Evidence That Palmitic Acid Is Absorbed as *sn*-2 Monoacylglycerol from Human Milk by Breast-Fed Infants

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Milk fatty acids consist of about 20–25% palmitic acid (16:0), with about 70% of 16:0 esterified to the *sn*-2 position of the milk triacylglycerols. Hydrolysis of dietary triacylglycerols by endogenous lipases produces *sn*-2 monoacylglycerols and free fatty acids, which are absorbed, reesterified, and then secreted into plasma. Unesterified 16:0 is not well absorbed and readily forms soaps with calcium in the intestine. The positioning of 16:0 at the *sn*-2 position of milk triacylglycerols could explain the high coefficient of absorption of milk fat. However, the milk lipase, bile salt-stimulated lipase, has been suggested to complete the hydrolysis of milk fat to free fatty acids and glycerol. These studies determined whether 16:0 is absorbed from human milk as *sn*-2 monopalmitin by comparison of the plasma triacylglycerol total and *sn*-2 position fatty acid composition between breast-fed and formula-fed term gestation infants. The human milk and formula had 21.0 and 22.3% of 16:0, respectively, with 54.2 and 4.8% 16:0 in the fatty acids esterified to the 2 position. The plasma triacylglycerol total fatty acids had 26.0 ± 0.6 and 26.2 ± 0.6% of 16:0, and the *sn*-2 position fatty acids had 23.3 ± 3.3 and 7.4 ± 0.7% of 16:0 in the three-month-old exclusively breast-fed (n = 17) and formula-fed (n = 18) infants, respectively. Marked differences were found in the plasma total and the 2 position phospholipid percentage of 20:4ω6, i.e., 11.6 ± 0.3 and 6.9 ± 0.6 (total), 17.7 ± 1.4 and 9.7 ± 0.6 (*sn*-2 position) and percentage of 22:6ω3, 4.6 ± 0.3 and 2.1 ± 0.3 (total), 5.6 ± 0.6 and 2.0 ± 0.2 (*sn*-2 position) for the breast-fed and formula-fed infants, respectively. These studies provide convincing evidence that 16:0 is absorbed from human milk as *sn*-2 monoacyl-glycerol. The metabolic significance of the differences in positional distribution of fatty acids in the plasma lipids of breast-fed and formula-fed infants is not known.

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Palmitic acid (16:0) usually represents 20–25% of human milk fatty acids, and about 70% of 16:0 is esterified to the *sn*-2 position in the triacylglycerols (1–3). The reason for the preferential esterification of 16:0 to the 2 position of glycerol during triacylglycerol synthesis in the mammary gland, and any potential physiological significance of this to infant nutrition, is not yet clear. It is known that the products of gastric and pancreatic colipase-dependent lipase hydrolysis of dietary fat are free fatty acids, released from the *sn*-1,3 positions and *sn*-2 monoacylglycerols (4). The efficiency of absorption of unesterified 16:0 by infants is relatively low, only about 63% compared to over 90% for the unsaturated fatty acids oleic acid (18:1ω9) and linoleic acid (18:2ω6) (5). This is believed to be due in part to a melting point above body temperature (63°) and a tendency for rapid formation of hydrated fatty acid soaps at the pH of the intestine (4). Consistent with this, studies have been reported that showed that fat absorption is higher in infants fed fats with 16:0 esterified to the triacylglycerol *sn*-2 rather than the *sn*-1,3 positions (6–8). This supports the hypothesis that the high efficiency of human milk fat absorption may be the result of the specific positioning of milk 16:0 at the *sn*-2 position of the triacylglycerol moiety (8).

The significance of *sn*-2 monoacylglycerol absorption in infants fed fresh human milk is, however, controversial because of the hypothesized role of the milk lipase, bile salt-stimulated lipase, in milk fat digestion. This enzyme is synthesized in the mammary gland, survives the acid environment of the stomach, and, at least *in vitro*, is able to complete the hydrolysis of milk triacylglycerols to form glycerol and free fatty acids (9–13). Additional hydrolysis of the products of endogenous lipase digestion of milk fat by bile salt-stimulated lipase has been suggested to explain the high efficiency of absorption of human milk (9–13). Hydrolysis to release 16:0 from *sn*-2 monoacylglycerols formed by endogenous lipase activity, however, does not seem consistent with the specific positioning of 16:0 at the *sn*-2 position of human milk triacylglycerols, the low coefficient of absorption of unesterified 16:0, or the high coefficient of absorption of human milk fat in infants.

