

Mandatory *trans* fat labeling regulations and nationwide product reformulations to reduce *trans* fatty acid content in foods contributed to lowered concentrations of *trans* fat in Canadian women's breast milk samples collected in 2009–2011^{1–3}

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ABSTRACT

Background: Recent efforts in Canada to reduce industrial *trans* fatty acids (TFAs) in foods include mandated inclusion of TFA content on food labels and recommendations by Health Canada that encourage the food industry to voluntarily limit TFA content in all vegetable oils and soft margarines and in all other prepackaged foods to <2% and <5% of total fat, respectively.

Objective: To assess the impact of these efforts, we measured the concentration of TFAs in human breast milk samples.

Design: The TFA content in 639 breast milk samples collected in 2009, 2010, and 2011 from breastfeeding mothers in 10 major cities across Canada was analyzed by gas chromatography.

Results: The mean (\pm SD) TFA contents were $2.7 \pm 0.9\%$ ($n = 153$, range: 1.4–7.2%), $2.2 \pm 0.7\%$ ($n = 309$, range: 1.0–6.8%), and $1.9 \pm 0.5\%$ ($n = 177$, range: 0.9–3.4%) of total milk fat for samples collected in 2009, 2010, and 2011, respectively. These values are considerably lower than the value of $7.2 \pm 3.0\%$ (range: 0.1–17.2%) found previously for Canadian human milk in 1992. On the basis of a linear correlation between the percentage of TFAs in the diet and human milk fat established by Craig-Schmidt et al, and assuming that 30% of energy of a lactating mother's diet is derived from fat, we estimated from the TFA human milk fat data that TFA intake of Canadian breastfeeding mothers was 0.9%, 0.5%, and 0.3% of total energy in 2009, 2010, and 2011, respectively. These estimated values are lower than the WHO's maximum recommended intake of 1% of total energy for a healthy diet.

Conclusions: The results suggest that the *trans* fat labeling regulations introduced in 2003 and recommendations by Health Canada in 2007 instructing the food manufacturers and restaurants to limit TFAs in foods have resulted in significant reductions in TFAs in the diets of Canadian breastfeeding mothers and their breast milk. *Am J Clin Nutr* 2014;100:1036–40.

INTRODUCTION

Partially hydrogenated vegetable oils (PHVOs)⁴ have traditionally been used in Canada in frying and in the preparation of margarines and breads, as well as in a number of other processed foods (1–8). Partial hydrogenation results in the formation of *trans* fatty acids (TFAs). Consumption of TFAs increases the risk of coronary artery disease (CAD) (9, 10).

In the 1990s, it was estimated, through analysis of TFA content in human milk, that the average TFA intake in Canada

was 8.4 g/d or 3.7% of total energy (11). Since the early 2000s, a number of measures to limit the dietary concentrations of TFAs have been implemented in Canada. In 2003, the Canadian government passed legislation requiring mandatory TFA food labeling, which came into force by 2005 (8, 12). Furthermore, in 2007, Health Canada called on the food industry to voluntarily reduce concentrations of TFAs to <2% of total fat in vegetable oils and soft spreadable margarines and to <5% of total fat in all other prepackaged foods. These limits do not apply to food products for which the fat originates exclusively from ruminant meat or dairy products. It was estimated that these limits, established by a Canadian task force on *trans* fats (13), would result in a reduction in the TFA concentrations in the Canadian diet to the WHO-recommended limit of <1% of total energy (14).

Health Canada has been performing a national assessment of the fatty acid profile of prepackaged foods and restaurant foods to closely monitor the efforts of industry to ensure that substantial progress is being made to achieve the recommended limits. The results have shown that food companies have made substantial progress in reducing the TFA content in their products to below the 2% or 5% limits (15–17). The most important implication of these changes was that, by 2008, the average intake of TFAs in Canada significantly dropped from the high value of 8.4 g/d in the mid-1990s (18) to 3.4 g/d (or 1.4% of total energy) (16).

Breast milk is a convenient biological sample for establishing dietary concentrations of TFAs (19–21). This is because the TFA content in human milk reflects the mother's previous-day diet.

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⁴ Abbreviations used: CAD, coronary artery disease; GC, gas chromatogram; MIREC, Maternal-Infant Research on Environmental Chemicals; PHVO, partially hydrogenated vegetable oil; TFA, *trans* fatty acid.

