

trans Fatty acids in human milk are inversely associated with concentrations of essential *all-cis* n-6 and n-3 fatty acids and determine *trans*, but not n-6 and n-3, fatty acids in plasma lipids of breast-fed infants¹⁻³

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ABSTRACT

Background: Human milk fatty acids vary with maternal dietary fat composition. Hydrogenated dietary oils with *trans* fatty acids may displace *cis* n-6 and n-3 unsaturated fatty acids or have adverse effects on their metabolism. The effects of milk *trans*, n-6, and n-3 fatty acids in breast-fed infants are unclear, although n-6 and n-3 fatty acids are important in infant growth and development.

Objective: We sought to determine the relations between *trans* and *cis* unsaturated fatty acids in milk and plasma phospholipids and triacylglycerols of breast-fed infants, and to identify the major maternal dietary sources of *trans* fatty acids.

Design: We collected milk from 103 mothers with exclusively breast-fed 2-mo-old infants, blood from 62 infants, and 3-d dietary records from 21 mothers.

Results: Mean (\pm SEM) percentages of *trans* fatty acids were as follows: milk, $7.1 \pm 0.32\%$; infants' triacylglycerols, $6.5 \pm 0.33\%$; and infants' phospholipids, $3.7 \pm 0.16\%$. Milk *trans* fatty acids, α -linolenic acid (18:3n-3), arachidonic acid (20:4n-6), docosahexaenoic acid (22:6n-3) ($P < 0.001$), and linoleic acid (18:2n-6) ($P = 0.007$) were each related to the same fatty acid in infant plasma phospholipids. Milk *trans* fatty acids were inversely related to milk 18:2n-6 and 18:3n-3, but not to milk or infant plasma 20:4n-6 or 22:6n-3. *trans* Fatty acids represented 7.7% of maternal total fat intake (2.5% of total energy); the major dietary sources were bakery products and breads (32%), snacks (14%), fast foods (11%), and margarines and shortenings (11%).

Conclusions: There were comparable concentrations of *trans* fatty acids in the maternal diet, breast milk, and plasma triacylglycerols of breast-fed infants. Prepared foods were the major dietary source of *trans* fatty acids. *Am J Clin Nutr* 1999;70:383-90.

KEY WORDS Arachidonic acid, *trans* fatty acids, docosahexaenoic acid, human milk, breast-fed infants, breast milk, plasma lipids, n-6 fatty acids, n-3 fatty acids

INTRODUCTION

Recent interest in the health effects of *trans* fatty acids has centered largely around potential adverse effects of *trans* fatty acids on lipid risk factors for cardiovascular disease, as well as metabolism of the essential (*all-cis*) n-6 and n-3 fatty acids, particularly

in relation to infant growth and development (1-7). The major dietary sources of *trans* fatty acids in Western countries are hydrogenated fats and oils. Smaller amounts are consumed in foods from ruminants (eg, dairy fats, beef, and lamb) due to biohydrogenation of unsaturated fatty acids in the rumen. The extent of hydrogenation of dietary fats and oils depends on the unsaturated fatty acid content of the oil and the desired stability and physical properties of the product. Hydrogenation increases stability and the melting point by reducing the content of naturally occurring *cis* double bonds to give a complex mixture of geometric and positional isomers (8). The resulting fats and oils, in addition to containing *trans* fatty acids, have reduced amounts of linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3). *trans* Fatty acids are well absorbed and are incorporated into tissue lipids, although predominantly into triacylglycerols rather than into phospholipids (8, 9). Estimates of average daily intakes by adults in the United States, Canada, Europe, and Australia based on food usage, food-frequency questionnaires, or duplicate portion analysis range from ≈ 3 to 17 g/person (8, 10-14).

Considerable evidence from published studies shows that the proportions of fatty acids in human milk are influenced by the *trans*, n-6, and n-3 fatty acid composition of the maternal diet (15-17). The composition of fatty acids in breast milk consumed by young infants is of concern because of the important roles of n-6 and n-3 fatty acids in infant growth and development (18-20). Some studies have noted an inverse relation between concentrations of *trans* fatty acids in fetal or infant tissue and measures of growth (5-7). It has also been suggested that inverse associations between *trans* fatty acids and arachidonic acid (20:4n-6), as well as the ratio of 20:4n-6 to 18:2n-6, reflect inhibition of desaturation of 18:2n-6 to 20:4n-6 (5-7). Although some animal and in vitro studies are consistent with the hypothesis that *trans* fatty acids may interfere with the desat-

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uration of 18:2n-6 (4, 21, 22), it is possible that diets high in *trans* fatty acids are also low in *all-cis* n-6 and n-3 fatty acids.

Several studies have documented the presence of *trans* fatty acids in human milk (15, 16, 23-26), with concentrations possibly higher in Canada (25) than in Europe (23, 24, 26). Whether high milk concentrations of *trans* fatty acids result in high *trans* fatty acids and reduced 18:2n-6, 18:3n-3, 20:4n-6, or docosahexaenoic acid (22:6n-3) concentrations in the plasma lipids of breast-fed infants has not been clearly established. The objective of this study, therefore, was to determine in ≈ 100 breast-feeding mothers and their term infants the relations between *all-cis* n-6 and n-3 and *trans* fatty acid concentrations in the mothers' milk and in the plasma phospholipids and triacylglycerols of the breast-fed infants.

