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ARTICLE

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Quantification of breast milk *trans* fatty acids and *trans* fat intake by Hong Kong lactating women

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Abstract

Background/objectives Diet westernization in Hong Kong may increase *trans* fat intake, whereas it may decrease intake of polyunsaturated fatty acids. The present study was to determine the current breast milk fatty acid composition and *trans* fat intake by Hong Kong lactating mothers.

Methods Sixty lactating women were recruited for the study. Each participant donated 15 ml of milk at week 4 and 6 after delivery. Dietary data were collected using a 3-day food record method, covering 2 weekdays and 1 weekend day. Milk fatty acids were analyzed using a gas chromatography method. *Trans* fat consumption was assessed using the Nutrition Data System for Research or calculating the intake based on percentage of *trans* fatty acids in the breast milk.

Results Linoleic acid, α-linolenic acid, arachidonic acid, and docosahexaenoic acid in breast milk were 16.23%, 1.52%, 0.59%, and 0.66% total milk lipids, respectively. Mean daily intakes of total fat, saturated fatty acids, monounsaturated fatty acids, were 79, 24, 29, and 18 g, respectively. Total *trans* fat intake was estimated to be 1.15–1.20 g daily and accounted for 0.50–0.52% total energy.

Conclusions Breast milk of Hong Kong Chinese lactating women contained relatively higher contents of arachidonic and docosahexaenoic acids compared with those of Western countries. Compared with the previous study conducted in 1995, breast milk total *trans* fatty acids in 2018 still remained low. Total *trans* fat intake by Hong Kong lactating women in 2018 was a half of WHO's recommendation that total *trans* fat intake should be <1% total energy.

Introduction

The consumption of total *trans* fatty acids increases the risk of heart diseases and may affect the growth and development of infants [1–3]. Human studies have revealed that *trans* fatty acid consumption raises plasma cholesterol and triacylglycerol level. Arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) are considered essential fatty acids for pre-term infants. These long-chain polyunsaturated fatty acids (LC-PUFA) can be endogenously synthesized if diet contains linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3) through a pathway of desaturation and elongation. Inverse correlations are found between *trans* fatty acid consumption and LC-PUFA in the venous cord blood lipids in full-term infants [4]. Although adverse effect of *trans* fatty acids on infant growth and development has not been established, it must exercise caution in this aspect.

Hong Kong is a city where the East meets the West. Diet westernization is taking place due to extensive cultural exchanges, industrialization, and urbanization [5, 6]. With improvement in educational attainment and an increase in job opportunities, there is an increase in the percentage of Hong Kong women joining the labor force in the past 20 years [7]. A smaller family size, recently due to a decrease in fertility rates for working couples, and an increase in opportunity cost of women's time, may lead to eat outside home more frequently and increase the demand of

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convenient processed foods, such as fast foods, and ready-made meals [7, 8].

Breast milk is the primary nutrient source for infants and serve as an indicator for dietary practices of the lactating mothers. It is known that humans do not produce *trans* fatty acids and maternal dietary *trans* fatty acids are able to transfer to the breast milk [9]. In this regard, the presence of *trans* fatty acids in breast milk is mainly come from maternal diet [10]. Our previous research in 1995 showed that the total *trans* fatty acids in breast milk of Hong Kong lactating women accounted for 0.88% total milk lipids [11]. Accompanied with diet westernization in Hong Kong and an increase in consumption of convenient processed foods, *trans* fat intake may increase accordingly, thus possibly leading to an increase in breast milk *trans* fatty acid content. Although the fatty acid composition of human milk has been well reported in various countries [12–17], there is very limited updated information on human milk fatty acid composition of Hong Kong Chinese lactating women in the recent years. The aims of the present study were (i) to quantify *trans* fatty acids and LC-PUFA in diets of Hong Kong lactating mothers using a method of 3-day food records; (ii) to quantify *trans* fatty acids and LC-PUFA in breast milk of Hong Kong lactating women using a method of gas chromatography; and (iii) to compare the current breast milk *trans* fatty acid composition with that obtained in 1995 [11].

Materials and methods

Study design

A 3-day food record method including 2 weekdays and 1 weekend day within a week was used to collect dietary intake data. Breast milk samples were collected to investigate its fatty acid composition. Data collection started in September 2017 and completed in December 2018. Ethical approval was obtained from the New Territories East Cluster Clinical Research Ethics Committee of the Chinese University of Hong Kong prior to initiation of the study. A signed consent was obtained from all lactating women who agreed to participate in this study.

Subjects and recruitment

Pregnant women were recruited mainly from obstetrics department in hospitals in Hong Kong. During their antenatal checkups, invitation letter and study information were distributed. After the delivery, those who were interested in participating the study were contacted to arrange home visits and provide training on documenting food record and materials for collecting breast milk. A signed consent was collected at the first home visit. Inclusion criteria of subjects included: (i) they were able to complete a 3-day food record at week 4 and 6 after the delivery; (ii) they were willing to donate breast milk sample after completion of week 4 and 6 food records; (iii) they had no chronic diseases that require food restriction or follow specific diets; and (iv) they had no breast disease such as mastitis.

Dietary data collection and analyses

The lactating women were instructed how to document their food intake by a registered dietitian. Each subject was required to record and describe the names, consumption amount, time, and location, where the foods and drinks were consumed. No food record was conducted 3 days before and after festivals that might affect one's regular eating habits (e.g., Mid-autumn Festival or Chinese New Year). Each lactating mother recorded her food intake for 3 days, 2 weekdays, and 1 weekend day, at week 4 and 6 after delivery. Thus, a total of 6 days of food records from each subject was collected.

