Original Article

The *trans* fatty acid content in human milk and its association with maternal diet among lactating mothers in Malaysia

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Excessive intake of *trans* fatty acids (TFA) could reduce the fat density of human milk and impair the desaturation of essential fatty acids. Because the mammary glands are unable to synthesize TFA, it is likely that the TFA in human milk come from dietary intake. Thus, the aim of this study was to investigate the sources of TFA intake for lactating mothers in one of the urban areas in Selangor. In this cross-sectional study, anthropometric measurements, FFQ including 7 food groups and dietary consumption data were collected from 101 lactating mothers. Five major TFA isomers (palmitoelaidic acid (16:1*t*9), petroselaidic acid (18:1*t*6), elaidic acid (18:1*t*9), vaccenic acid (18:1*t*11) and linoelaidic acid (18:2*t*9,12) in human milk were measured by gas chromatography (GC). The relationship between food consumption and TFA levels was assessed using the non-parametric Spearman's rho test. The TFA content in human milk was 2.94±0.96 (SEM) % fatty acid; this is considered low, as it is lower than 4%. The most abundant TFA isomer was linoelaidic acid (1.44±0.60% fatty acid). A sub-experiment (analyzing 3 days of composite food consumption) was conducted with 18 lactating mothers, and the results showed that linoelaidic acid was the most common TFA consumed (0.07±0.01 g/100 g food). Only 10 food items had an effect on the total TFA level and the isomers found in human milk. No association was found between TFA consumption and the TFA level in human milk.

Key Words: human milk, TFA, elaidic acid, vaccenic acid, linoelaidic acid

INTRODUCTION

Prospective epidemiological studies and case control studies clearly demonstrate that chronic diseases are linked to *trans* fatty acid (TFA) consumption.[1,2] Studies performed by Innis *et al*,[3] Semma,[4] Craig-Schmidt,[5] and Saunders *et al*[6] have also shown a positive association between serum TFA levels and chronic diseases, such as cardiovascular disease, diabetes and arteriosclerosis. Diets high in TFA are also linked to an increased risk of inflammation and may trigger allergic symptoms, such as asthma, allergic rhinitis, conjunctivitis and atopic eczema.[4,7,8] Mothers who consume excessive TFA may also reduce the fat density of their milk and impair the desaturation of essential fatty acids, such as linoleic acid (LA) and alpha linolenic acid (ALA). The inhibition of the essential fatty acid desaturation process could prevent the formation of long chain polyunsaturated fatty acids (LC-PUFA), such as arachidonic acid (AA) (20:4) and docosahexaenoic acid (DHA) (22:6), in the mammary glands.[9] However, it is likely that the effects of TFA on health depend on the type of TFA isomer. The TFA isomers that originate from industry, such as elaidic acid (18:1*t*9) and linoelaidic acid (18:2*t*9,12), are strongly associated with cardiovascular diseases, diabetes and cancer. In contrast, there was no association between the TFA that originate from ruminants, such palmitoelaidic acid (16:1*t*9) and vaccenic acid (18:1*t*11), and heart disease.[10] Thus, based on scientific evidence, the TFA isomers of ruminant origin have a less deleterious effect on human health than the TFA from industrial sources.

Most of the TFA content in Malaysian food is still underreported, and there is not yet a requirement to list TFA content on food labels in Malaysia. Due to limited information about TFA in Malaysian food, the intake of TFA by lactating mothers could be excessive, potentially leading to the health effects stated above. Because the mammary glands are unable to synthesize TFA, the TFA that accumulate in the mammary glands must come from dietary intake.[11] Thus, the aim of this study was to investigate the sources of TFA intake for lactating mothers by evaluating the consumption of 42 food items (from 7 food

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groups) and measuring 5 dietary parameters related to TFA content in human milk. In addition, a sub-experiment (analyzing 3 days of composite food consumption) was conducted with 18 lactating mothers to measure the actual intake of TFA on a daily basis.

MATERIALS AND METHODS

Subjects

A higher intake of TFA is usually associated with urban areas. High accessibility of food such as bakery products, snacks and fast food are increasing TFA consumption of the population in that area. A cross-sectional study was carried out in the Health Centre in Bandar Baru Bangi, one of the urban areas in Selangor, Malaysia. Based on a calculation using a sample formula,[12] One hundred and one Malay respondents were recruited between May 2009 and October 2009. The inclusion criteria for participation in the study were as follows: lactating women with mature milk (between 15 days and 6 months postpartum) who had delivered a full-term infant. The exclusion criteria were factors related to health status: women diagnosed with chronic diseases (eg, heart disease, cancer or diabetes) or communicable diseases (eg, HIV/AIDS, hepatitis or tuberculosis) were excluded. Subjects were given an oral explanation of the study including its benefits and procedure, and were asked to read and sign an informed consent document. Approval was obtained from the Malaysia Ministry of Health, the Hulu Langat District Health Office and the Research Medicine Ethics Committee at Universiti Putra Malaysia.

The anthropometric measurements for height (m), weight (kg), BMI (kg/m^2) and body fat (%) were carried out. Seca body meter (Vogal & Halke) (GmbH, Germany) and electronic weighing scale (Tanita) (Japan) were used to measure height and weight, respectively. Both of height and weight are recorded to the nearest 0.1 cm or 0.1kg. The fat analyzer (Omron) (Japan) was used to measure values of BMI and body fat. The value of BMI and body fat (%) were classified based on WHO (2004).[13] The FFQ were also completed. In addition, samples of human milk were collected from all the respondents, and the data for the sub-experiment (3 days of composite food consumption) were collected from 18 respondents.

