factors and fatty acid composition in human milk from river/lake, coastal and inland regions of China. *Br J Nutr.* 2013 Jun;109(11):1949–61.
- 15 Jiang J, Wu K, Yu Z, Ren Y, Zhao Y, Jiang Y, et al. Changes in fatty acid composition of human milk over lactation stages and relationship with dietary intake in Chinese women. *Food Funct.* 2016 Jul 13;7(7):3154–62.
- 16 Liu G, Ding Z, Li X, Chen X, Wu Y, Xie L. Relationship between polyunsaturated fatty acid levels in maternal diets and human milk in the first month post-partum. *J Hum Nutr Diet.* 2016;29(4):405–10.
- 17 Tian H-M, Wu Y-X, Lin Y-Q, Chen X-Y, Yu M, Lu T, et al. Dietary patterns affect maternal macronutrient intake levels and the fatty acid profile of breast milk in lactating Chinese mothers. *Nutrition.* 2019 Feb;58:83–8.
- 18 Wong V, Ng Y-F, Chan S-M, Su Y-X, Kwok K, Chan H-M, et al. Positive relationship between consumption of specific fish type and omega-3 polyunsaturated fatty acids in milk of Hong Kong lactating mothers. *Br J Nutr.* 2019 Jun;121(12):1431–40.
- 19 Huang Z, Hu Y. Dietary patterns and their association with breast milk macronutrient composition among lactating women. *Int Breastfeed J.* 2020 Jun 5;15(1):52.
- 20 Peng Y, Zhou T, Wang Q, Liu P, Zhang T, Zetterstrom R, et al. Fatty acid composition of diet, cord blood and breast milk in Chinese mothers with different dietary habits. *Prostaglandins Leukot Essent Fatty Acids.* 2009 Nov–Dec;81(5–6):325–30.
- 21 Hu R, Fei J, Zhai Y, Feng Y, Warren J, Jin Y, et al. The dietary intake of two groups of lactating women in Shanghai during the puerperium. *Asia Pac J Clin Nutr.* 2019;28(1):106–15.
- 22 National Institute of Nutrition and Food Safety CC. *China food composition.* Beijing: University Medical Press; 2009.
- 23 Nayak U, Kanungo S, Zhang D, Ross Colgate E, Carmolli MP, Dey A, et al. Influence of maternal and socioeconomic factors on breast milk fatty acid composition in urban, low-income families. *Matern Child Nutr.* 2017;13(4):e12423.
- 24 Miliku K, Duan QL, Moraes TJ, Becker AB, Mandhane PJ, Turvey SE, et al. Human milk fatty acid composition is associated with dietary, genetic, sociodemographic, and environmental factors in the CHILD Cohort Study. *Am J Clin Nutr.* 2019 Dec 1;110(6):1370–83.
- 25 Poh BK, Wong YP, Karim NA. Postpartum dietary intakes and food taboos among Chinese women attending maternal and child health clinics and maternity hospital, Kuala Lumpur. *Mal J Nutr.* 2005;11(1):1–21.
- 26 Yang YX, Wang XL, Leong PM, Zhang HM, Yang XG, Kong LZ, et al. New Chinese dietary guidelines: healthy eating patterns and food-based dietary recommendations. *Asia Pac J Clin Nutr.* 2018;27(4):908–13.
- 27 Li YC, Jiang B, Zhang M, Huang ZJ, Deng Q, Zhou MG, et al. Vegetable and fruit consumption among Chinese adults and associated factors: a nationally representative study of 170,847 adults. *Biomed Environ Sci.* 2017 Dec;30(12):863–74.
- 28 Koletzko B, Cetin I, Brenna JT. Dietary fat intakes for pregnant and lactating women. *Br J Nutr.* 2007 Nov;98(5):873–7.