In contrast to human milk, 16:0 in vegetable oils and nonmilk-derived animal fats, other than lard, is predominantly esterified to the triacylglycerol *sn*-1,3 positions, and unsaturated fatty acids occupy the *sn*-2 position (2). Since endogenous lipases do not hydrolyze the *sn*-2 ester bond of dietary fats (4,9), it is reasonable to assume that infants fed formula will absorb *sn*-2 monoacylglycerols containing unsaturated fatty acids. The composition of monoacylglycerols absorbed from the intestinal lumen is important to the fatty acid distribution of circulating lipids because about 70% of the fatty acids absorbed as *sn*-2 monoacylglycerols are conserved in the original position during reesterification to form triacylglycerols in the intestinal cells (4). Several studies have shown that the rate of hydrolysis and composition of remnant particles formed by lipoprotein and hepatic lipase is influenced by the plasma triacylglycerol fatty acid composition and positional distribution (4,14,15). It is possible, therefore, that the distribution of fatty acids in milk or formula triacylglycerols could have important metabolic effects beyond that on fat absorption. As a first step in understanding the metabolic significance of the fatty acid distribution in milk fat, the present studies sought to determine if *sn*-2 monoacylglycerol containing 16:0 is absorbed from human milk. This was done by the comparing of the composition of total and *sn*-2 position fatty acids in plasma lipids between breast-fed infants and infants fed formula containing

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Abbreviation: LCAT, lecithin:cholesterol acyltransferase.

similar amounts of 16:0 to milk but predominantly esterified to the formula fat *sn*-1,3 positions.

MATERIALS AND METHODS

Subjects and methods. Infants were eligible for this study if they were full-term (>37 wk gestation at birth), appropriate weight for gestational age, and if the mother had chosen to exclusively breast-feed or feed formula for at least 3 mon. The infants were enrolled into the study by 14 d of age following written, informed parental consent prior to 14 d of age. Infants assigned to the formula group were fed a ready-to-feed liquid formula (Enfalac; Mead Johnson Nutritionals, Evansville, IN) containing amounts of 16:0 similar to those in human milk (Table 1). Body weight, length, head circumference and skin-fold thickness (subscapular, abdominal and triceps) were recorded for all infants at 14 d and at 1,2 and 3 mon of age. Breast-feeding mothers provided a mid-feed sample of breast milk at 14 d and 3 mon *postpartum*. Blood samples were obtained by venipuncture from each infant at 3 mon (90 ± 4 d) of age during an out-patient visit to the BC's Children's Hospital. Infants were not fasted, and had been fed between 1 and 3 h prior to blood sampling. The protocol and procedures involving the infants were approved by the University of British Columbia Screening Committee for Research Involving Human Subjects, and by the Research Screening Committee of the BC's Children's Hospital.