Food frequency questionnaires (FFQ)

One hundred and one respondents were asked to indicate the frequency of their consumption of various foods over a 1-year period. The intake frequency of approximately 42 food items in 7 food groups was measured. These food items were selected because we suspect that these foods have a high amount of TFA. The TFA content of these 42 food items was analyzed in an earlier study.[14] The food on the FFQ included bakery products (cakes, doughnuts, croissants, white bread, whole grain bread and pizza); snack foods (cream crackers, chocolate biscuits, potato chips, chocolate bars and chocolate wafers); breakfast cereal; semi-solid fat and cooking oil (mayonnaise, shortening, blended oil, palm oil, corn oil, olive oil and soybean oil); milk and dairy products (cheese, yogurt, fresh milk and powdered milk); fast food (fried chicken, chicken nuggets, fries, beef burgers, chicken burgers, cheeseburgers and fish burgers) and miscellaneous foods (*roti canai*, chapatti, thosai, sweetened milk, ice cream and drinking cereal). The frequency of food consumption was classified using the following response scale: never, 1 time/month, 2-3 times/ month, 1 time/week, 2-3 times/week, ≥4 times/week, 1 time/day, 2-3 times/day and ≥4 times/day.[15]

$$\text{Score FFQ for each food items} = \frac{R_1S_1 + R_2S_2 + ... + R_9S_9}{9}$$

Where; $R_{1.....}$ R_9 are the percentages of respondents who select a point scale, $S_{1.....}$ S_9 are the point scale ranging from 1- never, 2- once/month, 3- two to three times/ month, 4- once/week, 5- two to three times/week, 6- more than three times/week, 7- once/day, 8- two to three times /day and 9- more than three times/day. Nine is the maximum value for point scale.

Sub-experiments (3-day analysis of composite food consumption)

The respondents who participated in the sub-experiment kept track of all the food they ate for a 3-day period by saving similar-sized portions of the food in plastic containers in their refrigerators. After 3 days, the samples were brought to the laboratory to be homogenized and stored at -80°C until future analysis.

Dietary consumption parameters

The 101 participants reported how frequently they ate fruits, vegetables, deep-fried food, steamed or boiled food over the last year; they also reported how frequently they dined out. The frequency responses used to measure these data were never, 1 time/month, 2-3 times/month, 1 time/ week, 2-3 times/week, >3 times/week, 1 time /day, 2-3 times/day and >3 times/day.

Human milk collection

Human milk was collected from all respondents and stored in capped amber glass bottles. Each participant was asked collect milk for 3 days and stored the milk in her own refrigerator until the samples were collected by a researcher. Approximately 16-17 mL of self-expressed human milk were collected each day, and after 3 days, the bottles were transported to the laboratory, immediately homogenized and stored at -80°C before being analyzed.

Quantification of TFA in human milk and 3 days of composite foods

Fat extraction, fatty acid methyl ester (FAME) and gas chromatography (GC)

Fat was extracted from the human milk samples and the composite foods using Bligh and Dyer's method with the slight modifications made by Philips and colleagues.[16] Lipids can be converted into FAME through boron trifluoride (BF3) transesterification.[17] Two ml of boron trifluoride (12%) in methanol and 1 ml of an internal standard (Tridecanoic acid 13:0, 0.01 g/100 mL heptanes) were added to 20 µl (0.02 g lipid) aliquots of the samples. The mixture was incubated for 1.5 hr at 55 °C and shaken vigorously every 20 min to allow the complete *trans*-esterification. To terminate the process, 2 mL of anhydrous sodium carbonate (8.5 g/100 mL in distilled water) was added. An additional 1.98 mL of heptane was added before the solution was centrifuged at 3600 rpm for 15 min. The heptane layer containing FAME was collected for GC analysis. The fatty acid composition was assessed in duplicate by separating the FAME by gas chromatography (Hewlett Packard HP 6890 Network GC-systems, USA) equipped with a 100 m × 250 µm × 0.2 µm (film thickness) HP-88 column (USA: split ratio 10:1) for cis/*trans* separation. Helium was used as a carrier gas with a flow rate 1.0 ml/min, and the total flow was 13 ml/min. The temperature program lasted a total of 57.9 min and consisted of the following: 0.50 min at 40°C, an increase of 25°C/min to 192°C, 25 min at 192°C, an increase of 15°C/min to 205°C, 3 min at 205°C, an increase of 10°C/min to 230°C and 20 min at 230°C. Fatty acids were identified with HP Chemstation software (Hewlett Packard, California, USA). *Trans* fatty acid isomers (palmitoelaidic acid (16:1*t*9), petroselaidic acid (18:1*t*6), elaidic acid (18:1*t*9), vaccenic acid (18:1*t*11) and linoelaidic acid (18:2*t*9,12) were determined by comparing the retention time with the authentical standard. Each TFA isomer was determined using the formula below:[18]

$$\text{TFA isomers (g/100 g lipid)} = \frac{\text{Area}_{\text{FA isomers}} \times \text{Conc}_{\text{IS}} \times \text{RRF}_{\text{FA isomers}}}{\text{Area}_{\text{IS}} \times \text{Conc}_{\text{fat}} \times 1.04}$$

In this formula, 1.04 is the conversion factor from methyl ester to fatty acid. The relative response factor (RRF) is obtained from an injection of TFA isomer standard, and the internal standard (IS) is 0.001 g of tridecanoic acid (13:0). RRF was determined as follows:

$$\text{RRF}_{\text{TFA isomers}} = \frac{\text{Area}_{\text{IS}} \times \text{Conc}_{\text{FA isomers}}}{\text{Area}_{\text{FA isomers}} \times \text{Conc}_{\text{IS}}}$$

Statistical analysis

The correlation between the TFA content in human milk and dietary consumption (ie, the consumption of 42 food items, the 5 dietary parameters and the 3-day composite food collection) was analyzed using the non-parametric Spearman's Rho (r_s) test. SPSS version 15 was used to run the statistical analysis. A correlation was considered significant at $p<0.05$ (two-tailed).