SUBJECTS AND METHODS

Subjects

This study involved 103 mothers who were exclusively breast-feeding their term (born at 37-41 wk gestation) infants. Infants with any evidence of a metabolic or physical abnormality, or who were given bottle feedings of formula or cow milk, were ineligible. A subset of the mothers ($n = 62$) agreed to allow collection of a venous blood sample from their infants at age 2 mo, concurrent with the provision of a milk sample. A separate subset of 21 mothers agreed to provide dietary information. No personal dietary instructions were given to any of the mothers. The procedures and methods used in this study were reviewed and approved by the Committee for Ethical Review of Research Involving Human Subjects at the University of British Columbia. Written, informed consent was obtained from all the women who participated.

Methods

Samples of breast milk (60-100 mL) were collected from all the women during the course of a feeding, at approximately the midpoint of the feeding, on the same day that the infants' blood samples or the mothers' 24-h dietary recalls were collected from subjects in the subsets providing this information.

The mothers who provided dietary information were interviewed by a nutritionist, who recorded their food intake for the preceding 24 h on the day when samples were collected. The 24-h dietary recall was used to train the mothers in how to keep a 3-d weighed food record. The importance of recording in detail the methods of food preparation, recipes, and brand names for all foods eaten was emphasized. The 3-d food records were completed during the following week, returned by mail, and reviewed by the nutritionist.

Venous blood samples were collected from the infants ($n = 62$) 2-3 h after a feeding, as close as possible to the anticipated time of the next feeding. Plasma was separated by low-speed centrifugation ($2000 g \times 15 \text{ min}$ at 5°C) immediately after blood collection. Milk and plasma samples were stored at -70°C until analyzed. The milk samples were thawed in ice-cold water and were directly transmethylated to avoid potential losses of medium-chain saturated fatty acids (27).

The infants' plasma total lipids were extracted and then the triacylglycerols, phospholipids, and cholesteryl esters were separated by using thin-layer chromatography (TLC). The lipid fractions were recovered and the fatty acids were converted to their respective methyl esters (28) for separation by gas chro-

matography (GC) with a Varian 3400 gas chromatograph (Varian Canada Inc, Mississauga, Ontario), as described by Chen et al (25), that used an SP-2560 capillary column (100 m \times 0.25 mm internal diameter, 20- μm film thickness) (Supelco, Bellefonte, PA). The CV of the GC method for the milk and infant plasma triacylglycerol and phospholipid fatty acid analyses was $<1\%$. The GC procedure that we used does not separate all possible *trans* and *cis-trans* positional isomers (25, 29). Therefore, for the purposes of this report, concentrations of *trans*, *cis-trans*, and unusual *cis* positional isomers of the naturally occurring *cis* unsaturated fatty acids were calculated by summation and designated as total *trans* fatty acids.

The 3-d food records were analyzed to determine the average daily intake of total energy, fat, and *trans* fatty acids for each individual by using the FOOD PROCESSOR program (version 7.02; ESHA Research, Salem, OR), modified to include the total fat and fatty acid contents of ≈ 300 specific brand name food products, which were analyzed in the present study.

Statistical analysis

The significance of any relation between the concentration of a given fatty acid in milk and the concentration of the same fatty acid, or other fatty acids, in the infants' plasma triacylglycerols or phospholipids was examined by using correlation analysis for the results of matched analyses for milk and infants' plasma. The relations among the concentrations of *trans* fatty acids, 18:2n-6, 18:3n-3, 20:4n-6, and 22:6n-3 in the milk samples were similarly explored by using correlation analysis. A P value ≤ 0.05 was considered significant. Because the fatty acid concentrations were not normally distributed, the data were logarithmically transformed before statistical analysis. All statistical analyses were performed with the Statistical Package for the Social Sciences (version 7.5; SPSS Inc, Chicago).

RESULTS

Human milk and infant plasma triacylglycerol and phospholipid fatty acids

The composition of the major saturated, *cis* n-9 and n-7 monounsaturated, n-6, and n-3 fatty acids, conjugated linoleic acid (CLA), and other *trans*, unusual *cis*, and *cis-trans* isomers of unsaturated fatty acids in breast milk are shown in **Table 1**. The percentage distribution of fatty acids in the Canadian milk samples reported here are generally similar to those reported by Chen et al (25) for milk collected from women in 9 provinces in Canada. Our results show wide ranges for the concentrations of all fatty acids in milk; the samples were collected from ≈ 100 women who were all at a similar stage postpartum and were all exclusively breast-feeding their infants. Whereas mean percentages of 18:2n-6, 18:3n-3, 20:4n-6, and 22:6n-3 were 12.1%, 1.4%, 0.4%, and 0.2% of the total fatty acids, respectively, individual values varied widely among the women; eg, from 6.0% to 21.5% for 18:2n-6, from $<0.1\%$ to 4.1% for 18:3n-3, from 0.2% to 0.8% for 20:4n-6, and from 0.1% to 2.6% for 22:6n-3. Similarly, whereas the mean concentration of total *trans* fatty acids was 7.1%, the concentrations varied from 2.2% to 18.7% of milk fatty acids among the 103 women studied. The results (Table 1) also clearly show similar, wide interindividual variation in the concentrations of 14:0, 16:0, 18:0, *cis* 18:1n-9, and *cis* 18:1n-7 in the milk fatty acids.