Following collection, blood samples were immediately placed on ice and transferred to the research laboratory no more than 15 min after collection. Plasma was separated by centrifugation, total lipids were extracted, and triacylglycerols, phospholipids and cholesteryl esters were separated by thin-layer chromatography (16,17). Analysis of the composition of fatty acids at the *sn*-2 position of the plasma phospholipids and triacylglycerols, and of the formula and milk fat was based on the methods published by Kuksis (18) and Christie (19), as described elsewhere (17). The enzymatic hydrolysis utilized pig pancreatic lipase (EC 3.1.1.3, type II) for the milk and formula fat and plasma triacylglycerols, and phospholipase A_2 (EC 3.1.1.4) (Sigma Chemical Co., St. Louis, MO) for plasma phospholipids. After hydrolysis, 17:0 monoacylglycerol was added as an internal standard, the sample was extracted, and the monoacylglycerols separated by thin-layer chromatography. Monoacylglycerol recovery and the composition of the fatty acids in the *sn*-2 position of the milk and formula diet, and in the plasma lipid fractions was determined by fatty acid methyl ester analysis on the monoacylglycerol fraction. The fatty acid compositions of the milk and formula fat were analyzed by the method of Lepage and Roy (20) without solvent extraction. Fatty acid methyl esters were analyzed using a Varian 3400 gas–liquid chromatograph equipped with an RTx 2330 capillary column, 30 m x 25 mm i.d. (Restek Corporation, Bellefonte, PA) and a Varian Star data system (Varian Canada Inc., Georgetown, Ontario, Canada) as described elsewhere (17). The flow rate of the carrier gas (helium) was 1 mL/min; the injector and flame-ionization detector were maintained at 240 and 260°C, respectively. The samples were injected with the column oven at 80°C. The temperature of the oven was programmed to increase to 170°C at 10°C/min after 2 min, then increase to 195°C at 20°C/min after 25 min, and to increase to 245°C at 20°C/min after 18 min. The fatty acid methyl esters were identified by comparison of their retention times with those of authentic standards.

Statistical analysis. Comparisons of results to determine the effect of breast-feeding vs. formula-feeding on the fatty acid compositions of the infants' plasma lipids were conducted using Student's *t*-test with a $P < 0.05$ as statistically significant. All calculations were performed using the GLM procedure in the Number Cruncher Statistical System, version 5.01 (Kaysville, UT).

TABLE 1

Composition of Human Milk and Infant Formula Fat Total and *sn*-2 Position Fatty Acids (wt%)[a]

Fatty acid (wt%)	Human milk		Formula	
	Total	*sn*-2 Position	Total	*sn*-2 Position
12:0	4.1 ± 0.4	2.5 ± 0.4	8.9	4.5
14:0	5.5 ± 0.4	6.2 ± 0.8	4.7	1.0
16:0	21.0 ± 0.5	54.2 ± 1.5	22.3	4.8
16:1	3.1 ± 0.2	3.5 ± 0.3	0.2	0.2
18:0	7.1 ± 0.3	2.9 ± 0.4	5.1	1.3
18:1	40.2 ± 0.7	17.1 ± 0.8	37.1	58.8
18:2ω6	13.4 ± 0.8	8.1 ± 0.7	17.9	27.1
18:3ω3	1.5 ± 0.1	0.9 ± 0.1	2.1	1.8
20:2ω6	0.4 ± 0.0	0.1 ± 0.0	nd	nd
20:3ω6	0.4 ± 0.0	0.2 ± 0.0	nd	nd
20:4ω6	0.5 ± 0.0	0.7 ± 0.1	nd	nd
20:5ω3	0.1 ± 0.0	0.1 ± 0.0	nd	nd
22:4ω6	0.1 ± 0.0	0.2 ± 0.0	nd	nd
22:5ω3	0.2 ± 0.0	0.3 ± 0.0	nd	nd
22:6ω3	0.2 ± 0.0	0.4 ± 0.0	nd	nd

[a]Values given are percentage of total fatty acids and for human milk represent means ± SEM (range) of mid-feed milk collected when the infants were 3 months old. Values for formula are the average of three separate analyses; <0.1 indicates value >0.00 and <0.10%; nd, not detected.

RESULTS

Formula and milk triacylglycerol composition and fatty acid distribution. The fatty acid composition of the human milk samples was similar to that reported in other recent studies (2) with mean levels of 21.0% 16:0, 13.3% 18:2ω6, 0.5% 20:4ω6 and 0.2% 22:6ω3 (Table 1). The fatty acids at the *sn*-2 position of the milk fat had 54.2% 16:0, indicating that over 80% of the milk total 16:0 was esterified to the *sn*-2 position. This result is consistent with the value of 51.2% 16:0 in fatty acids at the *sn*-2 position of mature human milk triacylglycerols reported by Martin *et al.* (3). In contrast to 16:0, only about 14% of the total 18:1 and 20% of the total 18:2ω6 in the milk fat was esterified to the *sn*-2 position (Table 1). The longer chain ω6 and ω3 fatty acids, 20:4ω6 and 22:6ω3, were approximately equally distributed between the *sn*-2 and the *sn*-1/-3 positions, with about 46%