RESULTS AND DISCUSSION

The data for socio-demographic characteristics of the subjects was not shown, but most of them graduated from university (50.5%) and 31.7% of them have monthly household income of more than RM 4,001. Moreover, the number of respondents who are working was also higher (58.4%) compared to number of fulltime housewife (41.60%). The anthropometric measurements for all respondents are shown in Table 1. The mean height and weight of the respondents were 1.56±0.06 (SD) m and 61.3± 13.2 (SD) kg, respectively. According to the WHO definition,[13] almost half of the respondents had a normal BMI (48.5%), some were overweight or obese (45.6%), and some were underweight (5.90%). Most of the participants had a high percentage of body fat (62.4%), while

Table 1. Anthropometry of lactating mothers

Anthropometry (n=101)	Mean (SD)	%
Height (m)	1.56 (0.06)	-
Weight (kg)	61.3 (13.20)	-
BMI (kg/m^2)	25.0 (5.24)	-
Underweight	-	5.9
Normal	-	48.5
Overweight/Obese	-	45.6
Fat in body (%)	33.2 (6.64)	
Unhealthy range (too low)	-	0.0
Acceptable range (lower end)	-	8.9
Acceptable (upper end)	-	26.7
Unhealthy (too high)		62.4

Table 2. Lipid composition of human milk

Milk fat/fatty acid	g/100 g lipid Mean (SEM)	g/100 g milk Mean (SEM)	% fatty acids Mean (SEM)
Milk fat/total fat	-	4.04 (0.32)	-
Lauric acid 12:0)	0.45 (0.25)	0.03 (0.00)	8.44 (0.97)
Myristic acid (14:0)	0.40 (0.05)	0.02 (0.00)	7.64 (0.60)
Palmitic acid (16:0)	1.81 (0.34)	0.06 (0.01)	29.0 (2.67)
Stearic acid (18:0)	0.10 (0.03)	0.01 (0.00)	5.64 (0.39)
ΣSFA	2.76 (0.35)	0.12 (0.01)	50.7 (1.28)
Palmitoleic acid (16:1c9)	0.15 (0.02)	0.01 (0.00)	3.02 (0.39)
Oleic acid (18:1c9)	2.11 (0.29)	0.08 (0.01)	32.5 (0.47)
ΣMUFA	2.26 (0.19)	0.09 (0.01)	35.5 (0.60)
Linoleic acid (18:2) (ω-6)	0.37 (0.05)	0.02 (0.00)	7.53 (0.25)
AA (20:4) (ω-6)	0.09 (0.02)	0.00 (0.00)	2.57 (0.40)
Σω-6	0.46 (0.05)	0.02 (0.00)	10.1 (0.48)
Linolenic acid (18:3) (ω-3)	0.01 (0.01)	0.00 (0.00)	0.35 (0.05)
EPA (20:5) (ω-3)	0.06 (0.02)	0.00 (0.00)	1.59 (0.54)
DHA (22:6) (ω-3)	0.00 (0.00)	0.01 (0.00)	0.82 (0.11)
Σω-3	0.07 (0.02)	0.01 (0.00)	1.76 (0.55)
ΣUFA	2.79 (0.20)	0.12 (0.00)	47.4 (2.76)
Palmitoelaidic acid (16:1*t*9)	0.03 (0.02)	0.00 (0.00)	0.46 (0.28)
Petroselaidic acid (18:1*t*6)	0.18 (0.18)	0.01 (0.01)	0.66 (0.65)
Elaidic acid (18:1 *t*9)	0.03 (0.03)	0.00 (0.00)	0.22 (0.16)
Vaccenic acid (18:1 *t*11)	0.00 (0.00)	0.00 (0.00)	0.15 (0.12)
Linoelaidic acid (18:2*t*9,12)	0.18 (0.08)	0.00 (0.00)	1.44 (0.60)
ΣTFA	0.42 (0.20)	0.02 (0.01)	2.93 (0.96)

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; UFA: Unsaturated fatty acid; TFA: *Trans* fatty acid; AA: Arachidonic acid; EPA:Eicosapentanoic acid; DHA: Docosahexaenoic acid; SEM: Standard error of the mean.

Table 3. *Trans* fatty acid (TFA) content in 3-days composite food

TFA isomers (n=18)	g/100 g of lipid Mean (SEM)	g/100 g of food Mean (SEM)	g/100 g lipid/day/person Mean (SEM)
Palmitoelaidic acid (16:1*t9*)	<0.001	<0.001	-
Petroselaidic acid (18:1*t6*)	<0.001	<0.001	-
Elaidic acid (18:1 *t9*)	1.29 (0.51)	0.03 (0.01)	-
Vaccenic acid (18:1 *t11*)	<0.001	<0.001	-
Linoelaidic acid (18:2*t9,12*)	2.52 (0.40)	0.07 (0.01)	-
Total TFA	3.82 (0.77)	0.10 (0.02)	1.27 (0.26)

SEM: Standard Error of Mean.

fewer participants had an acceptable body fat level at the upper end (26.7%) and an acceptable body fat level at the lower end (8.9%). Based on the definition by Lee and Nieman,[19] none of the respondents had a low percentage of body fat.