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Craig-Schmidt et al (20) generated a linear correlation between the percentage of TFAs in human milk fat and the percentage of TFAs in mothers' previous-day dietary fat.

Earlier studies detected very high concentrations of TFAs in Canadian human milk (18, 21, 22). National-level biomonitoring of human milk for TFAs would allow a time trend analysis and also help to further assess the impact of TFA labeling regulations and Health Canada's recommendation to the industry for a voluntary reduction of TFAs in prepackaged and restaurant foods. In this study, we determined the concentrations of TFAs in milk samples collected from breastfeeding mothers nationwide for 3 consecutive years from 2009 to 2011.

SUBJECTS AND METHODS

Subjects

The human milk samples used in this study were from the MIREC (Maternal-Infant Research on Environmental Chemicals) study (23). The MIREC study was established to obtain Canadian biomonitoring data for pregnant women and their infants. One objective of the MIREC study was to measure selected known or perceived beneficial substances in human milk, such as nutrients and minerals (eg, vitamins D and E, complete fatty acid profile). Full details of the MIREC study design and study population have been published recently (23). Recruitment of the subjects for the study started in February 2008.

In brief, 2001 women consented to participate in the MIREC Research Platform, and from this cohort, 1017 women provided breast milk samples. Mothers were asked to express 185 mL of mature milk (2–8 wk postpartum) manually after or during nursing to obtain an optimum combination of fore and hind milk. Samples were stored in the refrigerator for a maximum of 72 h or in the home freezer for time exceeding 72 h. The milk samples were then shipped on ice to Health Canada in Ottawa. Each milk sample was partitioned into smaller volumes to provide each participating laboratory at Health Canada with the amount of milk necessary to perform the required analysis. For fatty acid analysis, 10 mL was portioned into aliquots and stored in amber glass bottles at -80°C until analysis.

From the 1017 samples collected, 639 were randomly used in this study for the analysis of milk fatty acid composition. The 639 samples were from the 3-y consecutive collection period of 2009 ($n = 153$), 2010 ($n = 309$), and 2011 ($n = 177$) from mothers living in 10 major cities across Canada. The breakdown according to the 10 cities was as follows: Calgary ($n = 15$), Halifax ($n = 95$), Hamilton ($n = 75$), Kingston ($n = 65$), Montreal ($n = 96$), Ottawa ($n = 57$), Sudbury ($n = 30$), Toronto ($n = 94$), Vancouver ($n = 73$), and Winnipeg ($n = 39$). Compared with our previous nationwide study of 198 human milk samples in 1992 (21), it was thought that the 639 samples (average of 213 samples/y) would be adequate for a time trend comparison of TFA concentrations.

Fatty acid analysis

Milk samples were thawed at room temperature, and fat from a 1-g weighed subsample was extracted according to AOAC Official Method 996.06 (24) with some modifications. Briefly, to the 1-g sample, 1 mL 95% ethanol, 2 mL distilled water, and 1 mL

ammonium hydroxide were added, mixed for about 2 min, and heated in a shaking water bath at $70\text{--}80^{\circ}\text{C}$ for 10 min. After allowing the contents to cool to room temperature, 12 mL diethyl ether was added and the contents mixed for 5 min. Furthermore, 12 mL petroleum ether was added and the contents mixed again for another 5 min. The samples were then centrifuged for 2 min at $120 \times g$ and the organic solvent layer collected and evaporated to dryness under a stream of nitrogen. The extracted fat was dissolved in 2 mL toluene and converted to fatty acid methyl esters by using 1 mL of $\text{BF}_3\text{-CH}_3\text{OH}$.

The fatty acid methyl esters in hexane were analyzed on a gas chromatogram (GC) (Agilent 6890N system with an auto injector; Agilent) that was fitted with a flame ionization detector and a $100\text{-m} \times 0.25\text{-mm}$ (0.2-mm film) capillary column coated with SP-2560 (Supelco). The initial column oven temperature was 180°C , followed by 2 ramps at 38 min (215°C) and 69 min (225°C). The injector and the detector temperatures were 250°C . Ultra-high-purity hydrogen was the carrier gas and run at a flow rate of 0.8 mL/min. Peaks were identified by comparison with the GC traces published in the American Oil Chemists' Society Official Method Ce 1h-05 (25) and by Ratnayake (26). Identifications were further established by comparison with known fatty acid methyl ester standards (Nu-Chek Prep and Sigma). The fatty acid composition, as a percentage of total fatty acids, was calculated according to the American Oil Chemists' Society Official Method Ce 1h-05 (25). The direct GC procedure employed here gives a good resolution of all the fatty acids, including the *cis* and *trans* isomers of oleic acid (18:1 or octadecenoic acid), except for the minor overlap of 9*c*-18:1 with 15*t*-18:1 (25–28). Because of this overlap, the concentration of 15*t*-18:1 could not be measured from the direct GC procedure, but it is included with 9*c*-18:1 in the fatty acid composition data shown in **Table 1**. Because 15*t*-18:1 is always a minor component in most dietary fat ($<0.1\%$ of total fatty acids), the error in grouping 15*t*-18:1 with 9*c*-18:1 has only a minimal impact on the calculation of the total TFAs and 9*c*-18:1 (26).