Estimation of *trans* fat intake and nutrient intake using dietary data

Dietary data analyses were performed using a food composition database, Nutrition Data System for Research (NDSR) (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA). When a consumed food that could not be found in the NDSR database, a similar food would be used as an alternative, or created a new food item by using ingredients that are available in the NDSR database. The data were then exported to the Statistical Package for the Social Sciences version 23.0 (SPSS Inc, Chicago, IL, USA) for analysis. The mean 3-day food record of week 4 and 6 was accordingly computed. Results were compared with the recommendations for lactating women of the Chinese Nutrition Society (CNS) and the United States (US) Institute of Medicine (IOM). The recommendations by the World Health Organization (WHO) and Food and Agricultural Organization of the United Nations were also used when appropriate.

Milk collection and lipid extraction

Each participant expressed 15 ml of her milk using a manual milk pump on the assigned dates at week 4 and 6 after the delivery. The participants were instructed to collect the milk samples at the end of feeding in the morning. Babies were breastfed on demand and mothers look for signs of hungry and feed their babies whenever babies were hungry. Therefore, there was no a fixed time schedule for collecting the milk. Each time after collection, milk sample was placed in a residue-free tube and stored immediately into a freezer at −20 °C. Milk samples were thawed at room temperature (25 °C) before the analyses. Total fat from 6 g sample was extracted using 15 milliters of $CHCl_3$/MeOH (2:1, vol/vol) containing 6 mg/ml triheptadecanoin as an internal standard to quantify total milk fat.

Gas chromatographic analyses of milk fatty acids

The milk lipids were converted to fatty acid methyl esters (FAME) using a mixture of 14% BF_3/MeOH reagent (Sigma-Aldrich Co., St Louis, MO, USA) and toluene (1:1, vol/vol) at 90 °C for 45 min under nitrogen. FAME were analyzed on a SP-2560 flexible fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 µm film thickness; Supelco, Inc., Bellefonte, PA, USA) in a gas chromatograph (Hewlett-Packard 6890 Series with auto injector; Agilent, Palo Alto, CA, USA) equipped with a flame-ionization detector. Peaks were identified by comparison with authentic fatty acid standards (GLC reference standard GLC-674; Nu-Chek-Prep, Inc., Elysian, MN, USA). Column temperature was programmed from 150 °C to 180 °C at a rate of 0.5 °C/min, and then to 210 °C at a rate of 3 °C/min. Injector and detector temperatures were 220 °C and 220 °C, respectively. Hydrogen was used as the carrier gas at a head pressure of 20 psi. A typical GC chromatogram was shown in Supplementary Fig. 1.

Calculation of *trans* fat intake on its percentage in breast milk lipids

The *trans* fat intake by each lactating woman was also calculated on the basis of *trans* fatty acid content in breast milk, using a method of Craig-Schmidt et al. [18], who found that the *trans* fatty acid in the mother diet of previous day was well correlated with that in the breast milk. The *trans* fatty acid intake by mothers could be estimated using two equations shown below (Supplementary Fig. 2):

$$Y = -0.011X^2 + 0.707X; \quad (1)$$

$$T_{\text{intake}} = (E_{\text{total}} \times E_{\text{fat}} \times X) \div 9 \quad (2)$$

where Y is the percentage of *trans* fatty acids in breast milk fat; X is the percentage of *trans* fatty acids in the dietary fat of previous day consumed by mother; T_{intake} is the *trans* fatty acid intake of the mother; E_{total} is mother's total energy intake; E_{fat} is mother's energy percentage of fat intake; and 9 is kcal value per gram fat.

Statistical analysis

Data were expressed as mean ± standard deviation. Analysis of variance followed by Student's t-test (two tailed) where applicable was used for statistical evaluation of significant differences between groups. A p value < 0.05 was considered statistically significant.

Table 1 Characteristics of participants.

	N = 60		
	Mean	Range	N (%)
Age (years)	33.12	23–44	–
No. of child delivered	1.23	1–3	–
Postnatal care helpers were employed	–	–	31 (51.7)
Giving birth via natural delivery	–	–	26 (43.3)
Giving birth via C-section	–	–	34 (56.7)
Types of housing			
Public housing	–	–	8 (13.3)
Home ownership scheme housing	–	–	12 (20.0)
Private housing	–	–	28 (46.7)
Village house	–	–	6 (10.0)
Tenement building	–	–	5 (8.3)
Government quarters	–	–	1 (1.67)

Results

Participants

Total 72 lactating women were recruited in the present study. Among them, 60 met all of the inclusion criteria. The mean age of the participants was 33 years old, with a range of 23–44 years old. Characteristics of the participants were shown in Table 1.

Intakes of energy, carbohydrate, protein, and dietary fiber by Hong Kong lactating women in 2018

Mean energy intake was found to be 2069 kcal/day, lower than the estimated energy requirement (EER) of 2300–2900 kcal by CNS (Table 2). Mean carbohydrate intake was 215 g, accounting for 43% of total energy. As CNS only has estimated the average requirement (EAR) available for total grams of carbohydrate, IOM RDA was used for assessment. The mean carbohydrate intake among these Hong Kong lactating women was very close to the RDA of 210 g/day. Dietary data showed that 88% of the carbohydrate was from refined grains, whereas the mean intake of added sugar was ~27 g, which was about 5% of the total energy within the AMDRs (Table 2). Mean protein intake was 123 g, accounting for 24% of total energy intake. Compared with that set by IOM AMDR (10–35% energy), an average of 24% of the energy from protein among these lactating mothers was within the recommendation. The intake of animal proteins was prevalent, compared with that

Table 2 Calculated intakes of carbohydrate (CHO), protein (Pro), total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), *trans* fatty acids, using database Nutrition Data System for Research (NDSR) compared with the relevant recommendations.