TABLE 1
Major saturated and unsaturated *cis* and *trans* fatty acids in human milk¹

Fatty acid	Value
	% of total fatty acids
10:0	0.6 ± 0.03 (0.0–1.4)
12:0	4.1 ± 0.15 (0.5–9.3)
14:0	6.1 ± 0.21 (2.1–14.5)
16:0	19.4 ± 0.28 (12.1–27.1)
18:0	7.2 ± 0.15 (3.5–11.9)
20:0	0.2 ± 0.00 (0.1–0.4)
22:0	0.1 ± 0.00 (<0.1–0.2)
24:0	0.1 ± 0.00 (<0.1–0.2)
<i>cis</i> 14:1	0.2 ± 0.01 (0.1–0.5)
<i>cis</i> 16:1n–9	0.3 ± 0.01 (0.1–0.5)
<i>cis</i> 16:1n–7	2.2 ± 0.07 (0.5–4.9)
<i>cis</i> 18:1n–9 ²	33.9 ± 0.34 (18.2–40.6)
<i>cis</i> 18:1n–7	1.8 ± 0.07 (<0.1–3.7)
<i>cis</i> 20:1n–11	0.2 ± 0.03 (<0.1–0.5)
<i>cis</i> 20:1n–9	0.4 ± 0.02 (<0.1–1.0)
<i>cis</i> 22:1n–9	0.2 ± 0.02 (<0.1–1.1)
<i>cis</i> 24:1n–9	0.1 ± 0.01 (<0.1–0.4)
<i>cis</i> 18:2n–6	12.1 ± 0.35 (6.0–21.5)
<i>cis</i> 18:3n–6	0.1 ± 0.00 (<0.1–0.3)
<i>cis</i> 20:2n–6	0.3 ± 0.01 (0.2–0.5)
<i>cis</i> 20:3n–6	0.3 ± 0.01 (0.2–0.7)
<i>cis</i> 20:4n–6	0.4 ± 0.01 (0.2–0.8)
<i>cis</i> 22:4n–6	0.1 ± 0.00 (<0.1–0.1)
<i>cis</i> 18:3n–3	1.4 ± 0.07 (<0.1–4.1)
<i>cis</i> 20:5n–3	0.1 ± 0.01 (<0.1–0.8)
<i>cis</i> 22:5n–3	0.2 ± 0.02 (0.1–1.7)
<i>cis</i> 22:6n–3	0.2 ± 0.03 (0.1–2.6)
Total <i>trans</i> ³	7.1 ± 0.32 (2.2–18.7)
CLA	0.4 ± 0.01 (0.1–0.7)

¹ $\bar{x} \pm$ SEM; range in parentheses. Values are given for fatty acids present at >0.05% of total fatty acids for milk collected 2 mo after full-term delivery from 103 exclusively breast-feeding women. CLA, conjugated linoleic acid.

²May include some *t*-18:1 Δ 12– Δ 16 isomers.

³Includes all *trans* and mixed *cis-trans* fatty acids, excluding CLAs.

The analyses of plasma triacylglycerol and phospholipid fatty acids in the 2-mo-old exclusively breast-fed infants also showed considerable interindividual variability in the concentrations of *all-cis* n–6 and n–3 fatty acids and *trans* fatty acids (Table 2). Mean (\pm SEM) *trans* fatty acid concentrations were higher in the infants' plasma triacylglycerols (6.5 ± 0.33%; range: 1.9–15.6%) than in the phospholipids (3.7 ± 0.16%; 1.7–8.3%), consistent with the higher incorporation of *trans* fatty acids into human tissue triacylglycerols than into phospholipids (9). In contrast, the concentrations of *all-cis* n–6 and n–3 fatty acids, with the exception of 18:3n–3, were consistently higher in the infant plasma phospholipids than the triacylglycerols. Comparison of the results for the human milk and infant plasma phospholipid and triacylglycerol fatty acid analyses (shown in Tables 1 and 2, respectively) showed that the means and ranges for the percentages of the major saturated, *cis* unsaturated, and *trans* fatty acid, and CLA concentrations were similar.

Relation between milk and infants' plasma fatty acids

The concentrations of total *trans* fatty acids and *cis* 18:1, 18:2n–6, 18:3n–3, 20:4n–6, and 22:6n–3 in human milk each showed a significant positive relation to the concentration of the same fatty acid in both the plasma triacylglycerols and phospho-

TABLE 2
Major saturated and unsaturated *cis* and *trans* fatty acids in plasma phospholipids and triacylglycerols of 62 exclusively breast-fed infants at 2 mo of age¹