Fat is one of the most important nutrients found in human milk. It is needed to provide energy for breastfed infants. The results in Table 2 show that the percentage of milk fat in this study was higher (4.04%) than in previous studies (where percentages ranged from 3.2%[20] to 3.8-3.9%,[21] but it is still in the middle of the range reported by Jenness (3-5%).[22] The wide variation in the fat content of human milk can be found across the country and the region.[21,22] The variation could be partly due to biochemical changes in human milk. Over time, the nutrient content of human milk including the fat content, changes as infants (recipients) mature. Moreover, the diet of lactating mothers may also affect the fat composition of milk. As observed in previous studies, lactating mothers with a high fat intake have a higher fat content in their milk. The composition of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of Malay women in this study was similar to those reported earlier by Kneebone *et al*. in 1985.[23]

In addition to MUFA, PUFA and SFA, TFA is also one of the fatty acids that comprise the fat content in human milk. The TFA that lactating mothers consume in their diet could be transferred rapidly to the mammary glands. As reported by Lonnerdal,[24] women who have a strong preference for products that contain partially hydrogenated oil have a significantly higher amount of TFA in their milk. Thus, human milk can be used as a biomarker to measure the consumption of TFA. The measurement of TFA in human milk is important because high consumption of TFA could affect the cognitive abilities of infants.[21] The accumulation of TFA (as little as 3 g/day) can reduce milk fat by over 20%.[25] As shown in Table 2, this study found that the mean total TFA content is 2.94% of the total fatty acids in human milk (0.42 g/100 g lipid). This value seems to be near the low range reported in a German study (2.2-6.0% of total fatty acids).[26] This result, however, was lower than figures reported in Iran (11.3% of total fatty acids)[27] and Canada (7.1% of total fatty acids)[28] and higher than the value reported in Kuwait (2.8% of total fatty acids).[29] The TFA contents of the milk from lactating mothers in Hong Kong and in China in 1997 (0.88% and 0.22%, respectively) were also much lower than the value found in our study.[20] However, as

Table 4. Food Frequency Questionnaire (FFQ) score for 42 food items

Food groups	FFQ score
Bakery products	
Cake	17.3
Doughnut	19.6
Croissant	16.4
White bread	50.3
Whole grain	23.9
Bun	29
Pizza	19.4
Snack	
Crackers	52.3
Chocolate biscuits	17.7
Potato chips	23.3
Chocolate bar	31.3
Wafer chocolate	21.2
Milk and dairy products	
Cheese	17.5
Yogurt	22.4
Fresh milk	29.1
Powdered milk	41.7
Fast food	
Fried chicken	22.5
Chicken nugget	19
Fries	22.6
Beef burger	15.4
Chicken burger	17.5
Cheeseburger	14.8
Fish burger	13.2
Cooking oil and semisolid fat	
Olive oil	13.1
Blended oil (canola, soya bean, olive)	14.2
Soybean oil	13.2
Palm oil	85
Corn oil	15.3
Mayonnaise	22.8
Shortening	11.8
Breakfast cereal	
Chocolate coated cereal	17.3
Honey coated cereal	13.2
Corn cereal	15.5
Miscellaneous	
Roti canai	33.7
Chapatti	16.2
Thosai	15.3
Drinking cereal	27.3
Sweetened milk	47.3
Ice cream	28.4

Table 5. The association between food consumption and TFA and its isomers in human milk

Food	TFA and its isomers in human milk											
	Palmitoelaidic acid (16:1*t*9)		Petroselaidic acid (18:1*t*6)		Elaidic acid (18:1*t*9)		Vaccenic acid (18:1*t*11)		Linoelaidic acid (18:2*t*9,12)		Total TFA	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
Bakery products												
Cakes	0.13	NS	0.02	NS	-0.17	NS	0.09	NS	-0.09	NS	-0.11	NS
Doughnut	0.09	NS	-0.01	NS	0.14	NS	-0.00	NS	-0.07	NS	0-.10	NS
Croissant	-0.01	NS	0.10	NS	-0.01	NS	0.11	NS	-0.08	NS	0.-01	NS
White bread	-0.07	NS	0.01	NS	0.19	NS	0.10	NS	0.16	NS	0.15	NS
Whole-grain bread	-0.04	NS	-0.09	NS	-0.09	NS	-0.04	NS	-0.03	NS	-0.10	NS
Bun	0.09	NS	0.11	NS	0.20	<0.05	-0.40	NS	0.13	NS	0.21	<0.05
Pizza	-0.02	NS	-0.02	NS	-0.05	NS	-0.09	NS	0.03	NS	-0.06	NS
Snack												
Cream crackers	0.07	NS	0.02	NS	0.00	NS	0.02	NS	0.10	NS	0.07	NS
Chocolate biscuits	-0.13	NS	-0.69	NS	-0.12	NS	-0.02	NS	-0.11	NS	-0.15	NS
Potato chips	0.09	NS	0.03	NS	0.02	NS	0.08	NS	0.06	NS	0.07	NS
Milk chocolate	-0.06	NS	0.04	NS	0.17	NS	0.02	NS	-0.04	NS	-0.04	NS
Wafer chocolate	0.06	NS	0.01	NS	-0.13	NS	0.04	NS	0.03	NS	0.02	NS
Milk and dairy products												
Cheese	-0.05	NS	-0.01	NS	-0.12	NS	-0.03	NS	-0.02	NS	0.02	NS
Yogurt	-0.02	NS	0.08	NS	-0.00	NS	0.15	NS	-0.01	NS	0.06	NS
Fresh milk	0.14	NS	0.11	NS	-0.06	NS	-0.15	NS	-0.08	NS	0.01	NS
Powdered milk	-0.04	NS	-0.23	<0.05	0.02	NS	-0.09	NS	-0.13	NS	-0.20	<0.05
Fast food												
Fried chicken	-0.10	NS	-0.30	NS	0.11	NS	0.08	NS	-0.03	NS	-0.05	NS
Chicken nugget	0.00	NS	-0.02	NS	0.12	NS	0.14	NS	0.06	NS	0.07	NS
Fries	-0.00	NS	0.04	NS	0.12	NS	0.08	NS	-0.10	NS	-0.10	NS
Beef burger	0.18	NS	0.03	NS	-0.10	NS	-0.12	NS	0.00	NS	-0.02	NS
Chicken burger	-0.02	NS	-0.04	NS	0.24	<0.05	-0.04	NS	0.09	NS	0.01	NS
Cheeseburger	0.02	NS	-0.12	NS	0.17	NS	-0.09	NS	0.24	<0.05	0.15	NS
Fish burger	-0.03	NS	-0.76	NS	0.11	NS	0.14	NS	0.02	NS	0.04	NS
Cooking oil and semisolid fats												
Olive oil	-0.06	NS	-0.08	NS	-0.06	NS	-0.06	NS	0.09	NS	0.03	NS
Corn oil	-0.09	NS	-0.09	NS	0.08	NS	-0.08	NS	0.25	<0.05	0.14	NS
Blended oil (canola, soybean and olive)	0.07	NS	0.04	NS	0.25	<0.05	-0.07	NS	0.12	NS	0.16	NS
Palm oil	0.11	NS	-0.02	NS	-0.13	NS	0.18	NS	-0.10	NS	-0.04	NS
Soybean oil	-0.04	NS	-0.03	NS	-0.04	NS	0.04	NS	0.04	NS	-0.12	NS
Mayonnaise	-0.07	NS	-0.08	NS	0.09	NS	0.26	<0.05	0.11	NS	0.07	NS
Shortening	-0.05	NS	-0.06	NS	-0.05	NS	-0.46	NS	0.28	<0.05	0.21	<0.05