Nutrients	*N* = 60												Recommendations	
	Week 4				Week 6				Overall					
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	CNS[a]	IOM[b]
Energy (kcal)	2085.96	624.59	877.40	4206.25	2052.52	600.29	739.72	3838.04	2069.24	611.94	739.72	4206.25	2300–2900[c]	ND
CHO (g)	218.44	69.44	44.44	510.66	210.97	64.46	67.64	413.96	214.71	67.01	44.44	510.66	160 g/d[d]	210 g/d[e]
Added sugar (g)	27.33	23.97	0.00	114.37	27.755	24.71	0.07	115.98	27.54	24.31	0.00	115.98	<10%E[f]	<25%E[f]
% kcal from CHO	43.06	10.05	18.75	73.68	42.08	8.51	16.73	62.84	42.57	9.31	16.73	73.68	50–65%E[f]	45–65%E[f]
Total PRO (g)	128.18	55.77	44.48	337.45	116.87	40.10	35.82	233.71	122.52	48.83	35.82	337.45	80 g/d[g]	70 g/d[f]
Animal PRO (g)	94.41	50.13	18.08	274.59	84.32	38.11	15.46	205.02	89.36	44.75	15.46	274.59	ND	ND
Vegetable PRO (g)	33.77	13.82	7.97	81.53	32.51	12.85	9.95	78.59	33.14	13.34	7.97	81.53	ND	ND
% kcal from PRO	24.74	6.29	10.31	46.19	23.61	6.09	11.71	43.35	24.18	6.21	10.31	46.19	ND	10–35%[f]
Total dietary fiber (g)	16.20	11.85	1.28	112.74	14.55	6.68	1.63	33.24	15.37	9.64	1.28	112.74	ND	29 g/d[h]
Soluble fiber (g)	3.10	2.77	0.12	29.11	3.07	1.84	0.01	9.85	3.09	2.35	0.01	29.11	ND	ND
Insoluble fiber (g)	13.01	10.45	0.98	103.47	11.37	5.37	1.62	27.87	12.19	8.34	0.98	103.47	ND	ND
Cholesterol (mg)	488.95	300.17	47.27	1694.79	464.79	295.58	65.34	3165.35	476.87	297.72	47.27	3165.35	ND	AL
Total fat (g)	76.11	31.43	12.80	177.14	81.09	33.64	15.19	178.34	78.60	32.61	12.80	178.34	ND	ND
% kcal from fat	31.98	7.40	10.09	53.24	34.17	7.08	14.05	53.52	33.07	7.31	10.09	53.52	20–30%E[f]	20–35%E[f]
Total SFA (g)	22.71	10.32	3.68	59.27	25.17	12.62	3.94	64.71	23.94	11.57	3.68	64.71	ND	ND
% kcal from SFA	9.55	2.82	2.18	17.80	10.49	3.35	3.52	22.49	10.02	3.13	2.18	22.49	<10% E[f]	AL
Total *trans* fat (g)	0.92	0.76	0.07	4.31	1.39	1.33	0.11	8.22	1.15	1.11	0.07	8.22	ND	AL
Total MUFA (g)	28.49	12.98	4.48	71.01	30.28	13.56	5.29	71.92	29.38	13.28	4.48	71.92	ND	ND
% kcal from MUFA	11.95	3.30	2.24	22.16	12.74	3.23	5.36	22.90	12.35	3.28	2.24	22.90	ND	ND
Total PUFA (g)	17.63	7.59	2.88	40.86	18.08	7.93	3.38	48.83	17.85	7.75	2.88	48.83	ND	ND
% kcal from PUFA	7.46	2.39	2.31	16.92	7.73	2.25	2.75	14.25	7.59	2.32	2.31	16.92	ND	ND

SD standard deviation, *Min* minimum, *Max* maximum, *kcal* kilocalories, *ND* recommendation not determined, *g/d* grams per day, *%E* percent of total energy, *AL* as low as possible.

[a]Recommendations by the Chinese Nutrition Society (CNS).

[b]Recommendation by the Institute of Medicine (IOM), National Academies.

[c]Estimated energy requirement (EER).

[d]Estimated average requirement (EAR).

[e]Recommended dietary allowance (RDA).

[f]Acceptable macronutrient distribution range (AMDR).

[g]Reference nutrient intakes (RNI).

[h]Adequate intake (AI).

Table 3 *Trans* fatty acid intake by Hong Kong Chinese lactating women in 2018[a].

	Week 4	Week 6	All stages of lactation
Total milk fat in breast milk (g)[b]	22.54 (9.81)	23.24 (9.83)	22.89 (9.79)
Total *trans* fat in breast milk (g)[b]	0.22 (0.12)	0.27 (0.13)	0.24 (0.13)
% total *trans* fat in breast milk fat[b]	0.98 (0.31)	1.17 (0.36)	1.08 (0.35)
% *trans* fat in dietary fat consumed by lactating women in previous day[c]	1.39 (0.44)	1.66 (0.52)	1.52 (0.49)
Daily *trans* fat intake (g)[c]	1.05 (0.45)	1.35 (0.76)	1.20 (0.64)

[a]Data are mean (SD); $n = 60$.

[b]Data are calculated from GC analysis of breast milk.

[c]Data are calculated by equations adapted from Craig-Schmidt et al. [18].

of vegetable protein in these women. Dietary fiber intake was 15 g, with 3 g of soluble fiber and 12 g of insoluble fiber (Table 2). Mean intake of dietary fiber was lower than the AI of 29 g/day set by IOM. Hong Kong Department of Health (DH) recommends daily fiber intake of at least 25 g. This indicates that the intake of dietary fiber by these Hong Kong lactating women was insufficient.

