Plasma lipoprotein fatty acids are altered by the positional distribution of fatty acids in infant formula triacylglycerols and human milk[1–3]

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ABSTRACT

Background: Triacylglycerol digestion involves hydrolysis of fatty acids esterified at the glycerol 1,3 positions by gastric and pancreatic lipase to produce 2-monoacylglycerols and unesterified fatty acids, which are then absorbed, reesterified to triacylglycerol, and secreted in chylomicrons. Palmitic acid (16:0) is predominantly esterified to the 2 position of human milk triacylglycerol but to the 1,3 positions in the oils used in infant formulas.

Objective: We aimed to determine whether the position of 16:0 in human milk and infant formula triacylglycerol influences the position of fatty acids in postprandial plasma chylomicron triacylglycerol.

Design: Full-term infants were fed formula with 25–27% 16:0 with either 39% of the 16:0 (synthesized triacylglycerol) or 6% of the 16:0 (standard formula) esterified at the triacylglycerol 2 position, or were breast-fed (23% 16:0, 81% at the triacylglycerol 2 position) from birth to 120 d of age. Chylomicron fatty acids and plasma lipids were assessed at 30 and 120 d of age.

Results: Infants fed the synthesized triacylglycerol formula, standard formula, or breast milk had 15.8%, 8.3%, and 28.0% 16:0 in the chylomicron triacylglycerol 2 position ($P < 0.05$). These results suggest that ≥50% of the dietary triacylglycerol 2-position 16:0 is conserved through digestion, absorption, and chylomicron triacylglycerol synthesis in breast-fed and formula-fed infants. Infants fed the synthesized triacylglycerol formula had significantly lower HDL-cholesterol and apolipoprotein A-I and higher apolipoprotein B concentrations than infants fed the standard formula.

Conclusion: Dietary triacylglycerol fatty acid distribution may alter lipoprotein metabolism in young infants. *Am J Clin Nutr* 1999;70:62–9.

KEY WORDS Infant formula, palmitic acid, 16:0, triacylglycerol structure, fatty acids, chylomicron, high-density lipoprotein, apolipoprotein, milk

INTRODUCTION

The normal pathway of dietary triacylglycerol digestion involves hydrolysis by gastric and pancreatic lipase to form 2-monoacylglycerols and unesterified fatty acids (1). The 2-monoacylglycerols and fatty acids are absorbed, reassembled to triacylglycerol via the 2-monoacylglycerol or 3-glycerophosphate pathways, and secreted in chylomicrons. The 2-monoacylglycerol pathway, in which fatty acids are reesterified with little or no specificity to the glycerol 1 and 3 positions, predominates in the fed state (2, 3). Consequently, the distribution of fatty acids in the dietary triacylglycerol determines not only whether fatty acids are absorbed as 2-monoacylglycerols or fatty acids but also, in part, the subsequent position of fatty acids in the chylomicron triacylglycerol.

The fatty acids hydrolyzed from the 1 and 3 position of chylomicron triacylglycerol by lipoprotein lipase are taken up by adipose, muscle, and other tissues (1, 4). The fate of the monoacylglycerol products, however, is less clear but may involve further hydrolysis or uptake by the liver (4, 5). Several studies have provided evidence that the metabolism of chylomicrons is influenced by the distribution of the component fatty acids. Chylomicrons with palmitic acid (16:0) in the triacylglycerol 2 position may be transported faster into lymph (6), whereas plasma clearance appears to be slower for chylomicrons with saturated rather than unsaturated fatty acids in the triacylglycerol 2 position (7, 8). However, in vitro hydrolysis of chylomicrons with 1,3-dioleoyl-2-palmitoylglycerol, however, was not different from that of 1-palmitoyl-2,3-dioleoyl glycerol (9).

The distribution of fatty acids in human milk triacylglycerol is unusual in that palmitic acid is preferentially found at the 2 position, whereas unsaturated fatty acids such as oleic acid (18:1) and linoleic acid (18:2n−6) are preferentially esterified at the 1 and 3 positions (10). In contrast, vegetable oils and nonmilk fats used in infant formula have 16:0 esterified at the 1 and 3 positions of the triacylglycerol (11). Previous studies that found ≈23% and 7% 16:0 in the 2 position of plasma triacylglycerol from breast-fed and formula-fed infants, respectively (12), suggest that the composition of absorbed 2-monoacyglycerols and fatty acids may differ between infants fed human milk and those fed formula. However, it is possible that the higher 16:0 found in the plasma triacylglyc-

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[2] Supported by a grant from the Medical Research Council of Canada, Industry Award, with industry support from Ross Laboratories, Columbus, OH.