Fatty acid	Phospholipids	Triacylglycerols
	% of total fatty acids	
12:0	0.0 ± 0.0 (0.0–0.0)	0.5 ± 0.06 (0.0–2.4)
14:0	0.2 ± 0.0 (0.1–0.4)	2.6 ± 0.13 (1.0–7.0)
16:0	22.9 ± 0.3 (17.6–26.6)	24.3 ± 0.43 (15.4–32.1)
18:0	17.7 ± 0.2 (15.4–26.7)	6.6 ± 0.15 (3.8–10.0)
20:0	0.2 ± 0.01 (0.1–0.3)	0.2 ± 0.01 (0.1–0.4)
22:0	0.2 ± 0.01 (0.1–0.6)	0.2 ± 0.01 (0.1–0.6)
24:0	0.1 ± 0.01 (0.1–0.2)	0.1 ± 0.01 (0.0–0.3)
<i>cis</i> 16:1n–9	0.2 ± 0.01 (0.1–0.3)	0.4 ± 0.02 (0.1–0.8)
<i>cis</i> 16:1n–7	0.3 ± 0.01 (0.1–0.3)	1.9 ± 0.08 (0.8–3.5)
<i>cis</i> 18:1n–9 ²	9.1 ± 0.21 (6.8–18.9)	34.8 ± 0.43 (28.7–45.6)
<i>cis</i> 18:1n–7	1.6 ± 0.03 (1.1–2.4)	1.9 ± 0.11 (0.3–3.5)
<i>cis</i> 20:1n–9	0.2 ± 0.01 (0.0–0.0)	0.4 ± 0.01 (0.3–0.6)
<i>cis</i> 22:1n–9	0.0 ± 0.00 (0.0–0.1)	0.1 ± 0.00 (0.0–0.2)
<i>cis</i> 24:1n–9	0.4 ± 0.02 (0.2–0.7)	0.0 ± 0.00 (0.0–0.1)
<i>cis</i> 18:2n–6	20.4 ± 0.33 (15.4–26.9)	14.2 ± 0.43 (9.2–23.0)
<i>cis</i> 20:2n–6	0.4 ± 0.01 (0.3–0.6)	0.4 ± 0.01 (0.2–0.6)
<i>cis</i> 20:3n–6	3.1 ± 0.09 (1.7–4.7)	0.4 ± 0.02 (0.2–1.4)
<i>cis</i> 20:4n–6	11.8 ± 0.21 (8.1–15.8)	1.1 ± 0.05 (0.6–2.6)
<i>cis</i> 22:4n–6	0.4 ± 0.02 (0.2–0.6)	0.1 ± 0.01 (0.0–0.3)
<i>cis</i> 22:5n–6	0.3 ± 0.01 (0.1–0.5)	0.1 ± 0.01 (0.0–0.2)
<i>cis</i> 18:3n–3	0.1 ± 0.01 (0.1–0.4)	1.1 ± 0.06 (0.5–2.6)
<i>cis</i> 20:5n–3	0.5 ± 0.03 (0.2–1.6)	0.2 ± 0.03 (0.0–1.5)
<i>cis</i> 22:5n–3	0.9 ± 0.02 (0.6–1.4)	0.3 ± 0.02 (0.1–0.6)
<i>cis</i> 22:6n–3	4.6 ± 0.13 (2.2–8.0)	0.6 ± 0.07 (0.2–3.5)
Total <i>trans</i> ³	3.7 ± 0.16 (1.7–8.3)	6.5 ± 0.33 (1.9–15.6)
CLA	0.2 ± 0.01 (0.1–0.3)	0.4 ± 0.03 (0.1–0.7)

¹ $\bar{x} \pm$ SEM; range in parentheses. Values are given for fatty acids present at >0.05% of total fatty acids for milk collected 2 mo after full-term delivery from 103 exclusively breast-feeding women. CLA, conjugated linoleic acid.

²May include some *t*-18:1 Δ 12– Δ 16 isomers.

³Includes all *trans* and *cis-trans* fatty acids, excluding CLAs.

lipids of the infants (Table 3, Figure 1). Similarly, the milk concentrations of the major saturated fatty acids (16:0 and 18:0) and other *cis* unsaturated fatty acids (16:1n–9, 16:1n–7, 20:2n–6, 20:3n–6, 22:4n–6, and 20:5n–3) each showed a significant positive relation to the concentration of the same fatty acid in both the plasma triacylglycerols and plasma phospholipids of the infants (Table 3).

The concentrations of *trans* fatty acids in milk showed significant inverse relations to the milk concentrations of 18:2n–6 ($P = 0.02$) and 18:3n–3 ($P = 0.02$) but not *cis* 18:1n–9, 20:4n–6, or 22:6n–3 (data not shown). There were no significant relations between the concentrations of *trans* fatty acids in milk and the concentrations of 20:4n–6 or 22:6n–3 in the infants' plasma triacylglycerols or phospholipids. Similarly, there were no significant relations between the milk concentration of 18:2n–6 and the infants' plasma triacylglycerol or phospholipid concentrations of 20:4n–6 or 22:6n–3, or between milk 18:3n–3 concentrations and the infants' plasma triacylglycerol 20:4n–6 or 22:6n–3 concentrations. In contrast, the concentration of 18:3n–3 in the milk showed a significant inverse relation to the 20:4n–6 concentration ($P = 0.05$) and a positive relation to the 22:6n–3 concentration ($P = 0.02$) in the infants' plasma phospholipids.

TABLE 3

Relations between concentrations of major saturated and unsaturated *cis* and *trans* fatty acids in human milk and in plasma triacylglycerols and phospholipids of 62 breast-fed infants¹

Fatty acid	Triacylglycerols		Phospholipids	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
10:0	0.41	NS	0.33	0.07
12:0	0.00	NS	0.26	0.04
14:0	0.60	<0.001	0.00	NS
16:0	0.60	<0.001	0.26	0.04
18:0	0.53	<0.001	0.00	NS
20:0	0.00	NS	0.14	NS
<i>cis</i> 16:1n-9	0.35	0.007	0.48	<0.001
<i>cis</i> 16:1n-7	0.84	<0.001	0.67	<0.001
<i>cis</i> 18:1n-9 ²	0.62	<0.001	0.28	0.02
<i>cis</i> 18:1n-7	0.10	NS	0.28	0.03
<i>cis</i> 20:1n-11	0.39	0.005	0.17	NS
<i>cis</i> 20:1n-9	0.56	<0.001	0.14	NS
<i>cis</i> 18:2n-6	0.84	<0.001	0.33	0.007
<i>cis</i> 18:3n-6	0.49	<0.001	0.10	NS
<i>cis</i> 20:2n-6	0.41	0.002	0.30	0.019
<i>cis</i> 20:3n-6	0.65	<0.001	0.44	<0.001
<i>cis</i> 20:4n-6	0.50	<0.001	0.55	<0.001
<i>cis</i> 22:4n-6	0.60	<0.001	0.30	0.02
<i>cis</i> 18:3n-3	0.83	<0.001	0.66	<0.001
<i>cis</i> 20:5n-3	0.71	<0.001	0.44	<0.001
<i>cis</i> 22:5n-3	0.51	<0.001	0.20	NS
<i>cis</i> 22:6n-3	0.77	<0.001	0.50	<0.001
Total <i>trans</i> ³	0.82	<0.001	0.67	<0.001
CLA	0.22	0.08	0.36	0.004