Calculation of intakes of cholesterol, total fat and *trans* fat by Hong Kong lactating women in 2018 using NDSR

Results from NDSR showed that Hong Kong lactating women had a mean intake of 477 mg cholesterol. CNS does not have a recommendation for cholesterol. IOM recommends daily cholesterol intake to be as low as possible. From the result of the 3-day food records, mean intakes of total fat, saturated fatty acids (SFA), *trans*-fatty acids, monounsaturated fatty acids (MUFA), and PUFA were 79, 24, 1.2, 29, and 18 g, respectively. Energy from total fat was 33%, exceeding the CNS recommendation of 20–30%, but within 20–35% recommended by IOM (Table 2). Energy from SFA was ~10% energy, which was at the borderline of the CNS recommendation of <10% in a day. Result from NDSR analysis showed that the Hong Kong lactating women consumed 1.15 g *trans* fat daily (0.50% energy), which was below WHO's recommendation of <1.0% total energy. Table 2 also showed the intakes of other fatty acids collected by 3-day food records.

Calculation of *trans* fat intake by Hong Kong women in 2018 using gas chromatographic analysis

Results from gas chromatographic analysis showed total fat and *trans* fat in breast milk were 22.89 and 0.24 g/kg breast milk (Table 3). The gas chromatographic analysis found that *trans* fatty acids accounted 1.08% total milk fat. Using the first equation $Y = -0.011X^2 + 0.707X$, an average of 1.52% *trans* fatty acids as total milk fat was calculated. Using the second equation $T_{intake} = (E_{total} \times E_{fat} \times X) \div 9$

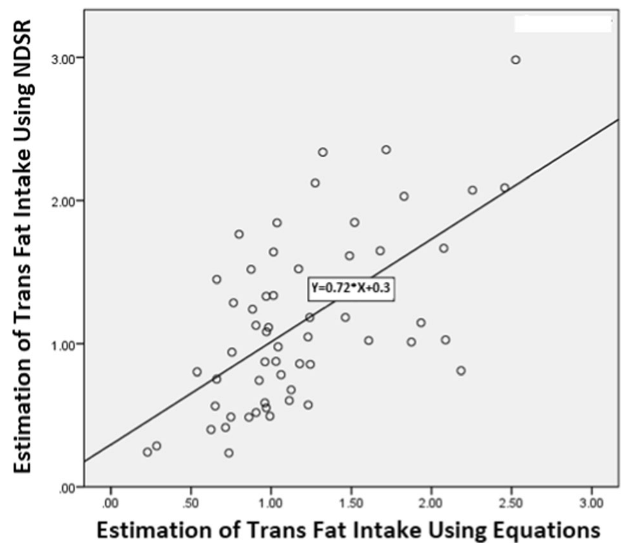

Fig. 1 Estimation of trans fat intake by two methods. Correlation of *trans* fat intake (g) of Hong Kong lactating women calculated by using data base Nutrition Data System for Research (NDSR) and using the equations described in "Materials and methods" adapted from [18] ($p < 0.01$).

described above [18], the Hong Kong lactating women consumed 1.20 g *trans* fat (Table 3), which was close to 1.15 g calculated using database NDSR (Table 2). Figure 1 shows a good correlation of Hong Kong lactating women's *trans* fat intake calculated using database NDSR and its intake calculated using the percentage of *trans* fatty acids in the breast milk determined by gas chromatographic analysis [18].

Comparison of milk fatty acid composition between week 4 and 6

Milk fatty acid composition in week 4 and 6 was shown in Table 4. In general, no differences were seen except for several minor fatty acids. The breast milk samples from week 4 had slightly but significantly higher amounts of 20:2n-6, 20:3n-6, and total n-6 long chain fatty acids than

Table 4 Milk fatty acid composition (wt% total fatty acids) of Hong Kong Chinese women at different stages of lactation in 2018[a].