TFA: *Trans* fatty acids; rs: Spearman Rho value; *p*: Significant level; NS: Not significant

Table 5. The association between food consumption and TFA and its isomers in human milk (cont.)

Food	TFA and its isomers in human milk											
	Palmitoelaidic acid (16:1*t9*)		Petroselaidic acid (18:1*t6*)		Elaidic acid (18:1*t9*)		Vaccenic acid (18:1*t11*)		Linoelaidic acid (18:2*t9,12*)		Total TFA	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
Breakfast cereal												
Chocolate coated cereal	-0.14	NS	0.00	NS	-0.12	NS	-0.12	NS	-0.15	NS	-0.19	NS
Honey coated cereal	0.07	NS	0.04	NS	-0.06	NS	-0.06	NS	0.15	NS	0.05	NS
Corn cereal	-0.01	NS	-0.02	NS	-0.10	NS	0.00	NS	-0.09	NS	-0.27	NS
Miscellaneous												
Roti canai	-0.27	NS	0.10	NS	0.16	NS	-0.10	NS	0.11	NS	0.13	NS
Chapatti	0.01	NS	0.04	NS	0.16	NS	-0.11	NS	0.11	NS	0.13	NS
Thosai	0.07	NS	-0.05	NS	0.18	NS	0.02	NS	0.03	NS	0.84	NS
Drinking beverages	-0.01	NS	-0.02	NS	-0.10	NS	0.00	NS	-0.09	NS	-0.27	NS
Sweetened milk	0.19	NS	0.07	NS	0.18	NS	0.16	NS	0.11	NS	0.20	<0.05
Ice cream	0.26	<0.05	-0.08	NS	-0.09	NS	0.04	NS	0.30	NS	0.63	NS

TFA: *Trans* fatty acids; rs: Spearman Rho value; p: Significant level; NS: Not significant

Table 6. The association between TFA in 3-days composite food and TFA and its isomers in human milk

TFA in 3-days composite food	TFA and isomers in human milk											
	Palmitoelaidic acid (16:1*t9*)		Petroselaidic acid (18:1*t6*)		Elaidic acid (18:1*t9*)		Vaccenic acid (18:1*t11*)		Linoelaidic acid (18:2*t9,12*)		Total TFA	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
Palmitoelaidic acid (16:1*t9*)	-	-	-	-	-	-	-	-	-	-	-	-
Petroselaidic acid (18:1 *t6*)	-	-	-	-	-	-	-	-	-	-	-	-
Elaidic acid (18:1 *t9*)	0.19	NS	0.24	NS	0.45	NS	0.35	NS	0.12	NS	0.48	<0.05
Vaccenic acid (18:1 *t11*)	-	-	-	-	-	-	-	-	-	-	-	-
Linoelaidic acid (18:2 *t9,12*)	0.07	NS	-0.07	NS	0.16	NS	0.18	NS	0.04	NS	0.09	NS
Total TFA	0.07	NS	-0.02	NS	0.35	NS	0.26	NS	0.07	NS	0.22	NS

TFA: *Trans* fatty acid; r_s: Spearman Rho value; *p*: Significant level; NS: Not significant (*p*-value more than 0.05).

reported by Fu *et al* in 2008,[30] the TFA content of popular Western-style products in China was high. As China has become a modern country and lifestyle patterns have changed, it is possible that the TFA content of human milk has also increased. Overall, the total amount of TFA in human milk in this study was less than 4% of the total fatty acids, which is considered low.[3]

Recent evidence suggests that different isomers from different food sources may elicit different effects, either beneficial or adverse.[31] For example, TFA isomers produced by industry, such as elaidic acid and linoelaidic acid, could increase the risk of health problems, such as cardiovascular disease.[10] In contrast, natural TFA such as vaccenic acid, which is an antiatherosclerosis and antidiabetic agent,[32] could be beneficial. Vaccenic acid, however, could cause separation of the milk fat from human milk; thus breastfed infants would consume less energy-dense food.[26] Therefore, it is important to differentiate between the TFA isomers in human milk. Based on our study, linoelaidic acid was found to be most abundant isomer in the human milk of the respondents, followed by petroselaidic acid, palmitoelaidic acid, elaidic acid and vaccenic acid (Table 2).