of the 20:4ω6 and 66% of the 22:6ω3 being recovered in the *sn*-2 monoacylglycerols (Table 1). Martin *et al.* (3) used chemical degradation to show that about 49% of the 20:4ω6 and 66% of the 22:6ω3 was in the *sn*-2 position, and about 45% of the 20:4ω6 and 33% of the 22:6ω3 was esterified to the *sn*-3 position of human milk triacylglycerols. The infant formula contained an amount of 16:0 (22.3%) similar to that in human milk. The formula, however, contained predominantly 18:1 and 18:2ω6, and had only 4.8% of 16:0 in the fatty acids esterified to the 2 position of the formula fat.

Total and sn-2 *fatty acid composition of plasma triacylglycerols from breast-fed infants and infants fed formula.* These studies provide unambiguous evidence that 16:0 esterified to the *sn*-2 position of human milk is absorbed intact by human infants and re-esterified to triacylglycerols for secretion into plasma (Table 2). The triacylglycerol total fatty acids of the breast-fed infants had significantly higher levels of 14:0, 16:1, 18:0, 20:4ω6, 20:5ω3 and 22:5ω6 and lower levels of 18:2ω6 than the formula-fed infants. The significantly higher percentage of 16:0 in the triacylglycerol *sn*-2 position fatty acids of the three-month-old breast-fed compared to formula-fed infants (23.3 ± 3.3 and 7.4 ± 0.7% 16:0, respectively) was accompanied by significantly lower levels of 18:1 and 18:2ω6. The formula-fed infants also had significantly lower levels of 16:1, 20:3ω6, 20:4ω6, 20:5ω3, 22:5ω3 and 22:6ω3 in their plasma triacylglycerol *sn*-2 position fatty acids than did the breast-fed infants.

Total and sn-2 *fatty acid composition of plasma phospholipids from breast-fed infants and infants fed formula.* The plasma phospholipid total fatty acids of the breast-fed infants had significantly lower levels of 16:0 and 18:2ω6, but higher levels of 18:0, 20:2ω6, 20:3ω6, 20:4ω6, 20:5ω3, 22:4ω6, 22:5ω3 and 22:6ω3 than those of infants who were fed formula (Table 3). Similarly, the phospholipid *sn*-2 position fatty acids of the breast-fed infants had significantly lower levels of 18:2ω6 and 18:3ω3 and higher 20:4ω6, 20:5ω3, 22:5ω3 and 22:6ω3 than those of the formula-fed infants.

Fatty acid composition of plasma cholesteryl esters from breast-fed infants and infants fed formula. The major difference in the plasma cholesteryl ester fatty acid composition due to feeding formula rather than breast feeding was in the percentage of 20:4ω6 and 22:6ω3. Levels of 20:4ω6 and 22:6ω3 in the plasma cholesteryl esters of the infants who were fed formula were approximately one-third of the levels in the infants who were breast-fed. The cholesteryl ester percentages of 14:0, 16:0, 16:1 and 20:5ω3 were also significantly lower, whereas the percentage of 18:2ω6 and 18:3ω3 were significantly higher in the formula-fed than breast-fed infants.

TABLE 2

Composition of Plasma Triacylglycerol Total and *sn*-2 Position Fatty Acids in Three-Month-Old Breast-Fed and Formula-Fed Gestation Infants[a]