Fatty acids	Week 4				Week 6				Total				*p* value
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
Total milk fat (g/L)	22.54	9.81	3.75	40.57	23.24	9.83	3.62	44.91	22.89	9.79	3.62	44.91	NS
10:0	1.67	0.82	0.59	4.65	1.46	0.59	0.37	3.43	1.57	0.72	0.37	4.65	NS
11:0	0.02	0.01	0.01	0.07	0.02	0.01	0.00	0.07	0.02	0.01	0.00	0.07	NS
12:0	6.61	2.84	0.00	12.63	5.97	1.98	2.49	11.75	6.29	2.46	0.00	12.63	NS
13:0	0.04	0.01	0.02	0.08	0.04	0.02	0.00	0.10	0.04	0.02	0.00	0.10	NS
14:0	5.31	1.94	2.07	9.41	4.95	1.52	1.79	8.45	5.13	1.75	1.79	9.41	NS
15:0	0.18	0.04	0.11	0.31	0.19	0.06	0.06	0.43	0.19	0.05	0.06	0.43	NS
16:0	19.86	2.23	15.23	27.02	20.03	2.16	15.47	24.93	19.95	2.19	15.23	27.02	NS
18:0	5.17	0.76	3.62	7.51	5.42	1.13	0.00	8.43	5.29	0.97	0.00	8.43	NS
20:0	0.14	0.03	0.09	0.22	0.15	0.04	0.07	0.28	0.15	0.03	0.07	0.28	NS
24:0	0.07	0.02	0.02	0.13	0.06	0.02	0.02	0.13	0.06	0.02	0.02	0.13	NS
14:0br	0.01	0.01	0.00	0.03	0.01	0.01	0.00	0.04	0.01	0.01	0.00	0.04	NS
16:0br	0.04	0.01	0.02	0.07	0.05	0.02	0.01	0.09	0.04	0.02	0.01	0.09	NS
Total saturated	39.11	5.23	30.83	50.69	38.35	4.69	25.99	48.64	38.73	4.96	25.99	50.69	NS
14:1Δ9c	0.12	0.06	0.05	0.26	0.13	0.07	0.02	0.37	0.13	0.06	0.02	0.37	NS
16:1Δ7c	0.48	0.07	0.32	0.71	0.47	0.06	0.30	0.62	0.48	0.07	0.30	0.71	NS
16:1Δ9c	2.73	0.70	1.17	4.00	2.53	0.64	0.52	3.81	2.63	0.67	0.52	4.00	NS
17:1Δ10c	0.17	0.04	0.11	0.31	0.18	0.05	0.01	0.30	0.17	0.04	0.01	0.31	NS
18:1Δ9c	32.54	3.93	22.24	40.87	33.16	3.12	26.43	40.34	32.85	3.55	22.24	40.87	NS
18:1Δ11c	2.00	0.29	1.30	2.96	2.07	0.87	1.43	8.44	2.04	0.65	1.30	8.44	NS
18:1Δ12c	0.03	0.03	0.00	0.13	0.05	0.11	0.00	0.87	0.04	0.08	0.00	0.87	NS
18:1Δ13c	0.08	0.02	0.01	0.12	0.08	0.02	0.03	0.17	0.08	0.02	0.01	0.17	NS
18:1Δ15c	0.01	0.01	0.00	0.05	0.02	0.02	0.00	0.06	0.01	0.01	0.00	0.06	NS
20:1Δ11c	0.46	0.11	0.26	0.93	0.48	0.15	0.26	1.13	0.47	0.13	0.26	1.13	NS
20:1Δ13c	0.08	0.05	0.03	0.31	0.09	0.04	0.03	0.23	0.09	0.05	0.03	0.31	NS
22:1Δ13c	0.12	0.06	0.06	0.30	0.12	0.06	0.05	0.26	0.12	0.06	0.05	0.30	NS
24:1c	0.16	0.16	0.08	1.34	0.20	0.38	0.06	2.48	0.18	0.29	0.06	2.48	NS
Other C18:1c	2.12	0.30	1.36	3.15	2.22	0.98	1.56	9.44	2.17	0.72	1.36	9.44	NS
Total cis-monounsaturated	39.03	4.41	26.12	47.34	39.61	3.33	31.20	46.38	39.32	3.90	26.12	47.34	NS
18:2Δ9c,15c	0.01	0.01	0.00	0.07	0.01	0.01	0.00	0.06	0.01	0.01	0.00	0.07	NS
20:2Δ8c,14c	0.06	0.05	0.00	0.28	0.06	0.05	0.02	0.30	0.06	0.05	0.00	0.30	NS
20:3Δ5c,8 c.11c	0.03	0.03	0.01	0.21	0.03	0.01	0.01	0.05	0.03	0.02	0.01	0.21	NS
20:3Δ5c.11 c.14c	0.06	0.02	0.01	0.11	0.06	0.03	0.03	0.17	0.06	0.02	0.01	0.17	NS
Total unusual cis-polyunsaturated	0.16	0.07	0.07	0.40	0.16	0.06	0.09	0.39	0.16	0.07	0.07	0.40	NS
18:2n-6	16.22	3.05	10.97	25.80	16.24	2.32	10.81	21.02	16.23	2.70	10.81	25.80	NS
18:3n-6	0.12	0.05	0.04	0.29	0.12	0.06	0.04	0.45	0.12	0.06	0.04	0.45	NS
20:2n-6	0.45	0.09	0.16	0.66	0.41	0.08	0.06	0.63	0.43	0.09	0.06	0.66	$p < 0.05$
20:3n-6	0.40	0.12	0.01	0.68	0.35	0.08	0.18	0.52	0.38	0.10	0.01	0.68	$p < 0.05$
20:4n-6	0.61	0.12	0.38	0.97	0.57	0.12	0.34	0.81	0.59	0.12	0.34	0.97	NS
22:4n-6	0.10	0.04	0.05	0.23	0.10	0.05	0.04	0.24	0.10	0.04	0.04	0.24	NS
22:5n-6	0.00	0.01	0.00	0.08	0.00	0.02	0.00	0.11	0.01	0.02	0.00	0.11	NS
Total n-6 LCP	1.56	0.26	1.06	2.18	1.44	0.23	0.87	1.97	1.50	0.25	0.87	2.18	$p < 0.01$
Total n-6 polyunsaturated	17.90	3.13	12.33	27.50	17.80	2.38	11.99	22.86	17.85	2.77	11.99	27.50	NS
18:3n-3	1.47	0.39	0.69	2.61	1.56	0.42	0.82	2.72	1.52	0.41	0.69	2.72	NS
20:4n-3	0.01	0.01	0.00	0.06	0.00	0.00	0.00	0.02	0.01	0.01	0.00	0.06	NS
20:5n-3	0.17	0.09	0.01	0.47	0.18	0.10	0.05	0.45	0.17	0.10	0.01	0.47	NS

Table 4 (continued)