The data collected in the sub-experiment (3-day com posite food collection) (Table 3) show that the total TFA intake among lactating mothers in our study was lower (1.27 g/day) than the dietary intake of lactating women in Turkey (2.16 g/day),[33] Canada (2.2 g/day)[28] and the state of Georgia in the U.S. (>4.5 g/day).[34] These data indicate that the respondents were acquiring less TFA from their diets. The results also showed that linoelaidic acid was found to be the most abundant isomer in the 3-day collection of composite food, similar to the finding in human milk.

The consumption of food items listed in the FFQ was considered low if nearly all FFQ scores were less than 60 (Table 4). To further assess the association between the TFA content in human milk and the consumption of the 42 food items, Spearman's *rho* was calculated. As shown in Table 5, the consumption of buns, shortening, powdered milk and sweetened milk were found to elevate the total TFA level in human milk. In terms of individual TFA isomers, elaidic acid in human milk may come from buns, chicken burgers and blended oil (canola, soya bean and olive oil) that are consumed by lactating mothers. During food processing, the partial hydrogenation process produces more elaidic acid than it produces other isomers.[35] It is likely that linoelaidic acid, which has health effects similar to the effects of elaidic acid, comes from cheeseburgers, corn oil and shortening. The deodorization process and heating edible oil above 180 °C would predominately produce this TFA isomer. The density of fat in human milk can be reduced by consuming a large amount of vaccenic acid. Vaccenic acid is also able to inhibit the lipogenesis process and impair milk synthesis; this process is called milk fat depression (MFP). The findings from this study show that mayonnaise consumption could increase this TFA isomer. In general, of the 42 food items included in this study, only 10 food items were found to reflect the total TFA level and the level of various isomers in human milk. This conclusion could be attributed to the low amount of TFA in these foods.[14] However, the limited number of analyzed samples of each food item may lead to an underestimation of TFA exposure in human milk. The content may also be underreported, as the food preparation methods used and the portion sizes consumed by lactating mothers were also unknown. Thus, it is recommended that future researchers select a larger sample size and focus on food preparation methods, as these data could provide detailed information on TFA exposure. Anderson *et al*[36] also reported that they found reduced fat in milk due to TFA intake in leaner lactating mothers but not in obese mothers. They suggested that women with smaller fat stores may have less substrate to mobilize and utilize as milk fat. Most of the respondents in our study had relatively high levels of body fat, so it is likely that the fat content in their milk was minimally affected by TFA intake.

Another aim of this study is to determine whether there is any association between TFA consumption (from the 3-day composite food collection) and the TFA content in human milk. The findings showed that linoelaidic acid did not affect the TFA level in human milk, even though linoelaidic acid is the primary isomer in human milk (Table 6). Instead, elaidic acid is positively associated with total TFA level in human milk. This result could be explained by the fact that the human body can differentiate between linoelaidic acid and elaidic acid, incorporating specific isomers into the mammary glands via a specific mechanism. Furthermore, a previous study reported that elaidic acid is preferentially incorporated into human cells.[37] However, the 3-day composite food analysis was conducted for only 18 respondents; thus, the results may underestimate the effect of linoelaidic acid on the composition of human milk. Further investigation should be

Table 7. The associations between dietary parameters and TFA and its isomers in human milk

Dietary parameters	TFA and isomers in human milk											
	Palmitoelaidic acid (16:1*t9*)		Petroselaidic acid (18:1*t6*)		Elaidic acid (18:1*t9*)		Vaccenic acid (18:1*t11*)		Linoelaidic acid (18:2*t9,12*)		Total TFA	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Healthy dietary parameters												
Intake of fruits	0.13	NS	0.13	NS	0.05	NS	0.08	NS	0.17	NS	0.17	NS
Intake of vegetables	0.01	NS	0.10	NS	-0.27	NS	0.22	<0.05	0.00	NS	-0.04	NS
Intake of steamed/boiled food	0.13	NS	0.06	NS	0.03	NS	-0.04	NS	0.10	NS	0.09	NS
Unhealthy dietary parameter												
Intake of deep-fried food	0.03	NS	0.03	NS	0.12	NS	-0.21	<0.05	-0.04	NS	-0.06	NS
Eating out	0.10	NS	-0.08	NS	-0.09	NS	0.02	NS	-0.18	NS	-0.21	<0.05

TFA: *Trans* fatty acid; r_s: Spearman Rho value; p: Significant level; NS: Not significant (p-value more than 0.05).

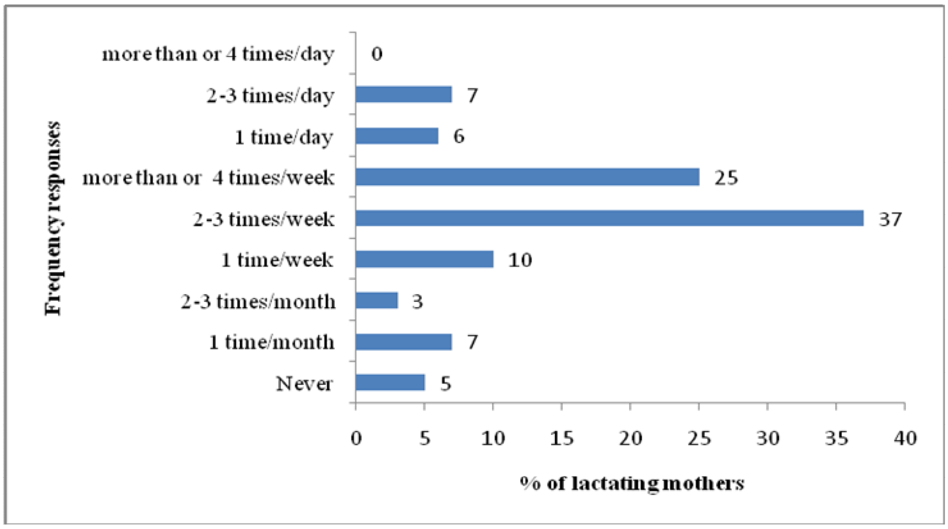

Figure 1. Fruit intake among lactating mothers.