Fatty acid (wt%)	Triacylglycerol total		*sn*-2 Position	
	Breast-fed	Formula-fed	Breast-fed	Formula-fed
12:0	0.5 ± 0.1	1.0 ± 0.2	0.2 ± 0.2	0.5 ± 0.1
14:0	2.9 ± 0.3	2.2 ± 0.2[b]	1.8 ± 0.8	0.7 ± 0.2
16:0	26.0 ± 0.6	26.2 ± 0.6	23.3 ± 3.3	7.4 ± 0.7[c]
16:1	2.7 ± 0.1	1.2 ± 0.0[b]	2.6 ± 0.3	1.3 ± 0.2[c]
18:0	6.6 ± 0.3	5.2 ± 0.2[b]	4.4 ± 0.5	6.0 ± 1.0
18:1	44.0 ± 0.6	42.7 ± 0.6	41.8 ± 2.5	53.3 ± 1.6[c]
18:2ω6	12.8 ± 0.8	17.7 ± 0.4[b]	19.5 ± 1.7	27.4 ± 0.6[c]
18:3ω3	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
20:2ω6	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
20:3ω6	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.0[c]
20:4ω6	0.8 ± 0.1	0.3 ± 0.0[b]	2.0 ± 0.3	0.5 ± 0.1[c]
20:5ω3	0.1 ± 0.0	0.0 ± 0.0[b]	0.1 ± 0.0	0.0 ± 0.0[c]
22:4ω6	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
22:5ω6	0.1 ± 0.0	0.0 ± 0.0[b]	0.0 ± 0.0	0.0 ± 0.0
22:5ω3	0.2 ± 0.0	0.2 ± 0.1	0.7 ± 0.2	0.1 ± 0.0[c]
22:6ω3	0.3 ± 0.0	0.2 ± 0.0	0.9 ± 0.1	0.1 ± 0.0[c]

[a]Values given are means ± SEM for seventeen breast-fed and eighteen formula-fed infants.
[b,c]Value for total or *sn*-2 fatty acids, respectively, significantly different from respective values for breast-fed infants; $P < 0.05$.

TABLE 3

Composition of Plasma Phospholipid Total and *sn*-2 Position Fatty Acids (wt%) in Infants Fed Human Milk or Formula[a]

Fatty acid (wt%)	Phospholipid total		*sn*-2 Position	
	Breast-fed	Formula-fed	Breast-fed	Formula-fed
12:0	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.2	0.3 ± 0.1
14:0	0.4 ± 0.1	0.4 ± 0.0	1.0 ± 0.2	0.8 ± 0.1
16:0	26.1 ± 0.6	28.4 ± 0.6[b]	13.4 ± 1.5	12.4 ± 1.0
16:1	0.5 ± 0.1	0.4 ± 0.0	0.8 ± 0.1	0.7 ± 0.1
18:0	18.4 ± 0.4	17.7 ± 0.2[b]	10.8 ± 1.3	9.5 ± 0.7
18:1	12.5 ± 0.3	12.6 ± 0.2	14.4 ± 0.6	15.3 ± 0.3
18:2ω6	19.8 ± 0.7	26.4 ± 1.0	25.0 ± 1.4	40.2 ± 1.6[c]
18:3ω3	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0[c]
20:2ω6	0.4 ± 0.0	0.3 ± 0.0[b]	0.5 ± 0.0	0.4 ± 0.0
20:3ω6	2.8 ± 0.1	2.3 ± 0.1[b]	4.5 ± 0.3	3.6 ± 0.1[c]
20:4ω6	11.6 ± 0.3	6.9 ± 0.6[b]	17.7 ± 1.4	9.7 ± 0.6[c]
20:5ω3	0.4 ± 0.0	0.2 ± 0.0[b]	0.6 ± 0.1	0.2 ± 0.1[c]
22:4ω6	0.4 ± 0.0	0.3 ± 0.0[b]	0.8 ± 0.0	0.9 ± 0.1
22:5ω6	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.1
22:5ω3	1.0 ± 0.0	0.5 ± 0.1[b]	1.5 ± 0.1	0.6 ± 0.0[c]
22:6ω3	4.6 ± 0.3	2.1 ± 0.3[b]	5.6 ± 0.6	2.0 ± 0.2[c]

[a]Values given are means ± SEM for seventeen breast-fed and eighteen formula-fed infants.
[b,c]Value for total or *sn*-2 fatty acids, respectively, significantly different from respective infants; $P < 0.05$.