¹ Values are given for fatty acids present at >0.05% of total fatty acids for milk collected 2 mo after full-term delivery from 103 exclusively breast-feeding women. There were no significant relations between milk and plasma concentrations of 22:0, 24:0, 22:1, or 24:1. CLA, conjugated linoleic acids.

² May include some *t*-18:1Δ12-Δ16 isomers.

³ Includes all *trans* and mixed *cis-trans* fatty acids, excluding conjugated 18:2.

Major dietary sources of *trans* fatty acids

Mean (\pm SEM) concentrations of the major saturated and unsaturated fatty acids were not significantly different between the mothers who provided 3-d weighed dietary records and the mothers who did not (data not shown). Total *trans* fatty acids in the milk of women who provided dietary records was $8.0 \pm 0.80\%$ (range: 3.4–18.7%) of total fatty acids. Fat represented $\approx 31.8\%$ of the total daily energy intake (\bar{x} : 10496 ± 597 kJ) with a range of 12.1–44.8% of daily energy (27.7–140.6 g) from fat among the mothers who provided dietary records (Table 4). Two of the 21 mothers reported that they ate no meat, fish, shellfish, or eggs and 1 mother reported no meat or eggs but did eat fish during the 3 d for which records were kept. Nine of the 21 mothers had at least one serving of fish or other seafood. The estimated daily intake of *trans* fatty acids was 6.87 g/person, representing $\approx 7.7\%$ of total fat and $\approx 2.46\%$ of daily energy intake. Of the total *trans* fatty acid intake, $\approx 78\%$ (5.34 g/d) was derived from prepared and processed foods, with the major sources being bakery products such as cakes, cookies, pies, and muffins ($\approx 22\%$ of daily *trans* fatty acid intake), snack foods ($\approx 14.4\%$), fast foods ($\approx 11.1\%$), and breads and rolls ($\approx 10.5\%$). Margarines contributed $\approx 11.1\%$ of daily *trans* fatty acids, but this varied widely among the mothers. The use of tub (soft) margarines as a table spread or in cooking ranged from 0 g fat/d ($n = 9$) to 23.64 g fat/d.