Statistical analysis

Data are expressed as mean \pm SD and ranges (minimum–maximum values). Analysis of variance followed by the Holm-Sidak test was used for statistical evaluation of significant differences between fatty acids. Relations were declared significant at $P \leq 0.05$. Statistical analyses were performed by using SigmaPlot version 11.2 (Systat Software).

RESULTS

The composition of major SFAs; *cis*-MUFAs; *n*-6 and *n*-3 PUFAs; *trans* isomers of octadecenoic acid (18:1*t*), linoleic acid (18:2*t*), and α -linolenic acid (18:3*t*); and the totals of these fatty acid classes are shown in Table 1 for milk samples collected in 2009, 2010, and 2011. For comparison purposes, our previously published fatty acid data of Canadian human milk samples collected in 1992 nationwide (21) are also shown in Table 1. The total TFA concentration decreased from 1992 to 2011, such that the milk collected in 2011 was 74% lower in TFAs than the milk collected in 1992 (21). The mean concentration of total TFAs in milk collected in 2011 was 1.9 g/100 g of human milk fatty acids (range: 0.9–3.9 g/100 g milk fatty acids), whereas the milk

TABLE 1Fatty acid composition of human milk samples collected in 1992 and the 3 consecutive years of 2009, 2010, and 2011¹