Fatty acids	Week 4				Week 6				Total				*p* value
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
22:5n-3	0.24	0.09	0.10	0.53	0.25	0.09	0.10	0.48	0.25	0.09	0.10	0.53	NS
22:6n-3	0.67	0.30	0.22	1.43	0.65	0.32	0.21	1.51	0.66	0.31	0.21	1.51	NS
Total n-3 LCP	1.09	0.45	0.45	2.36	1.08	0.47	0.41	2.23	1.08	0.46	0.41	2.36	NS
Total n-3 polyunsaturated	2.56	0.68	1.29	4.26	2.64	0.66	1.59	4.41	2.60	0.67	1.29	4.41	NS
14:1Δ9t	0.04	0.01	0.03	0.04	0.07	0.05	0.01	0.19	0.06	0.05	0.01	0.19	NS
16:1Δ7t	0.10	0.04	0.03	0.20	0.12	0.05	0.04	0.23	0.11	0.04	0.03	0.23	$p < 0.01$
16:1Δ9t	0.01	0.01	0.00	0.06	0.00	0.01	0.00	0.07	0.00	0.01	0.00	0.07	NS
18:1(Δ7–Δ11)t	0.34	0.19	0.11	1.04	0.45	0.26	0.15	1.29	0.40	0.23	0.11	1.29	$p < 0.05$
18:2tt	0.04	0.02	0.00	0.09	0.05	0.03	0.00	0.14	0.04	0.02	0.00	0.14	$p < 0.05$
18:2Δ9c.13t + 18:2Δ8t.12c	0.04	0.05	0.00	0.40	0.04	0.02	0.00	0.10	0.04	0.04	0.00	0.40	NS
18:2Δ9c.12t	0.11	0.03	0.07	0.25	0.12	0.02	0.07	0.18	0.11	0.03	0.07	0.25	NS
18:2Δ9t,12c	0.09	0.03	0.00	0.20	0.10	0.02	0.06	0.16	0.09	0.03	0.00	0.20	NS
18:3t	0.21	0.09	0.06	0.46	0.24	0.13	0.04	0.88	0.23	0.11	0.04	0.88	NS
Total C16:1t	0.13	0.03	0.09	0.20	0.15	0.04	0.04	0.23	0.14	0.03	0.04	0.23	$p < 0.05$
Total C18:2t	0.28	0.10	0.14	0.76	0.31	0.08	0.14	0.56	0.30	0.10	0.14	0.76	NS
Total Trans	0.98	0.31	0.54	1.76	1.17	0.36	0.66	2.18	1.08	0.35	0.54	2.18	$p < 0.01$
18:2 conjugate	0.12	0.08	0.00	0.27	0.14	0.10	0.03	0.44	0.13	0.09	0.00	0.44	NS

[a]$n = 60$; NS ($p > 0.05$); *NS* not significant.

those from week 6. In contrast, the breast milk samples from week 4 had slightly but significantly lower amounts of total *trans* fatty acids mainly including18:1(Δ7–Δ11)t and 16:1Δ7t (Table 4).

Comparison of milk fatty acid composition between 2018 and 1995

The average fatty acid composition of milk pooled from week 4 and 6 of lactation in Hong Kong Chinese lactating women in 2018 was shown in Table 4. For comparison, the data of Hong Kong Chinese lactating women in 1995 were also shown in Table 5 [11]. A number of differences in the milk fatty acid profiles were observed between 1995's and 2018. The breast milk in 2018 contained higher amounts of total *trans*-fatty acids and total n-3 fatty acids than those from 1995 (Table 5). Regarding the major saturated and mono-unsaturated fatty acids, the breast milk samples in 2018 had lower amounts of palmitic acid (PA, 16:0), stearic acid (SA, 18:0), and oleic acid (OA, 18:1n-9). Regarding the major n-6 fatty acids, no difference in LA (18:2n-6) and ARA (20:4n-6) were seen between 2018 and 1995. Regarding the major n-3 fatty acids, the breast milk samples from 2018 had higher amounts of ALA (18:3n-3) and EPA (20:5n-5) than those from 1995 ($P < 0.01$). DHA (22:6n-3) in the breast milk samples derived from 2018 was marginally higher than that from 1995 ($P = 0.06$).

Discussion

Extensive consumption of *trans* fat from partially hydrogenated vegetable oils is associated with the risk of heart diseases in adults [1, 2]. Excessive consumption of *trans* fat by mothers may also affect the growth and development of infants [3]. To find out *trans* fat intake by Hong Kong people, we estimated the *trans* fat intake using NDSR, finding that Hong Kong lactating women consumed an average of 1.15 g *trans* fat daily in 2018 (Table 2). The present study was the first of its kinds to estimate the *trans* fat intake by lactating women based on the percentage of *trans* fatty acids in the breast milk using Eqs. 1 and 2 described in "Materials and Methods", finding that Hong Kong lactating women consumed an average of 1.20 g *trans* fat each day (Table 3). Results demonstrated the two methods were well correlated and reliable ($p < 0.01$; Fig. 1). A total daily *trans* fat consumption of 1.15–1.20 g *trans* fat accounted for 0.50–0.52% total calories, which was a half of WHO's recommendation of <1.0% total energy. If the data on *trans* fat intake by lactating women could be extrapolated to the rest members of their family or general population at this time, it would be concluded that the relative *trans* fat intake by Hong Kong people was low.

Trans fat intake across the lactation in Hong Kong lactating women was not constant. It was interesting to notice that daily *trans* fat intake increased from 0.92 g in week 4 to 1.39 g in week 6 using NDSR (Table 2), while it increased

Table 5 Milk fatty acid composition (wt% total fatty acids) of Hong Kong Chinese women at different stages of lactation in 2018 compared with that in 1995[a].