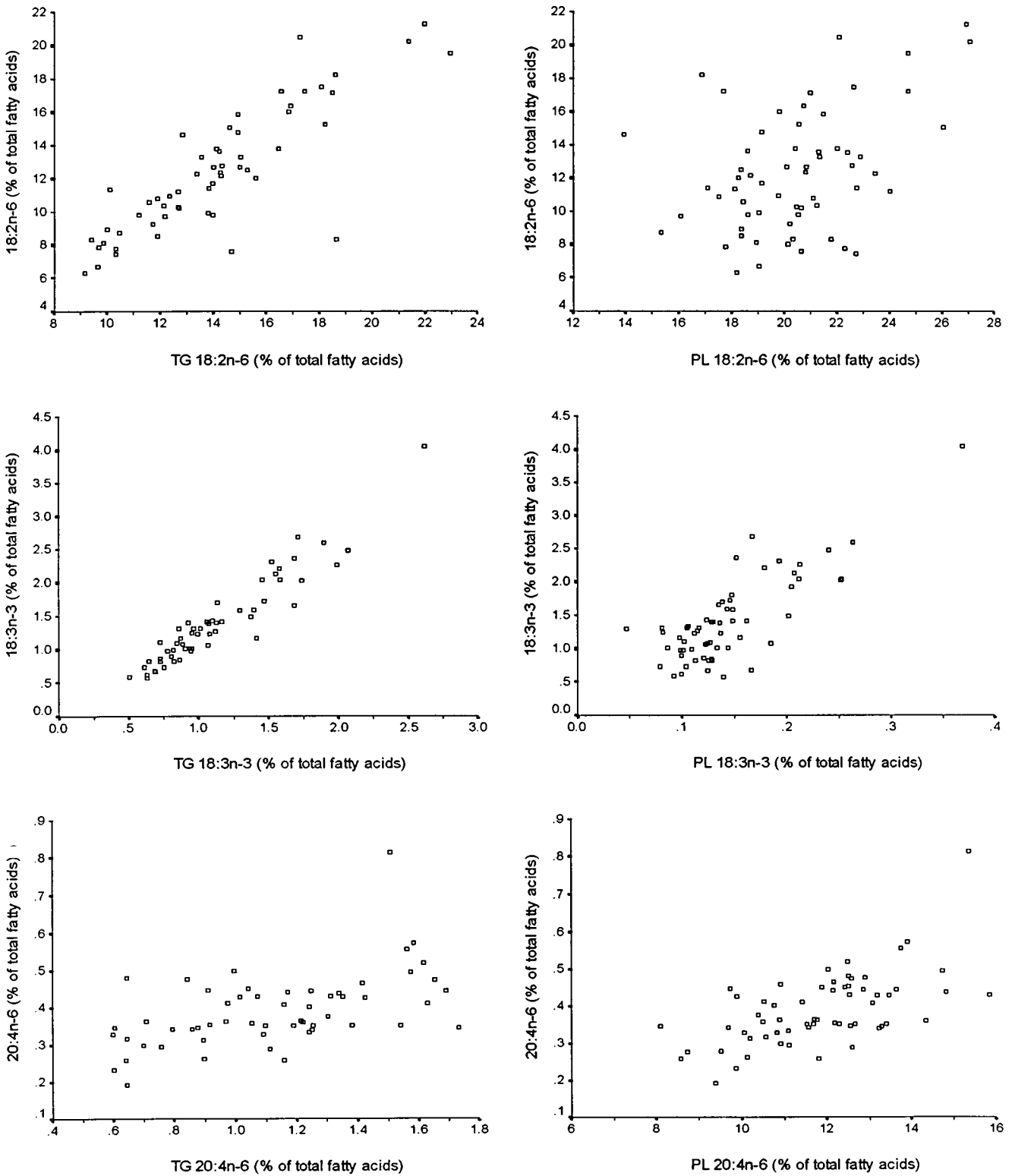
DISCUSSION

This study showed a wide range (up to 18.7%) of *trans* fatty acid concentrations in milk from breast-feeding Canadian mothers. The results also showed that the percentage of *trans* fatty acids in milk was closely paralleled ($P < 0.001$) by the percentages of *trans* fatty acids in the plasma triacylglycerols and phospholipids of the breast-fed infants. The percentage of *trans* fatty acids in plasma phospholipids was $\approx 40\%$ lower than that in plasma triacylglycerols, possibly because we collected blood samples when infants were in the fed state and because of the specificity of the acyltransferases involved in phospholipid synthesis. Similarly, the percentages of 18:2n-6, 18:3n-3, 20:4n-6, and 22:6n-3 in human milk were each significantly related to the percentage of the same fatty acid in the infants' plasma triacylglycerols and phospholipids. The percentage of *trans* fatty acids in milk was inversely related ($P < 0.05$) to the percentages of 18:2n-6 and 18:3n-3 in milk. Similar inverse relations between *trans* fatty acids and 18:2n-6 and 18:3n-3 in milk were noted previously (25). It is possible that women with high intakes of foods containing hydrogenated oils have lower intakes of *all-cis* 18:2n-6 and 18:3n-3, either from salad and cooking oils or from margarines.

Similar to our study, another study found a range for *trans* fatty acids in human milk of 0.1–17.2% (\bar{x} : 7.2%) for breast-feeding women in different regions of Canada (25). However, studies in Spain ($n = 38$) found mean concentrations of $\approx 1\%$ *trans* 18:1 (23), whereas milk from women in France ($n = 10$) had $\approx 2\%$ (range: 1.2–3.0%) *trans* fatty acids (24) and milk from women in Germany had a median of 4.4% (range: 2.2–6.0%) *trans* fatty acids (26). The intake of hydrogenated fats and oils in baked and other prepared foods may possibly be higher in Canada than in Europe.

In contrast with our results, Chen et al (25) found an inverse relation between *trans* 18:1 and *cis* 18:1n-9 in Canadian human milk. One explanation for the lack of a relation between *trans* fatty acids and *cis* 18:1n-9 in our study may be incomplete separation of *trans* 18:1 from *cis* 18:1n-9 by GC. Studies using partially hydrogenated margarines and synthetic unsaturated fatty acids have shown 9–30% overlap of *t*-18:1Δ12 to *t*-18:1Δ16 with *cis* 18:1 in GC with 100-m capillary columns, with the degree of overlap dependent on the total *trans* fatty acid content (29). The most prevalent isomer of 18:1 in human milk is *t*-Δ11 (23% 18:1 isomers), followed by *t*-Δ10 (20%) and *t*-Δ9 (16%), with smaller amounts of *t*-Δ12 and *t*-Δ13. Similarly, $\approx 43\%$ of 18:1 *trans* isomers in cow milk fat are *t*-18:1Δ11, with low proportions of other isomers (25, 29). Our analysis did not include separation of 18:1 isomers (eg, by AgNO₃-TLC) before GC. However, because the most common 18:1 isomers in hydrogenated vegetable oils are *t*-Δ11, *t*-Δ10, and *t*-Δ9, with relatively small amounts of *t*-Δ12 to *t*-Δ16, it seems probable that any overestimation of *cis* 18:1n-9 (or underestimation of *trans* fatty acids) was relatively small. Indeed, comparison of direct GC and AgNO₃-TLC followed by GC of milk fatty acids showed mean (\pm SD) *cis* 18:1n-9 values of $32.7 \pm 3.2\%$ and $30.6 \pm 2.7\%$, respectively, and total *trans* 18:1 values of $4.6 \pm 2.0\%$ and $5.9 \pm 2.5\%$ total fatty acids, respectively, with no differences in *cis* n-6 and n-3 fatty acids with the 2 methods (25). A more probable reason for the lack of a relation between milk *trans* fatty acids and *cis* 18:1n-9 was that the major food sources of *trans* fatty acids in the study were bakery products, snacks, and fast foods. It seems probable that in these foods, *trans* fatty acids replaced saturated fats (eg, butter, lard, or saturated vegetable oils) rather than *cis* 18:1n-9-rich margarines or oils.