Fatty acid	1992 ² (n = 198)	2009 (n = 153)	2010 (n = 309)	2011 (n = 177)
12:0	5.7 ± 2.0 ^b (2.3–11.7)	4.5 ± 1.7 ^a (1.3–10.8)	4.6 ± 1.7 ^a (0.0–12.3)	4.5 ± 1.6 ^a (1.5–9.7)
14:0	6.1 ± 1.7 (2.3–11.7)	5.7 ± 1.7 (2.7–11.1)	5.8 ± 1.7 (2.3–14.6)	5.7 ± 1.7 (1.5–12.2)
16:0	18.3 ± 2.3 ^a (12.9–24.1)	20.8 ± 2.4 ^b (12.3–28.2)	20.7 ± 2.2 ^b (13.1–27.0)	20.5 ± 2.4 ^b (15.1–27.3)
18:0	6.2 ± 1.0 ^{a,b} (3.5–9.9)	6.4 ± 1.1 ^{a,b} (3.8–9.4)	6.4 ± 1.2 ^b (3.9–14.0)	6.1 ± 1.0 ^a (3.5–9.8)
Total SFAs ³	38.5 ± 7.9 ^a (21.6–63.5)	39.8 ± 4.8 ^{a,b} (28.3–50.5)	39.9 ± 4.6 ^b (27.7–54.3)	38.9 ± 4.6 ^{a,b} (26.0–52.2)
Total 18:1 ⁴	5.9 ± 2.5 ^c (0.1–15.4)	1.8 ± 0.8 ^b (0.7–6.1)	1.5 ± 0.6 ^a (0.6–5.7)	1.3 ± 0.4 ^a (0.5–3.2)
Total 18:2 ⁵	0.9 ± 0.5 ^d (0.0–2.4)	0.6 ± 0.2 ^c (0.0–1.6)	0.5 ± 0.2 ^b (0.0–1.1)	0.4 ± 0.1 ^a (0.2–0.7)
Total 18:3 ⁶	0.1 ± 0.1 ^a (0.0–0.3)	0.2 ± 0.1 ^b (0.0–0.6)	0.2 ± 0.1 ^b (0.0–0.8)	0.1 ± 0.0 ^a (0.0–0.3)
Total TFAs ⁷	7.2 ± 3.0 ^d (0.1–17.2)	2.7 ± 0.9 ^c (1.4–7.2)	2.2 ± 0.7 ^b (1.0–6.8)	1.9 ± 0.5 ^a (0.9–3.9)
7c-16:1	0.4 ± 0.1 ^a (0.2–0.7)	0.4 ± 0.1 ^a (0.0–0.7)	0.5 ± 0.1 ^b (0.2–0.7)	0.5 ± 0.1 ^b (0.4–0.7)
9c-16:1	2.3 ± 0.6 ^b (1.1–3.9)	2.2 ± 0.6 ^{a,b} (1.1–4.1)	2.1 ± 0.6 ^a (0.6–4.6)	2.3 ± 0.6 ^b (1.0–4.6)
9c-18:1 ⁸	30.7 ± 2.7 ^a (23.6–40.6)	34.3 ± 3.3 ^b (25.5–43.9)	34.9 ± 3.7 ^b (25.5–50.2)	36.1 ± 3.3 ^c (27.9–44.4)
11c-18:1	1.9 ± 0.2 ^b (1.3–2.3)	1.8 ± 0.3 ^a (1.1–2.8)	1.8 ± 0.3 ^a (1.1–2.9)	1.8 ± 0.3 ^a (1.3–2.7)
Total 18:1 ⁹	34.9 ± 3.1 ^a (26.3–46.9)	36.8 ± 3.4 ^b (27.7–46.4)	37.1 ± 3.7 ^b (27.4–51.9)	38.3 ± 3.4 ^c (30.1–46.6)
Total MUFAs ¹⁰	38.6 ± 4.1 ^a (27.8–53.8)	40.1 ± 3.4 ^b (31.2–49.0)	40.6 ± 3.8 ^b (30.0–54.7)	42.0 ± 3.4 ^c (33.8–49.4)
18:2n-6	10.5 ± 2.6 ^a (5.8–21.4)	13.3 ± 2.7 ^b (6.9–19.9)	13.5 ± 2.8 ^b (0.0–21.8)	13.5 ± 2.5 ^b (7.4–19.6)
18:3n-6	0.1 ± 0.1 (0.0–0.2)	0.1 ± 0.1 (0.0–0.4)	0.1 ± 0.1 (0.0–0.3)	0.1 ± 0.0 (0.0–0.3)
20:3n-6	0.3 ± 0.1 ^a (0.0–0.5)	0.4 ± 0.1 ^b (0.0–0.7)	0.4 ± 0.1 ^b (0.2–0.7)	0.4 ± 0.1 ^b (0.2–0.8)
20:4n-6	0.4 ± 0.1 (0.1–0.7)	0.4 ± 0.1 (0.0–0.9)	0.4 ± 0.1 (0.0–0.8)	0.4 ± 0.1 (0.2–0.7)
22:4n-6	0.0 ± 0.1 ^a (0.0–0.2)	0.1 ± 0.1 ^b (0.0–0.4)	0.1 ± 0.0 ^b (0.0–0.3)	0.1 ± 0.0 ^b (0.0–0.2)
22:5n-6	0.0 ± 0.0 ^a (0.0–0.2)	0.1 ± 0.0 ^b (0.0–0.3)	0.1 ± 0.0 ^d (0.0–0.3)	0.0 ± 0.0 ^b (0.0–0.1)
Total LC n-6 PUFAs ¹¹	0.7 ± 0.3 ^a (0.1–1.5)	1.0 ± 0.2 ^b (0.4–2.3)	1.0 ± 0.2 ^b (0.5–1.9)	1.0 ± 0.2 ^b (0.6–1.4)
18:3n-3	1.2 ± 0.4 ^a (0.6–1.9)	1.4 ± 0.5 ^b (0.0–3.1)	1.5 ± 0.5 ^c (0.5–4.3)	1.6 ± 0.4 ^d (0.7–3.3)
20:5n-3	0.1 ± 0.1 (0.0–0.2)	0.1 ± 0.1 (0.0–0.6)	0.1 ± 0.1 (0.0–0.6)	0.1 ± 0.1 (0.0–0.6)
22:5n-3	0.1 ± 0.1 ^a (0.0–0.4)	0.2 ± 0.1 ^b (0.0–0.5)	0.2 ± 0.1 ^b (0.0–0.4)	0.2 ± 0.1 ^b (0.1–0.5)
22:6n-3	0.1 ± 0.1 ^a (0.0–0.5)	0.3 ± 0.2 ^b (0.0–1.6)	0.3 ± 0.2 ^b (0.0–1.0)	0.3 ± 0.2 ^b (0.1–1.2)
Total LC n-3 PUFAs ¹²	0.3 ± 0.2 ^a (0.0–1.2)	0.6 ± 0.3 ^c (0.1–2.4)	0.5 ± 0.3 ^b (0.1–2.0)	0.5 ± 0.3 ^b (0.2–2.3)