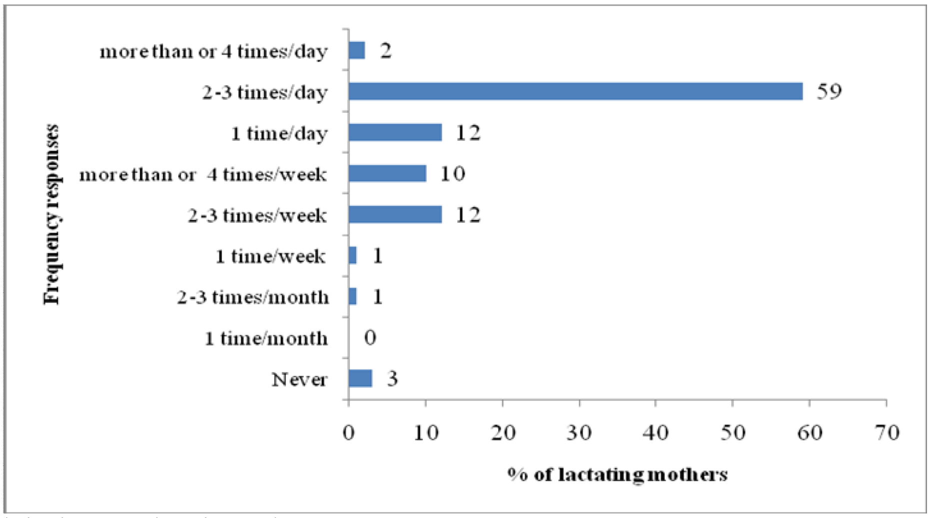

Figure 2. Vegetable intake among lactating mothers

conducted with a larger number of respondents to confirm this fatty acid mechanism.

Several dietary parameters were also studied to determine the food preferences of lactating mothers. Vegetable and fruit intake was found to be associated with a lower risk of chronic diseases.[38,39] The Malaysian Food Guide Pyramid[40] recommends that vegetables and fruits should be eaten daily to maintain personal health. Although vegetable intake met the guidelines of the Food Guide Pyramid, the intake of fruits was insufficient (Figure 1 and Figure 2). About one-quarter of adults eat more vegetables than fruits in their daily diet, according to the Centers for Disease Control and Prevention (CDC).[41] The Malaysian Dietary Guidelines[40] also recommend preparing food by boiling and steaming. The present study, however, showed that only a small number of respondents consumed boiled and steamed food on a daily basis (Figure 3). In comparison to boiled and steamed food, deep-fried food was consumed at a higher frequency by the respondents (Figure 4). Dining out was also common among the respondents (Figure 5). Most people prefer food with a substantial amount of fat because fat increases the palatability of food and encourages people to eat more.[42] Busy schedules, combined with the availability of fast food, may encourage people to purchase food outside the home, which is easier than cooking at home. Our findings, however, showed no associations between any dietary parameters and the amount of TFA in human milk. A possible explanation could be the relatively low intake of food containing a substantial amount of partially hydrogenated oil among the respondents. The extensive use of palm oil among Malaysians may be one of the reasons why the amount of TFA is low in food.[43] The formation of TFA during food production can be avoided due to the naturally solid structure of palm oil.

Conclusion

Of the 42 food items we studied, only buns, chicken burgers, cheeseburgers, shortening, powdered milk, sweetened milk, blended oil, mayonnaise, corn oil and ice cream had effects on the TFA level of human milk. Of the various TFA isomers, only elaidic acid (18:1*t*9) intake contributed to the elevation of total TFA in human milk. Although consuming deep-fried foods and dining out seemed to be common among lactating mothers, these two unhealthy dietary habits did not contribute to the