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TABLE 1
Major fatty acids in human milk and formula triacylglycerols and in position 2 of triacylglycerol[1]

	Total triacylglycerols			Position 2 of triacylglycerol		
Fatty acid	Human milk (n = 25)	Standard formula	Synthesized triacylglycerol formula	Human milk (n = 15)	Standard formula	Synthesized triacylglycerol formula
		% by wt			*% by wt*	
14:0	7.2 ± 0.3	3.2	3.7	7.5 ± 0.9	0.9	1.6
16:0	23.1 ± 0.6	27.2	24.8	56.4 ± 1.1	5.0	29.1
18:0	7.4 ± 0.2	5.3	5.2	1.9 ± 0.1	0.8	2.2
18:1	43.8 ± 1.8	41.1	39.5	15.3 ± 0.8	56.8	34.6
18:2n−6	14.2 ± 0.5	22.3	23.4	9.4 ± 0.9	32.7	28.4
20:2n−6	0.4 ± 0.0[2]	ND	ND	0.2 ± 0.0	ND	ND
20:3n−6	0.3 ± 0.0	ND	ND	0.2 ± 0.0	ND	ND
20:4n−6	0.5 ± 0.0	ND	ND	0.8 ± 0.1	0.1	0.1
22:4n−6	0.1 ± 0.0	ND	ND	0.3 ± 0.0	ND	ND
22:5n−6	0.1 ± 0.0	ND	ND	0.1 ± 0.0	ND	ND
18:3n−3	1.9 ± 0.1	2.2	2.6	1.3 ± 0.2	2.0	2.6
20:5n−3	0.1 ± 0.0	ND	ND	ND	ND	ND
22:5n−3	0.2 ± 0.0	ND	ND	0.3 ± 0.0	ND	ND
22:6n−3	0.3 ± 0.0	ND	ND	0.6 ± 0.1	ND	ND

[1] The human milk, standard formula, and synthesized triacylglycerol formula had 81%, 6%, and 39% of the total 16:0 esterified in position 2 of the triacylglycerol, respectively. Values for human milk are $\bar{x}$ ± SEM; values for formulas are the means of triplicate assays. ND, not detected.
[2] 0.0 indicates value ≥0.01 but <0.05.

erol 2 position in infants fed human milk than in infants fed a standard formula reflected differences in hepatic lipoprotein metabolism rather than effects of the positional distribution of fatty acids in the dietary triacylglycerol. Furthermore, information suggesting that the milk enzyme, bile salt–stimulated lipase, completes the hydrolysis of the milk triacylglycerol to fatty acids and glycerol (13) implies that the positional distribution of fatty acids in human milk triacylglycerol is not related to plasma chylomicron triacylglycerol fatty acids in breast-fed infants.

The objective of the current study was to determine the effects of feeding an infant formula with 16:0 in the triacylglycerol 2 position, a conventional formula with similar amounts of 16:0 esterified predominantly at the triacylglycerol 1 and 3 positions, and breast-feeding on plasma lipid concentrations and the distribution of fatty acids in plasma triacylglycerol-rich lipoproteins (predominantly chylomicrons). Results concerning the plasma, red blood cell, and other lipoproteins and lipid fatty acids will be the subject of a subsequent report.

SUBJECTS AND METHODS

Infants and diets

This was a prospective study of a group of infants randomly assigned to be fed 1 of 2 formulas and a group of nonrandomized infants who were breast-fed. Eligible study participants were full-term infants (37–42 wk gestation) with birth weights of 2500–4500 g, who were <72 h old and either breast-fed or formula fed exclusively. Infants were considered ineligible for the study if they were <37 or >42 wk gestation, weighed <2500 g or >4500 g, or had any suspected or known metabolic or physical problems that could interfere with feeding or normal metabolism. Mothers of breast-fed infants were requested to exclusively feed their own breast milk until the infant was ≥120 d old. Infants who were exclusively fed formula at 72 h of age were randomly assigned to be fed 1 of 2 formulas and then fed exclusively with the assigned formula until 120 d of age. The 2 formulas were prepared by Ross Laboratories, Columbus, OH, as ready-to-feed liquids and were identical in composition except for the fat blend (**Table 1**). One formula (standard formula) contained 37 g fat/L with 48% of total fat as palm olein oil, 26% as soybean oil, 14% as high-oleic acid sunflower oil, and 12% as coconut oil. The other formula had a fatty acid composition similar to the standard formula but was made with synthesized triacylglycerols (Betapol-2; Loders Croklaan, Wormerveer, Netherlands) as described in previous reports (14). The standard formula and the formula with synthesized triacylglycerol both contained (of total fatty acids) <25–27% 16:0; the human milk contained 23.1% 16:0 (Table 1). The standard formula had ≈5.0% 16:0 at the 2 position of the triacylglycerol, compared with ≈30% and 57% in the formula with synthesized triacylglycerol and the human milk, respectively.