¹ Values are weight percentages of total fatty acids, expressed as means ± SDs; ranges in parentheses. Means in a row not sharing a common superscript letter are significantly different, $P \leq 0.05$ (Holm-Sidak test). LC, long chain; TFA, *trans* fatty acid.

² The fatty acid data are from our previous study of human milk collected and analyzed in 1992 (21).

³ Sum of all SFAs from 4:0 to 24:0, including the minor and branched-chain SFAs.

⁴ Sum of all *trans* isomers of 18:1 t from $\Delta 4t$ to $\Delta 16t$. For 2009–2011 human milk samples, the total 18:1 t does not include the 18:1 $\Delta 15t$ isomer, because it coeluted with 9c-18:1 on gas-chromatographic analysis.

⁵ Sum of 18:2 $\Delta 9t, 12t, 18:2\Delta 9c, 12t, 18:2\Delta 9t, 12c, 18:2\Delta 9c, 13t, 18:2\Delta 8t, 13c, 18:2\Delta 10t, 15c,$ and 18:2 $\Delta 9t, 15c$.

⁶ Sum of 18:3 $\Delta 9t, 12c, 15t, 18:3\Delta 9c, 12t, 15t, 18:3\Delta 9c, 12c, 15t,$ and 18:3 $\Delta 9t, 12c, 15c$.

⁷ Sum of all *trans* isomers of 16:1, 18:1, 18:2, and 18:3.

⁸ For human milk samples from 2009–2011, the concentration for 9c-18:1 includes 18:1 $\Delta 15t$, because these 2 isomers could not be separated by the direct gas-chromatographic method used in the present study.

⁹ Sum of all 18:1 *cis* isomers from 18:1 $\Delta 6c$ to 18:1 $\Delta 16c$. As noted, the 18:1 $\Delta 15t$ isomer is included in the sum above.

¹⁰ Sum of all *cis*-monounsaturated fatty acid isomers of chain lengths of C14–C22.

¹¹ Sum of all LC n-6 PUFA metabolites of 18:2n-6, which includes 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6.

¹² Sum of all LC n-3 PUFA metabolites of 18:3n-3, which includes 20:5n-3, 22:5n-3, and 22:6n-3.

collected in 1992 was 7.2 g/100 g milk fatty acids (range: 0.1–17.2 g/100 g milk fatty acids). The total TFA concentration also gradually and significantly decreased from 2009 to 2011. From 2009 to 2010, the total TFAs decreased from 2.7 to 2.2 g/100 g milk total fatty acids (ie, a decrease of 18%), and by 2011, it decreased further to 1.9 g/100 g milk fatty acids, an additional decrease of 14%. From 2009 to 2011, the total TFA concentration decreased by 30%. As illustrated in Table 1, the decrease in the mean concentration of total TFAs was associated with a decrease in the minimum–maximum range of total TFAs. The decrease in total TFAs is primarily attributable to a decrease of the 18:1 t isomer group. Significant regional differences in the TFA concentrations as well as the other fatty acids were not observed in this study (data not shown).

The decrease of TFAs from 1992 to 2009 and from 2009 to 2011 was associated with significant increases in oleic (9c-18:1), linoleic (18:2n-6), and α -linolenic (18:3n-3) acids (Table 1).

The SFA concentrations remained unchanged for the 3 consecutive years from 2009 to 2011 (Table 1) and are not different from the 1992 collection, except for the slightly higher value for the 2010 samples.

DISCUSSION

The most striking finding of this study is that the concentration of total TFAs in Canadian human milk decreased gradually and significantly for the 3 consecutive years from 2009 to 2011. Furthermore, the TFA concentrations for these 3 y are considerably lower compared with our previous nationwide human milk study in 1992 (21). These concentrations are also low compared with those reported by Innis and colleagues for human milk samples collected in 1998 (22) and between November 2004 and January 2006 (28) from breastfeeding mothers living in Western Canada. The mean total TFA concentration for the samples

collected in 2011, which is the most recent Canadian nationwide collection of human milk, was 1.9 g/100 g of milk fatty acids, which is 74% lower than the concentration of 7.2 g/100 g milk fatty acids found for the samples collected from different regions of Canada in 1992.