	2018 ($n = 60$)	1995 ($n = 51$)[b]	p value
Saturated			
10:0	1.57 (0.72)	0.53 (0.38)	$p < 0.001$
12:0	6.29 (2.46)	4.23 (2.15)	$p < 0.001$
14:0	5.13 (1.75)	5.50 (2.01)	$p = 0.302$
15:0	0.19 (0.05)	0.31 (0.10)	$p < 0.001$
16:0	19.95 (2.19)	21.29 (2.27)	$p = 0.002$
18:0	5.29 (0.97)	5.86 (0.98)	$p = 0.003$
20:0	0.15 (0.03)	0.20 (0.15)	$p = 0.013$
Cis-monounsaturated			
16:1n-7	2.63 (0.67)	2.23 (0.59)	$p = 0.001$
16:1n-9	0.48 (0.07)	0.56 (0.09)	$p < 0.001$
17:1n-7	0.17 (0.04)	0.22 (0.07)	$p < 0.001$
18:1n-9	32.85 (3.55)	34.40 (3.90)	$p = 0.031$
Other 18:1c	2.17 (0.72)	2.02 (0.78)	$p = 0.295$
20:1n-9	0.47 (0.13)	0.79 (0.29)	$p < 0.001$
22:1n-9	0.12 (0.06)	0.16 (0.14)	$p = 0.047$
n-6 polyunsaturated			
18:2n-6	16.23 (2.70)	15.80 (3.01)	$p = 0.429$
18:3n-6	0.12 (0.06)	0.15 (0.09)	$p = 0.039$
20:2n-6	0.43 (0.09)	0.68 (0.32)	$p < 0.001$
20:3n-6	0.38 (0.10)	0.41 (0.16)	$p = 0.232$
20:4n-6	0.59 (0.12)	0.61 (0.18)	$p = 0.487$
22:4n-6	0.10 (0.04)	0.23 (0.20)	$p < 0.001$
22:5n-6	0.01 (0.02)	0.09 (0.11)	$p < 0.001$
n-3 polyunsaturated			
18:3n-3	1.52 (0.41)	1.24 (0.54)	$p = 0.002$
20:5n-3	0.17 (0.10)	0.08 (0.09)	$p < 0.001$
22:5n-3	0.25 (0.09)	0.23 (0.14)	$p = 0.366$
22:6n-3	0.66 (0.31)	0.56 (0.23)	$p = 0.060$
Total saturated	38.73 (4.96)	38.07 (4.56)	$p = 0.470$
Total C10–C13	7.91 (3.07)	4.76 (2.45)	$p < 0.001$
Total C14–C16	28.75 (3.11)	26.79 (3.14)	$p = 0.001$
Total n-6	17.85 (2.77)	17.97 (2.94)	$p = 0.825$
Total n-3	2.60 (0.67)	2.17 (0.74)	$p = 0.002$
Total *trans*	1.08 (0.35)	0.88 (0.61)	$p = 0.033$

[a]Data are mean (SD) from week 4 and 6.

[b]Adapted from Chen et al. [11].

from 1.05 g in week 4 to 1.35 g in week 6 using Eqs. 1 and 2 (Table 3). This was probably due to changes in dietary pattern. Usually, Chinese lactating women stay and eat at home in the first month after delivery. Thereafter, they start to eat outside home, leading to an increase in consumption of *trans* fat-enriched foods. Compared with a value of 0.88% *trans* fatty acids in total lipids of breast milk in 1995, the *trans* fat content in breast milk in 2018 slightly but significantly increased to 1.08%, an 27% increase (Table 5). In this regard, the *trans* fat content in breast milk of Hong Kong lactating women was much lower than that recently reported from Malaysia (2.95% milk lipids) and Brazil (1.5% milk lipids), but higher than that from New Zealand (0.86% total milk lipids) [12, 16, 17].

The milk fatty acid composition in 2018 samples of Hong Kong lactating women was slightly different from that in 1995 samples. Regarding the saturated and MUFA, Hong Kong lactating women in 2018 had slightly lower contents of PA (16:0), SA (18:0) and OA (18:1n-9) but had a slight higher content of palmitioleic acid (16:1n-7) (Table 5). Among n-6 PUFA, no differences in LA (18:2n-6) and ARA (20:4n-6) were seen between 2018 and 1995. However, these minor n-6 PUFA including 18:3n-6, 20:2n-6, 22:4n-6, and 22:5n-6 in breast milk samples of 2018 were slightly higher than those of 1995. When total n-6 PUFA in the breast milk of 2018 were compared with those of 1995, no significant difference was seen (Table 5). Regarding n-3 PUFA, Hong Kong lactating women in 2018 had slightly higher contents of ALA (18:3n-3) and EPA (20:5n-3) in the breast milk compared with those in 1995. As a whole, the breast milk samples collected in 2018 had an intake of total n-3 PUFA 18% higher than that collected in 1995. The changes in these minor fatty acids of 2018 breast milk samples suggested that dietary fats consumed by Hong Kong lactating women in 2018 were slightly different from those in 1995.

Human breast milk fat contains various fatty acids derived from three sources: maternal fat stores, maternal dietary lipids, and *de novo* synthesis in the mammary gland [19–23]. Contents of PUFA in breast milk vary with countries [12–17]. Hong Kong Chinese lactating women have a higher ALA (18:3n-3) content of 1.52% in total milk lipids compared with those in Brazil, Malaysia, Switzerland, New Zealand, and Spain [12, 15–17, 20]. DHA (22:6n-3) in the breast milk originates from either maternal diet or *de novo* synthesis. Hong Kong lactating women have a DHA content of 0.66% in total breast milk lipid comparable to that among the lactating women in Malaysia and South Korea [13, 16], but much higher than that in Brazil, Switzerland, New Zealand, Canada, and Spain, probably due to high consumption of sea foods in Hong Kong [12, 15, 17, 19, 20] (Supplementary Table 1). Hong Kong people prefer vegetable oils like corn oil, sunflower oil, and peanut oil as cooking oils. This was reflected from a higher content of LA (18:2n-6) in the breast milk lipids of Hong Kong lactating women compared with that in the breast milk of Malaysia, Switzerland, New Zealand, Canada, and Spain [12, 15, 16, 19, 20]. ARA (20:4n-6) in the breast milk originates from either maternal diet or *de novo* synthesis. As LA is the precursor for the synthesis of ARA, it was expected that Hong Kong Chinese lactating women had a higher ARA content in the breast milk compared with those in Brazil, New Zealand, Switzerland, South Korea, Canada, and Spain [12, 13, 15, 17]. Breast milk contains five major saturated and MUFA including PA (16:0), OA (18:1n-9), and SA (18:0), myristic acid (14:0), and lauric acid (12:0). The contents of these saturated fatty acids in the breast milk lipids of Hong Kong lactating women were similar to those in the breast milk lipids among the other countries [12–17].