DISCUSSION

The results of these studies provide convincing evidence that 16:0 esterified to the *sn*-2 position of human milk is conserved throughout digestion and absorption, and the conversion to triacylglycerols in the enterocyte, and then secreted into plasma as lipoprotein triacylglycerols. *In vitro* studies have shown that the milk lipase, bile salt-stimulated lipase, can complete the digestion of *sn*-2 monoacylglycerols formed by gastric acid colipase-dependent lipase hydrolysis of triacylglycerols to give unesterified fatty acids and glycerol (9,13). Information

on the extent of hydrolysis of *sn*-2 monoacylglycerols with 16:0 *in vivo* in young infants has not been published. Strong circumstantial evidence that bile salt-stimulated lipase does not quantitatively hydrolyze 16:0 from the *sn*-2 position of human milk fat is provided by the results of these studies that show approximately 26% of 16:0 in fatty acids esterified to the plasma triacylglycerol *sn*-2 position of breast-fed infants compared to only about 7.4% 16:0 in infants fed a formula containing comparable amounts of 16:0 to the milk, but predominantly esterified to the glycerol *sn*-1,3 positions. These results are evidence in support of the hypothesis (8) that the preferential esterification of 16:0 to the *sn*-2 position of human milk triacylglycerols is one of the reasons for the high coefficient of absorption of human milk fat. Although the studies reported here indicate that bile salt-stimulated lipase does not quantitatively complete the hydrolysis of milk triacylglycerols to free fatty acids and glycerol in infants *in vivo*, other studies have suggested a possible role in the hydrolysis of milk long-chain ω6 and ω3 fatty acids. The positioning of a substantial proportion of the 20:4ω6 and 22:6ω3 at the *sn*-3 position in milk triacylglycerols (3) is believed to render these fatty acids relatively resistant to hydrolysis by pancreatic colipase-dependent lipase (21,22). Whether or not bile salt-stimulated lipase is important in the digestion of long-chain ω6 and ω3 fatty acids from human milk has still to be fully clarified.

The plasma phospholipid levels of 20:4ω6 and 22:6ω3 are known to be lower in infants fed formula than in infants fed human milk (23,24). The analyses reported here show that this difference involves substantially lower 20:4ω6 and 22:6ω3 levels, but much higher 18:2ω6 levels in the fatty acids esterified to the *sn*-2 position of plasma phospholipids in infants fed formula rather than breast-fed (mean ± SEM 18:2ω6, 40.2 ± 1.6 and 25.0 ± 1.4%, 20:4ω6, 9.7 ± 0.6 and 17.7 ± 1.4%; 22:6ω3, 2.0 ± 0.2 and 5.6 ± 0.6%; for the formula and breast-fed infants, respectively). The higher levels of 18:2ω6 at the *sn*-2 position of plasma phospholipids in the formula-fed compared to breast-fed infants seems to reflect the higher amount of 18:2ω6 at the 2 position of the formula compared to the human milk fat (27.1 and 8.1% 18:2ω6 for the formula and human milk, respectively), and not the small difference in 18:2ω6 between the formula and milk total fatty acids (17.7 and 12.8% 18:2ω6, respectively). Although recent studies have shown that phospholipid synthesis in intestinal cells may proceed *via* the monoacylglycerol pathway (25), it is not clear to what extent this occurs *in vivo* in young infants. It may also be of relevance that recent studies have shown that the incorporation of 20:4ω6 into rat lymph phospholipids was higher when 20:4ω6 was given with 18:1 and monopalmitin than when given with 18:2ω6 and monoolein (26). This may suggest that absorption of monopalmitin following hydrolysis of human milk fat could facilitate incorporation of 20:4ω6 into intestinal lipoprotein phospholipids of breast-fed infants. Future studies on the fatty acid composition and structure of intestinal chylomicron and very low density lipoprotein would provide more specific information on this.