These considerable differences most likely reflect the low concentration of TFAs in the current Canadian diet and, therefore, in current Canadian foods compared with the Canadian diet and foods sold in the 1990s and the mid-2000s (4–6, 8, 29). The retail prepackaged foods that were available in the Canadian market in the 1990s and to mid-2000s contained very high concentrations of TFAs compared with those foods sold in Canada in recent years (15, 16). The mandatory TFA food labeling regulation of prepackaged foods that came into effect in 2005 (12) and Health Canada's recommendation in 2007, based on the report of the Canadian task force on *trans* fatty acids (13) to the food industry to reduce the TFA content in soft margarines to <2% of total fat and to <5% of total fat in all other foods, resulted in significant reductions in TFAs in Canadian foods (15–17). For example, most brands of soft margarines sold in Canada in the 1990s were prepared by using PHVOs and contained TFAs ranging from 20% to 25% of total fatty acids (4–6, 8). In contrast, an increasing number of soft margarines sold since 2005 have been prepared by using nonhydrogenated vegetable oils containing <2% TFAs (7). The food industry also has made substantial progress since 2007 in reformulating other prepackaged foods by using nonhydrogenated vegetable oils and thereby reducing the TFA content in many products (15–17). Our study from 2005 to 2008 of the nationwide monitoring of the TFA content in retail foods, recognized in the 1990s as important contributors of TFA, found that 76% of all the prepackaged foods met the <2% or <5% target concentrations (16, 17). Many fast-food restaurant chains have also been successful in decreasing TFAs in French fries, fried chicken, fried fish, fried onions, and pizzas (16, 17). This is especially important given that the nutrition labeling regulations do not apply to foods sold in restaurants. At the end of 2008, more than 82% of the foods sampled in fast-food restaurants in the nationwide monitoring study had achieved the <5% *trans* fat target in their products.

The association of the decrease of TFAs with increases in the concentrations of 9c-18:1, 18:2n-6, and 18:3n-3 from 1992 to 2011 human milk samples was not unexpected and consistent with the replacement of PHVOs in retail foods with high oleic oils or with a blend of high oleic oil and liquid oils such as canola (16). This replacement is expected to decrease the TFAs and increase 9c-18:1, 18:2n-6, and 18:3n-3 concentrations in the Canadian diet. The lack of change in the SFA concentrations between 2009, 2010, and 2011 human milk shown in the present study further confirms that the PHVOs in retail foods were replaced by unsaturated oils, not by saturated fats and oils, such as lard, beef fat, butter, palm oil, or coconut oil.

By using the equation $Y = 1.49 + 0.42X$ established by Craig-Schmidt et al (20) for the relation between the percentage of 18:1t in milk fatty acids (Y in the equation) and the previous-day maternal diet (X in the equation), and assuming that breastfeeding mothers' intake is 2350 kcal/d, of which 30% is derived from fat (29), we estimated that TFA intakes were 4.0% of total energy, equivalent to 10.6 g/person/d, for the Canadian breastfeeding mothers who participated in our 1992 nationwide human milk study (21). By using the same approach, we estimated that

the mean (range) intake of TFAs by breastfeeding mothers in the present study was 2.3 (0–10.6), 1.3 (0–10.0), and 0.8 (0–4.5) g/person/d for the 3 consecutive years of 2009, 2010, and 2011, respectively. The corresponding values, as a percentage of total dietary energy, are 0.9 (0–4.1), 0.5 (0–3.8), and 0.3 (0–1.7) person/d. These data show a substantial decrease in the TFA content in the mothers' diets in the present study compared with the mothers' diets in the 1992 study. Furthermore, these values are also considerably lower than the 1.4% value of energy that we previously estimated (16) for the Canadian general population in 2008 by using TFA data of retail foods from Health Canada's Trans Fat Monitoring Program (30) and dietary intake data from the 2.2 Cycle of the Canadian Community Health Survey (31). These estimates suggest that the concentrations of TFAs in Canadian foods are continuing to gradually decrease since our last round of surveys of Canadian foods in 2008 (16). The estimated TFA intake values for the mothers in the present study are below the WHO's maximum recommended intake of 1% of total energy (14).