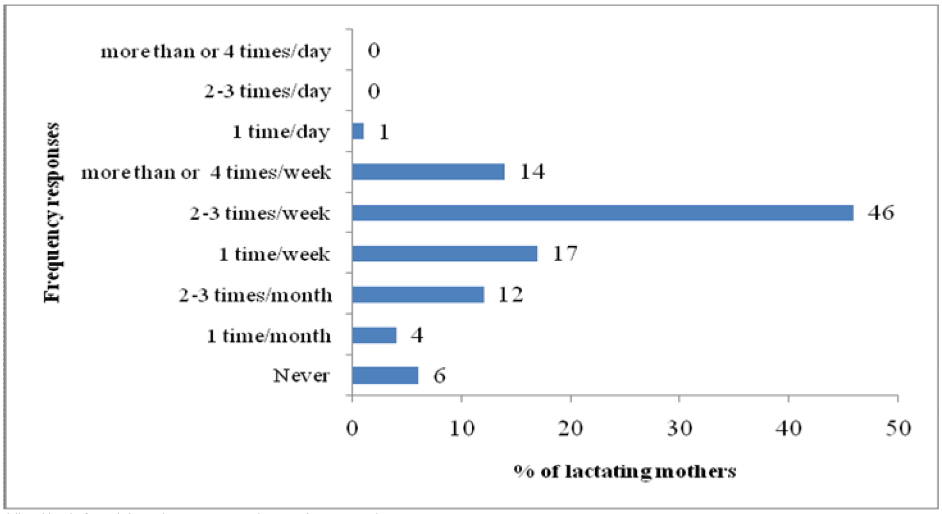

Figure 3. Steamed/boiled food intake among lactating mothers

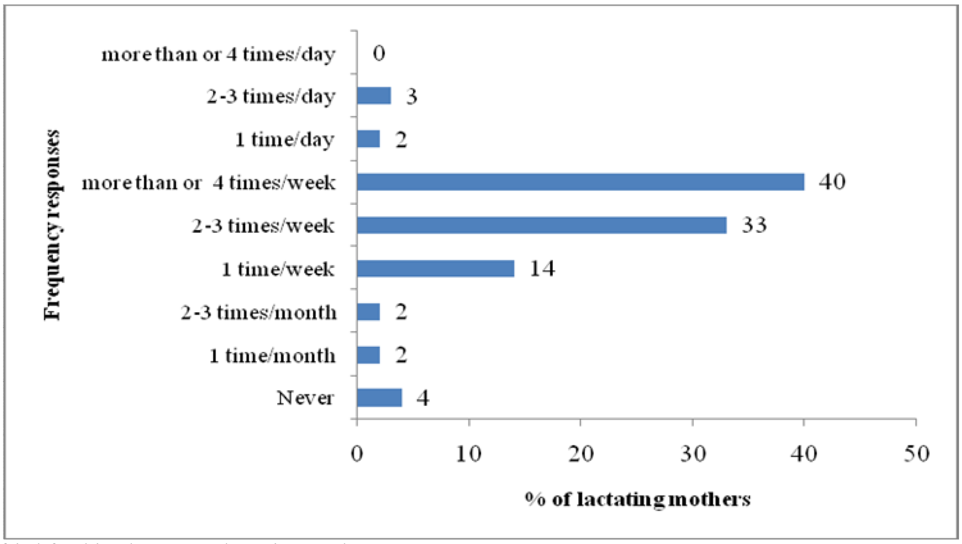

Figure 4. Deep-fried food intake among lactating mothers

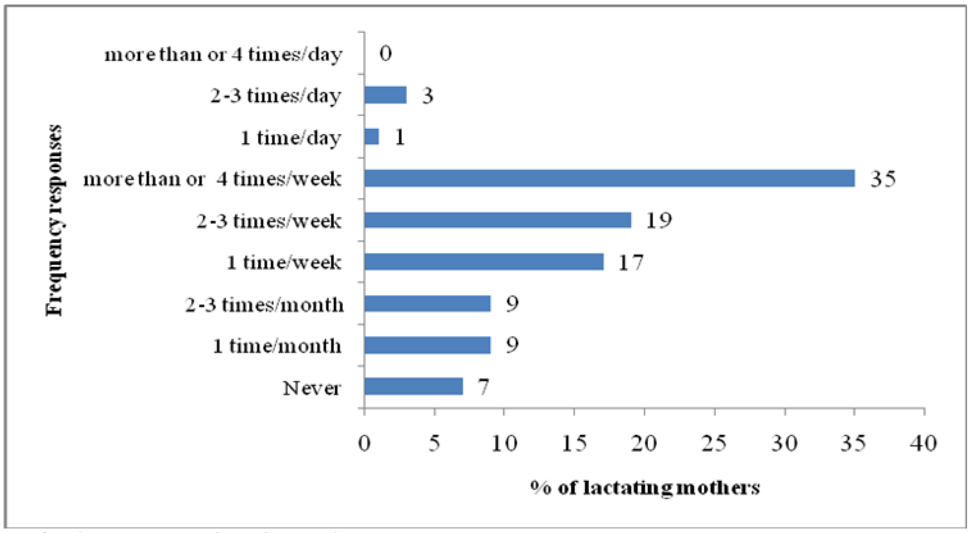

Figure 5. Patterns of eating out among lactating mothers

TFA content of their milk. This finding may be partially explained by the low amount of TFA in the food they consumed. The extensive use of palm oil in many Malaysian foods might offer health benefits to the Malaysian population.

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AUTHOR DISCLOSURES

The authors declare that they have no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Original Article

The *trans* fatty acid content in human milk and its association with maternal diet among lactating mothers in Malaysia

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馬來西亞婦女之哺乳期飲食與其母乳中反式脂肪酸含量的相關性

攝取過多反式脂肪酸會減少母乳的脂肪密度，且阻礙必需脂肪酸的去飽和作用。由於乳腺無法合成反式脂肪酸，故推測母乳中的反式脂肪酸可能來自於膳食攝取。此橫斷面研究旨在調查居住於雪蘭莪州都會區的哺乳婦女，其反式脂肪酸的攝取來源。調查資料包括 101 位哺乳母親的體位測量、含 7 類食物的飲食頻率問卷，並收集部分參與者 3 天的食物攝取樣本。利用氣相層析，分析母乳中五種主要的反式脂肪酸異構物，包括反棕櫚油酸(16:1t9)、六反十八碳烯酸(18:1t6)、反油酸(18:1t9)、十一反十八碳烯酸(18:1t11)、反亞油酸(18:2t9,12)。以無母數斯皮爾曼檢定測試食物攝取與反式脂肪酸之相關性。研究對象母乳中總反式脂肪酸的含量為 2.94%，低於一般認知的 4%；反亞油酸為 1.44%，含量最多。另外進行次研究，以 18 位哺乳婦女之 3 天飲食樣本進行成份分析，結果顯示攝取的反式脂肪酸，以反亞油酸最多。只有 10 項食物，會影響母乳中反式脂肪酸濃度，以及異構物的含量。反式脂肪酸的攝取量與母乳中反式脂肪酸的濃度並無相關。

關鍵字：母乳、反式脂肪酸、反油酸、十一反十八碳烯酸、反亞油酸