In recent studies, higher levels of 16:0 were found in plasma cholesteryl esters of piglets fed milk or formula containing 16:0 esterified to the 2 position of the fat than in piglets fed formula with 16:0 predominantly esterified to the triacylglycerol 1,3 positions (17). A similar, but smaller difference was found in the present studies with infants; that is, the percentage of 16:0 was about 15% higher in the cholesteryl esters of the breast-fed than of the formula-fed infants. Neither the origin nor metabolic significance of the relatively high amounts of cholesteryl palmitate in plasma seems to be known. Recent studies have suggested that lecithin:cholesterol acyltransferase (LCAT) from human plasma preferentially uses the *sn*-1 acyl group from phosphatidylcholines when 20:4ω6 or 22:6ω3 is esterified to the *sn*-2 position (26). Since molecular species of phosphatidylcholines containing 20:4ω6 and 22:6ω3 in the *sn*-2 position have 16:0 and 18:0 in the *sn*-1 position, this could explain the origin of 16:0 in plasma cholesteryl esters. The higher proportions of 20:4ω6 and 22:6ω3 in the plasma cholesteryl esters of infants who are breast-fed rather than fed formula (Table 4), however, suggests LCAT is able to use these fatty acids as substrates and form cholesteryl esters. Whether or not cholesteryl esters are quantitatively important in the transfer of fatty acids to tissues, possibly *via* low density lipoprotein receptors or *via* high density lipoprotein transfer, and the significance of the fatty acid composition to cholesteryl ester metabolism is not known.

The studies reported here provide *in vivo* data to show that the distribution of saturated and unsaturated fatty acids in human milk and infant formula is a determinant of the fatty acid distribution of infant plasma triacylglycerols and phospholipids. The high amount of 16:0 in the *sn*-2 position of plasma triacylglycerols in breast-fed infants shows that 16:0 is absorbed as *sn*-2 mono-

TABLE 4

Composition of Plasma Cholesteryl Ester Fatty Acids (wt%) in Infants Fed Human Milk or Formula[a]

Fatty acid (wt%)	Breast-fed	Formula-fed
12:0	0.2 ± 0.0	0.2 ± 0.0
14:0	1.1 ± 0.0	0.8 ± 0.0[b]
16:0	17.2 ± 0.5	15.0 ± 0.6[b]
16:1	2.6 ± 0.1	1.9 ± 0.1[b]
18:0	3.1 ± 0.3	3.2 ± 0.2
18:1	25.4 ± 0.6	27.1 ± 0.7
18:2ω6	40.1 ± 0.7	45.8 ± 0.8[b]
18:3ω3	0.4 ± 0.0	0.5 ± 0.0[b]
20:2ω6	0.1 ± 0.0	0.1 ± 0.0
20:3ω6	0.7 ± 0.0	0.5 ± 0.1
20:4ω6	6.3 ± 0.3	2.3 ± 0.2[b]
20:5ω3	0.2 ± 0.0	0.1 ± 0.0[b]
22:4ω6	0.3 ± 0.1	0.5 ± 0.1
22:5ω6	0.0 ± 0.0	0.1 ± 0.0
22:5ω3	0.1 ± 0.0	0.1 ± 0.0
22:6ω3	0.6 ± 0.1	0.2 ± 0.0[b]

[a]Values given are means ± SEM for seventeen breast-fed and eighteen formula-fed infants.
[b]Values significantly different from respective values for breast-fed infants.

palmitin by infants fed human milk. In contrast, it is probable that 16:0 is absorbed predominantly as an unesterified fatty acid from conventional infant formula. It is likely that analyses of isolated chylomicrons will show higher amounts of 16:0 in the triacylglycerol *sn*-2 position than were found in plasma total triacylglycerols. Lipoprotein lipase is stereospecific and is known to hydrolyze triacylglycerols with saturated fatty acids at the *sn*-2 position more slowly than triacylglycerols with unsaturated fatty acids at the *sn*-2 position (4,14,15). Whether or not hydrolysis of plasma triacylglycerols proceeds at similar rates in infants fed formula and infants fed human milk is not known. Future work, possibly involving analysis of the complete positional distribution of fatty acids in triacylglycerols and phospholipids in separated lipoproteins may help elucidate the implications of human milk fat structure to fatty acid metabolism and tissue delivery in the infant.

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