CAD is one of the leading causes of death in Canada. In 2008, cardiovascular disease accounted for 69,500 deaths, contributing to 29% of all deaths in Canada (32). It is well documented that TFAs adversely affect multiple CAD risk factors. TFAs lower HDL cholesterol and raise LDL cholesterol, total/HDL cholesterol ratio, lipoprotein(a), apolipoprotein B/apolipoprotein A1 ratio, C-reactive protein, and several circulating biomarkers of inflammation and endothelial dysfunction (33). CAD risk would also be variably decreased by different fats and oils replacing TFAs from PHVOs (33). Replacement of TFAs with MUFAs and PUFAs would reduce the CAD risk to a greater extent than by replacement of TFAs with SFAs (33). Previously, we conservatively estimated that reduction of TFA intake to 1% of energy would prevent 12,354 heart attack cases in Canada over a 19-y period from 2010 to 2029 (17). Providing an estimate of the CAD reduction associated with the current TFA intake concentration of 0.3% of energy estimated in this study for the Canadian breastfeeding mothers is beyond the scope of this study, but it can be safely suggested that the combined effect of a TFA intake concentration of 0.3% of energy with increased intake concentrations of MUFAs and PUFAs due to the replacement of PHVOs with liquid vegetable oils in reformulated prepackaged and restaurant foods would prevent more than 12,354 heart attacks in Canada from 2010 to 2029.

In summary, our results show a decrease in TFA concentrations in human milk, which indicates that the intake of TFAs among Canadian breastfeeding mothers has continued to decrease from 2009 to 2011. The TFA intake concentrations are lower than the maximum recommended intake concentration of 1% of energy (14). This decrease is most likely attributed to mandatory labeling of TFAs in prepackaged foods and replacement of PHVOs with liquid vegetable oils as the frying oils in fast-food restaurants and in the preparation of retail foods by the food industry.

Potential limitations of our study should be considered. It is not known whether human milk TFA data can be generalized to the Canadian population. TFA consumption may be higher or lower in the general population. Furthermore, the MIREC study, from which the milk samples were obtained, was not population based (23). However, the human milk samples were collected nationwide. Furthermore, in a given household, in general, all the family members, including breastfeeding mothers, eat the same

foods; therefore, it can be expected that intake of TFAs, as a percentage of total fatty acids, would remain the same for the entire household.

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The authors' responsibilities were as follows—WMNR: was responsible for the concept of the study, experimental design, interpretation of the data, and writing of the manuscript; ES: contributed to fatty acid analysis of human milk samples, compiling of data, and statistical analysis of the data and writing of the manuscript; RZ and CG: contributed to experimental design, fatty acid analysis of human milk samples, and compiling of data; and WL and PP: contributed to experimental design and fatty acid analysis. None of the authors had a conflict of interest.