In contrast with previous studies (5–7), we found no association between *trans* fatty acids and 20:4n-6 or 22:6n-3 in milk



(Continued)

FIGURE 1. Relations between milk concentrations of 18:2n-6, 18:3n-3, 20:4n-6, 22:6n-3, total *trans* fatty acids, and conjugated linoleic acid (CLA) and plasma triacylglycerol (TG) and phospholipid (PL) concentrations of the same fatty acids in breast-fed infants ($n = 62$). $P < 0.0001$ for all relations except for those between milk and PL 18:2n-6 ($P = 0.007$), milk and PL 22:6n-3 ($P = 0.02$), milk and TG CLA ($P = 0.08$), and milk and PL CLA ($P = 0.004$). The scale of the axes differs for the milk and plasma, and for the different fatty acids. Data for one mother who took fish oil supplements and who had 2.6% 22:6n-3 in milk fatty acids are not included in the plots, but the data for this mother-infant pair are included in Table 3.



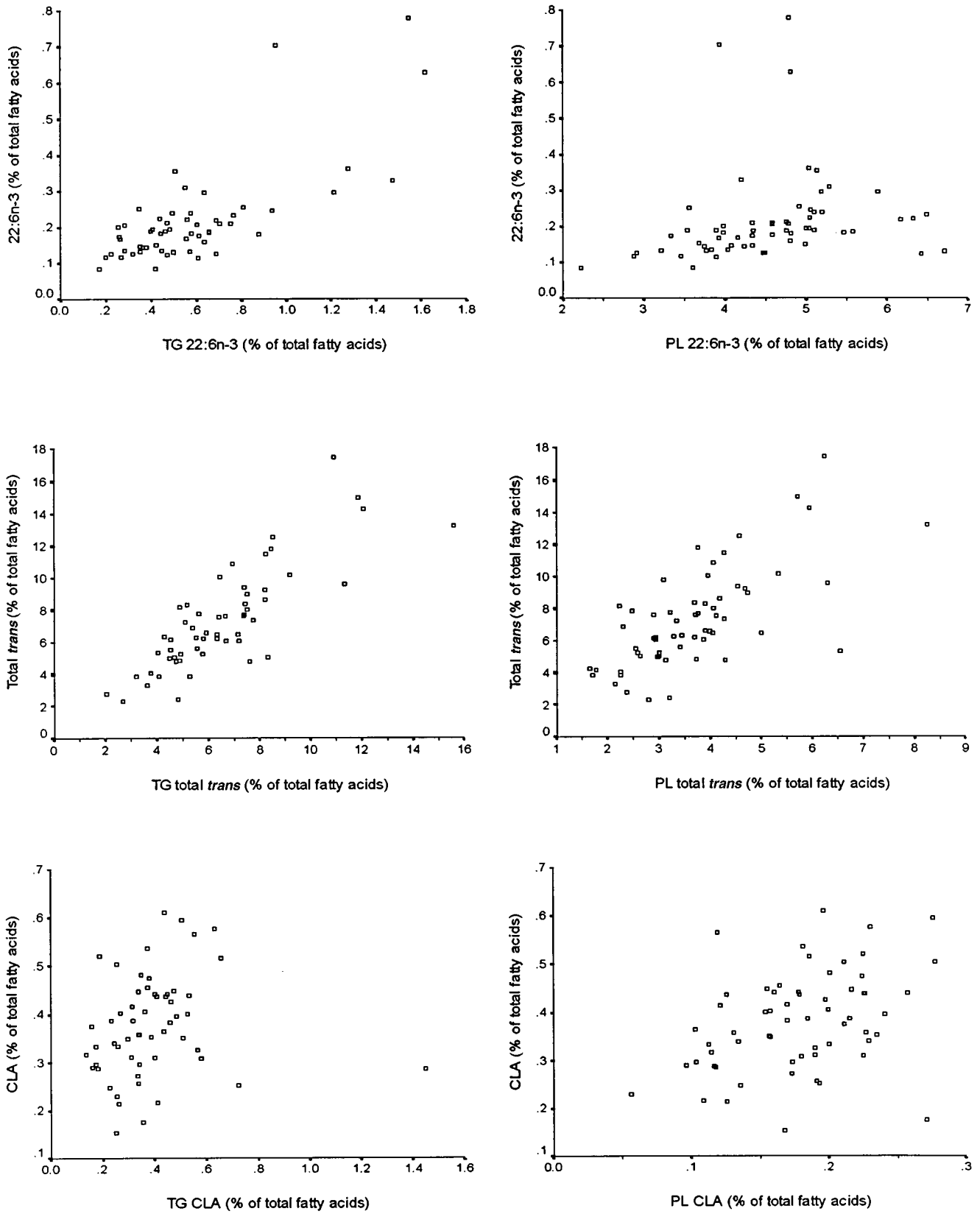


FIGURE 1. (Continued)



TABLE 4

Major sources of fat and *trans* fatty acids in the diets of 21 breast-feeding women in Canada¹