A strength of the current study was the first time to estimate the *trans* fat intake by Hong Kong lactating women using the two methods, namely NDSR database and calculation based on the percentage of *trans* fatty acids in the breast milk. Our results demonstrated that these two methods were well correlated and both methods were able to provide a reliable and accurate estimation of the *trans* fat intake by the lactating women. The second strength of the current study was to collect the breast milk twice at both week 4 and 6, ensuring the consistency and no bias of milk sampling. The third strength of the present study was to compare the fatty acid composition of breast milk collected in 2018 with that in 1995, demonstrating the fats consumed by Hong Kong Lactating women were slightly different from those in 1995 because the former had a relative high content of n-3 PUFA. However, the present study had three limitations. First, there was no a fixed time schedule for collecting the breastmilk due to avoid the disruption of on-demand feeding. Inconsistent timing of collecting milk sample might affect the results of milk fatty acid composition as the breast milk composition could change during each feeding. Second, all the lactating women were instructed to collect their milk sample at the end of feeding in the morning and were trained to record their dietary intake using 3-day food record, however, there was no actual measure on their compliance. Third, the blood samples of mothers were no taken so that the association between the *trans* fat concentrations in maternal plasma with that in breast milk could not be explored.

In conclusion, Hong Kong lactating women consumed a diet in which total *trans* fats accounted for 0.50–0.52% total calories. This *trans* fat intake remains in line with WHO's recommendation that total *trans* fat intake should not be over than 1.0% total energy. Compared with Western countries, the breast milk of Hong Kong Chinese lactating women had relatively higher contents of LA, ARA, ALA, and DHA, most likely due to the popularity in consuming vegetable oils and sea foods by Hong Kong lactating women.

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Author contributions PSPY and TFJC were responsible for collecting the milk samples and conducting the dietary survey. ZH was responsible for carrying out the statistical analyses. LKL and SFSL were responsible for recruiting the lactating mothers. ZYC supervised and led the project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Nestel P. Trans fatty acids: are its cardiovascular risks fully appreciated? Clin Ther. 2014;36:316–21.
2. Ooi EMM, Watts GF, Ng TWK, Barrett PHR. Effect of dietary fatty acids on human lipoprotein metabolism: a comprehensive update. Nutrients. 2015;7:4416–25.
3. Mennitti LV, Oliveira JL, Morais CA, Estadella D, Oyama LM, Oller do Nascimento CM, et al. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. J Nutr Biochem. 2015;26:99–111.
4. Decsi T, Burus I, Molnár S, Minda H, Veitl V. Inverse association between trans isomeric and long-chain polyunsaturated fatty acids in cord blood lipids of full-term infants. Am J Clin Nutr. 2001;74:364–8.
5. Food and Environmental Hygiene Department. Hong Kong population-based food consumption survey 2005–2007, Final Report. Hong Kong: Food and Environmental Hygiene Department, HKSAR; 2010. p. 48–50.
6. Woo KS, Chook P, Raitakari OT, McQuillan B, Feng JZ, Celermajer DS. Westernization of Chinese adults and increased subclinical atherosclerosis. Arterioscler Thromb Vasc Biol. 1999;19:2487–93.
7. Census and Statistics Department. Women and men in hong kong key statistics. Hong Kong: Census and Statistics Department, HKSAR; 2018. p. 90–3.
8. Elder S, Schmidt D. Global employment trends for women. Geneva: Employment Strategy Department, International Labour Office; 2004. p. 9–13.
9. Chen ZY, Pelletier G, Hollywood R, Ratnayake WMN. Trans fatty isomers in Canadian human milk. Lipids. 1994;30:15–21.
10. Mojska H. Influence of trans-fatty acids on infant and fetus development. Acta Microbiol Pol. 2003;52:67–74.
11. Chen ZY, Kwan KY, Tong KK, Ratnayake WMN, Li HQ, Leung SSF. Breast milk fatty acid composition: a comparative study between Hong Kong and Chongqing Chinese. Lipids. 1997;32:1061–7.
12. Butts C, Hedderley D, Herath T, 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;10:1231.
13. 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;117:556–61.
14. Tian HM, Wu YX, Lin YQ, Chen XY, 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;58:83–88.
15. Thakkar SK, De Castro CA, Beauport L, Tolsa JF, Fischer Fumeaux CJ, Affolter M, et al. Temporal progression of fatty acids in preterm and term human milk of mothers from Switzerland. Nutrients. 2019;11:112.

16. Daud AZ, Mohd-Esa N, Azlan A, Chan YM. The trans fatty acid content in human milk and its association with maternal diet among lactating mothers in Malaysia. Asia Pac J Clin Nutr. 2013;22:431–42.
17. de Souza Santos da Costa R, da Silva Santos F, de Barros Mucci D, de Souza TV, de Carvalho Sardinha FL, Moutinho de Miranda ChavesCR, et al. Trans fatty acids in colostrum, mature milk and diet of lactating adolescents. Lipids. 2016;51:1363–73.
18. Craig-Schmidt MC, Weete JD, Faircloth SA, Wickwire MA, Livant EJ. The effect of hydrogenated fat in the diet of nursing mothers on lipid composition and prostaglandin content of human milk. Am J Clin Nutr. 1984;39:778–86.
19. 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. https://doi.org/10.1093/ajcn/nqz229.
20. García-Ravelo S, Díaz-Gómez NM, Martín MV, Dorta-Guerra R, Murray M, Escuder D, et al. Fatty acid composition and eicosanoid levels (LTE_4 and PGE_2) of human milk from normal weight and overweight mothers. Breastfeed Med. 2018;13:702–10.
21. Mohammad MA, Haymond MW. Regulation of lipid synthesis genes and milk fat production in human mammary epithelial cells during secretory activation. Am J Physiol Endocrinol Metab. 2013;305:E700–716.
22. Smith S. Mechanism of chain length determination in biosynthesis of milk fatty acids. J Mammary Gland Biol Neoplasia. 2009;14:245–60.
23. Lönnerdal B. Effects of maternal dietary intake on human milk composition. J Nutr. 1986;166:499–513.