REFERENCES

- Sahasrabudhe MR, Kurian CJ. Fatty acid composition of margarines in Canada. *Can Inst Food Sci Technol J* 1979;12:140–4.
- Beare-Rogers JL. Trans and positional isomers of common fatty acids. In: Draper HH, ed. *Advances in nutrition research*. Vol 5. New York, NY: Plenum, 1983:171–200.
- Beare-Rogers JL, Hollywood R, O'Grady E. Fatty acids in Canadian margarines. *Can J Public Health* 1985;76:276–7.
- Ratnayake WMN, Hollywood R, O'Grady E. Fatty acids in Canadian margarines. *Can Inst Food Sci Technol J* 1991;24:81–6.
- Ratnayake WMN, Hollywood R, O'Grady E, Pelletier G. Fatty acids in some common food items in Canada. *J Am Coll Nutr* 1993;12:651–60.
- Ratnayake WMN, Pelletier G, Hollywood R, Bacler S, Leyte D. Trans fatty acids in Canadian margarines: recent trends. *J Am Oil Chem Soc* 1998;75:1587–94.
- Ratnayake WMN, Lillycrop W, Wong L, Meleta M, Calway P, Gagnon C. Trans fatty acid content of Canadian margarines prior to mandatory *trans* fat labelling. *J Am Oil Chem Soc* 2007;84:817–25.
- Ratnayake WMN, Zehaluk C. Trans fatty acids in foods and their labelling. In: Akoh CC, Lai OM, eds. *Healthful lipids*. Champaign, IL: AOCS Press, 2005:1–32.
- Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med* 2006;354:1601–13.
- Mozaffarian D, Aro A, Willett WC. Health effects of trans-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* 2009;63 (suppl 2):S5–21.
- Ratnayake WMN, Chen ZY. Trans fatty acids in Canadian breast milk and diet. In: Przybylski R, McDonald BE, eds. *Development and processing of vegetable oils for human nutrition*. Champaign, IL: AOCS Press, 1996:20–35.
- Health Canada. Regulations amending the food and drug regulations (nutrition labelling, nutrient content claims and health claims). *Canada Gazette* 2003;137(part II):154–6.
- Health Canada, Trans Fat Task Force. *TRANSforming the food supply*. Ottawa, Canada: Health Canada, 2006.
- World Health Organization. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser* 2003;916:i–viii, 1–149.
- Ratnayake WMN, L'Abbe MR, Mozaffarian D. Nationwide reformulations to reduce trans fatty acids in Canada: when trans fat goes out, what goes in? *Eur J Clin Nutr* 2009;63:808–11.
- Ratnayake WMN, L'Abbe MR, Farnworth S, Dumais L, Gagnon C, Lampi B, Casey V, Mohottalage D, Rondeau I, Underhill L, et al. *Trans* fatty acids: current contents in Canadian foods and estimated intake levels for the Canadian population. *J AOAC Int* 2009;92: 1258–76.
- Krenosky S, L'Abbe M, Lee N, Underhill L, Vigneault M, Godefroy S, Ratnayake N. Risk assessment of exposure to trans fat in Canada. *Int Food Risk Anal J* 2012;2:1–15.
- Ratnayake WMN, Chen ZY. Trans, n–3, and n–6 fatty acids in Canadian human milk. *Lipids* 1996;31:S279–82.
- Aitchison JM, Kunkley WL, Cantoly NL, Smith LM. Influence of diet on human milk trans fatty acids. *Am J Clin Nutr* 1977;30: 2006–15.
- 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.
- Chen ZY, Pelletier G, Hollywood R, Ratnayake WMN. Trans fatty acids isomers in Canadian human milk. *Lipids* 1995;30:15–21.
- Innis SM, King DJ. Trans fatty acids in human milk are inversely associated with concentrations of all-cis n–6 and n–3 fatty acids and determine trans, but not n–6 and n–3 fatty acids of plasma lipids of breast-fed infants. *Am J Clin Nutr* 1999;70:383–90.
- Arbuckle TE, Fraser WD, Fisher M, Davis K, Liang CL, Lupien N, Bastien S, Velez MP, Daelszen PV, Hemmings DG, et al. Cohort profile: the Maternal-Infant Research on Environmental Chemicals Research Platform. *Paediatr Perinat Epidemiol* 2013;27:415–25.
- AOAC International. *Official methods of analysis*. 18th ed. Gaithersburg, MD: AOAC International, 2005.
- American Oil Chemists' Society. *Official methods and recommended practices of the AOCS*. 5th ed. Champaign, IL: AOCS, 2005:Method Ce 1h-05.
- Ratnayake WMN. Overview of methods for the determination of trans fatty acids by gas chromatography, silver-ion thin layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. *J AOAC Int* 2004;87:523–39.
- Mendis S, Cruz-Hernandez C, Ratnayake WMN. Fatty acid profile of Canadian dairy products with special attention to the trans-octadecenoic acid and conjugated linoleic acid isomers. *J AOAC Int* 2008;91: 811–9.
- Friesen R, Innis SM. Trans fatty acids in human milk in Canada declined with the introduction of *trans* fat food labeling. *J Nutr* 2006;136: 2558–61.
- Elias SL, Innis SM. Bakery foods provide the major sources of trans fatty acids among pregnant women with diets providing 30% energy from fat. *J Am Diet Assoc* 2002;102:46–51.
- Health Canada. *Trans Fat Monitoring Program*. Available from: http://www.hc-sc.gc.ca/fn_an/nutrition/gras-trans-fats/index-eng/index-eng.php (cited 6 August 2013).
- Statistics Canada. *Canadian Community Health Survey, cycle 2.2, nutrition—general health (including vitamin and mineral supplement) & 24 hour dietary recall components user guide*. Ottawa, Canada: Health Statistics Division, Statistics Canada, 2008.
- Statistics Canada. *Mortality: summary list of causes, 2008*. Ottawa, Canada: Statistics Canada, 2011.
- Mozaffarian D, Clarke R. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *Eur J Clin Nutr* 2009;63 (suppl 2):S22–33.