	Fat	<i>trans</i> Fatty acids
	g/d	
Total from all foods	88.74 ± 8.40 ²	6.87 ± 1.07 ³
Bakery products	7.62 ± 2.07	1.51 ± 1.54
Snacks	4.87 ± 2.80	0.99 ± 0.89
Meat	15.37 ± 3.25	0.87 ± 0.18
Fast foods	6.29 ± 1.72	0.76 ± 0.25
Margarines and shortening	4.19 ± 2.39	0.76 ± 1.15
Breads and cereals	3.61 ± 0.69	0.72 ± 0.63
Dairy	24.61 ± 5.21	0.66 ± 0.14
Beverages	0.99 ± 0.29	0.35 ± 0.59
Confectionary	4.50 ± 1.98	0.19 ± 0.31
Nuts and nut products	1.52 ± 0.60	0.03 ± 0.07
Salad and cooking oils	9.50 ± 2.37	0.03 ± 0.06
Fish and seafood	0.83 ± 0.33	—
Eggs	2.59 ± 1.33	—
Fruit and vegetables	1.98 ± 0.33	—

¹ $\bar{x} \pm \text{SEM}$, based on analysis of 3-d dietary records; energy intake was 10496 ± 597 kJ/d. Breads and cereals included breads, rolls, buns, and breakfast cereals; bakery products included cakes, cookies, pies, muffins, and scones; snacks included potato and corn chips, pretzels, and crackers; fast foods included prepared meal items and convenience foods; beverages included chocolate, cocoa, and flavored drinks; nuts and nut products included nuts and nut butters; salad and cooking oils included salad dressings and mayonnaise.

²Represents 31.81 ± 3.01% of energy.


³Represents 2.46 ± 0.38% of energy.

or plasma phospholipids or triacylglycerols of breast-fed infants. Chen et al (25) also found no differences in the percentages of 20:4n-6 or 22:6n-3 in human milk samples grouped as milks with low (<4%), medium, or high (>10%) percentages of *trans* fatty acids. In our study, the percentages of 20:4n-6 and 22:6n-3, but not of *trans* fatty acids, in milk were significantly related to the percentages of 20:4n-6 and 22:6n-3, respectively, in the infants' plasma lipids. It may be that intakes of preformed 20:4n-6 and 22:6n-3 are a more important determinant of milk and blood lipid 20:4n-6 and 22:6n-3 than are any potential effects of inhibition of 18:2n-6 and 18:3n-3 desaturation by *trans* fatty acids. Some, but not all, studies have reported a relation between blood lipid 20:4n-6 and 22:6n-3 concentrations of young infants and measures of growth and visual development, respectively (19, 20). Whether the wide ranges of concentrations of 20:4n-6 (8.1–15.8%) and 22:6n-3 (2.2–8.0%) in the plasma phospholipids of breast-fed infants have any physiologic significance regarding growth or development is not known. However, because 20:4n-6 and 22:6n-3 are found in animal but not plant foods, mothers with higher 20:4n-6 and 22:6n-3 concentrations in their milk are likely to have intakes of animal, fish, and egg protein that are different from those of women with lower milk 20:4n-6 and 22:6n-3 concentrations. This suggests that a detailed analysis of nutrient intakes of a larger group of women than our sample is needed to investigate any potential relations of maternal diet to infant growth and development.

Our results also showed a relation between CLA concentrations in milk and in the plasma lipids of breast-fed infants. CLAs

are positional and geometric isomers of 18:2n-6 that occur naturally in several foods, particularly dairy products and beef, and that appear to have biological activity (30, 31). In contrast with other *trans* fatty acids, CLAs were preferentially accumulated, by ≥2-fold, in the infants' plasma phospholipids rather than in triacylglycerols. Animal studies have also suggested possible tissue specificity for CLA (32). Whether CLAs have any physiologic effects on breast-fed infants is not yet known.

Craig-Schmidt et al (16) found that the concentration of *trans* 18:1 in milk increased from 1.8% to 6.5% of total fatty acids in 8 women when *trans* 18:1 in the diet increased from 1.0% to 11.8% of fatty acids. Using the equation generated in that study (16) to describe the relation between the percentage of *trans* 18:1 in the milk and in the diet, Chen et al (25) estimated daily *trans* fatty acid intakes of 7.7%, 3.9%, and 1.1% of total energy (equivalent to 20.3, 10.1, and 3.0 g, respectively) for Canadian women with high, medium, and low concentrations of *trans* fatty acids in their milk. Our analysis of 3-d diet records estimated a mean (±SEM) *trans* fatty acid intake of 6.9 ± 1.1 g/d (range: 1.3–10.9 g/d), representing ≈2.5% of energy intake and 7.7% of total fat intake. The finding that the mean (±SEM) percentage of *trans* fatty acids in milk (7.1 ± 0.3%) was similar to that calculated for the diet suggests that the estimated value of 6.9 g *trans* fatty acids/d is a reasonable estimation of the *trans* fatty acid intake of the women in this study. The major food sources of *trans* fatty acids for the women in our study were bakery products, snacks, and fast foods, with margarine and shortening contributing only ≈11% of the mean daily *trans* fatty acid intake (0.76 g). Similarly, data for the United States suggest that breads, cakes, cookies, snacks, and fried foods are likely to be major, although variable, sources of dietary *trans* fatty acids (8).

Numerous studies have shown that human milk fatty acids, particularly monounsaturated, n-6, n-3, and *trans* fatty acids, change in response to changes in maternal dietary intake (15–17). Concerns about dietary fat and health or lifestyle issues that alter maternal food choices may therefore influence the quality of fatty acid nutrition of breast-fed infants. It is particularly important to note that our results identified baked and prepared foods and breads as major sources of *trans* fatty acids, providing ≈50% of the total *trans* fatty acid intake but <20% of the total fat intake. The lack of labeling regarding fat composition and the possibility that many of these foods may be perceived as healthy in the context of lower-fat diets warrant consideration. 

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