- 29 Antonakou A, Skenderi KP, Chiou A, Anastasiou CA, Bakoula C, Matalas AL. Breast milk fat concentration and fatty acid pattern during the first six months in exclusively breastfeeding Greek women. *Eur J Nutr.* 2013 Apr;52(3):963–73.
- 30 Kim H, Kang S, Jung BM, Yi H, Jung JA, Chang N. Breast milk fatty acid composition and fatty acid intake of lactating mothers in South Korea. *Br J Nutr.* 2017 Feb;117(4):556–61.
- 31 Barrera C, Valenzuela R, Chamorro R, Bascuñán K, Sandoval J, Sabag N, et al. The impact of maternal diet during pregnancy and lactation on the fatty acid composition of erythrocytes and breast milk of Chilean women. *Nutrients.* 2018 Jun 28;10(7):839.
- 32 Butts CA, Hedderley DI, Herath TD, Paturi G, Glyn-Jones S, Wiens F, et al. Human milk composition and dietary intakes of breastfeeding women of different ethnicity from the Manawatu-Wanganui Region of New Zealand. *Nutrients.* 2018 Sep 4;10(9):1231.
- 33 Aumeistere L, Ciproviča I, Zavadskā D, Andersons J, Volkovs V, Čelmalniece K. Impact of maternal diet on human milk composition among lactating women in Latvia. *Medicina.* 2019 May 20;55(5):173.
- 34 Richter CK, Bowen KJ, Mozaffarian D, Kris-Etherton PM, Skulas-Ray AC. Total long-chain n-3 fatty acid intake and food sources in the United States compared to recommended intakes: NHANES 2003–2008. *Lipids.* 2017 Nov;52(11):917–27.
- 35 United States Department of Agriculture. National nutrient database for standard reference nutrient data laboratory (NDL)/Food and Nutrition Information Center (FNIC) food composition database.
- 36 Institute of Medicine. *Nutrition during lactation.* Washington, DC: The National Academies Press; 1991.
- 37 Scopesi F, Ciangherotti S, Lantieri PB, Rizzo D, Bertini I, Campone F, et al. Maternal dietary PUFAs intake and human milk content relationships during the first month of lactation. *Clin Nutr.* 2001 Oct;20(5):393–7.
- 38 Visentainer JV, Santos OO, Maldaner L, Zappiello C, Neia V, Visentainer L, et al. Lipids and fatty acids in human milk: benefits and analysis. 2018.
- 39 Zhang Z, Fulgoni VL, Kris-Etherton PM, Mittlemeier SH. Dietary intakes of EPA and DHA omega-3 fatty acids among US childbearing-age and pregnant women: an analysis of NHANES 2001–2014. *Nutrients.* 2018 Mar 28;10(4):416.
- 40 EFSA panel on dietetic products, nutrition, and allergies (NDA); scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J.* 2010;8(3):1461.
- 41 Bzikowska-Jura A, Czerwonogrodzka-Senczyna A, Jasińska-Melon E, Mojska H, Olędzka G, Wesolowska A, et al. The concentration of omega-3 fatty acids in human milk is related to their habitual but not current intake. *Nutrients.* 2019;11(7):1585.
- 42 Bender R, Lange S. Adjusting for multiple testing: when and how? *J Clin Epidemiol.* 2001 Apr;54(4):343–9.
- 43 Wason JM, Stecher L, Mander AP. Correcting for multiple-testing in multi-arm trials: is it necessary and is it done? *Trials.* 2014 Sep 17;15:364.
- 44 Li G, Taljaard M, Van den Heuvel ER, Levine MA, Cook DJ, Wells GA, et al. An introduction to multiplicity issues in clinical trials: the what, why, when and how. *Int J Epidemiol.* 2017 Apr 1;46(2):746–55.
- 45 Subcommittee on Criteria for Dietary Evaluation – National Research Council. *Nutrient adequacy: assessment using food consumption surveys. Design, methods and first results.* Washington, DC: National Academies Press; 1986.