The protocol and procedures for this study were approved by the University of British Columbia Screening Committee for Research Involving Human Subjects and by the Research Screening Committee of the BC Children's Hospital and the Ethics Committee of Surrey Memorial Hospital. Informed, written consent was obtained from a parent for each infant before participation.

Anthropometric measures

Body weight, length, and head circumference at birth were obtained from hospital records. Body weight, length, and head circumference were measured at 30, 60, 90, and 120 d of age, as in previous studies (15). Venous blood was collected from the infants at 30 and 120 d of age by a registered phlebotomist at the Outpatient Laboratory of the BC Children's Hospital. The infants were fed ≈2–3 h before collection of blood samples with no fasting. A sample of breast milk was provided by each of the breast-feeding mothers enrolled in the study on the day of blood collection.

Biochemical assessments and analyses

Concentrations of plasma total cholesterol and triacylglycerol and nonesterified fatty acids were determined by using enzymatic

kits from Diagnostic Chemicals (Charlottetown, Canada) and from Wako Chemicals (Richmond, VA), respectively. Plasma HDL-cholesterol concentration was determined after precipitation of the apolipoprotein (apo) B–containing lipoproteins with heparin-manganese chloride (16). Apo A-I and B were determined by immunoprecipitation using reagents from Incstar Corp, Stillwater, MN.

The triacylglycerol-rich lipoproteins, essentially chylomicrons, were separated from plasma collected in the fed state at a density (d) = 10.06 g/L within 1 h of blood collection by ultracentrifugation (Beckman TL100 Tabletop Ultracentrifuge; Beckman Instruments, Inc; Palo Alto, CA) at 436 000 × g and 15 °C, with a TLA 100.2 rotor (Beckman). The separation of chylomicrons, LDL, and HDL was confirmed by agarose gel electrophoresis (Corning Universal, Palo Alto, CA).

Plasma and chylomicron lipids were extracted and the phospholipids, cholesterol esters, and triacylglycerols were separated by using thin-layer chromatography (17). The phospholipids, triacylglycerols, and cholesterol ester fatty acids were then converted to their respective methyl esters and analyzed by gas-liquid chromatography using a Varian 3400 gas chromatograph (Varian Canada, Inc, Mississauga, Canada) equipped with a 30 m × 0.25 mm (internal diameter) column (SP2330; Supelco, Inc, Bellefonte, PA) and STAR software (version 4.0; Varian Canada, Inc) (12). The identity of fatty acids esterified at the 2 position of chylomicron triacylglycerols was analyzed by using porcine pancreatic lipase (EC 3.1.1.3 type 11, crude; Sigma Chemicals, St Louis) followed by thin-layer chromatography to separate monoacylglycerol and fatty acid products, then gas-liquid chromatography, as described previously (18). The amounts and identities of the milk and formula fatty acids were analyzed by using similar procedures.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the effect of the formula fed and infant age, and their interaction on the plasma fatty acids and lipid concentrations of the formula-fed infants. Significant differences in the plasma fatty acid composition and lipid concentrations between the formula-fed and breast-fed infants were also determined by using two-way ANOVA. When a significant difference was found, Scheffe's test for pair-wise comparisons was used to determine which treatment means differed. Differences were considered significant at $P < 0.05$. All calculations were performed by using the Statistical Package for the Social Sciences (SPSS) (version 7.5.1; SPSS Inc, Chicago). Values given in the tables and figures are means ± SEMs and are shown with the results of the statistical analyses for the effects of the diet fed. Because of the limited volume of blood that could be obtained, not all analyses could be done for all infants.

RESULTS

Characteristics of infants

Eighty-seven infants were enrolled in this study, with 47 randomly assigned to be fed 1 of the 2 formulas, and 40 enrolled nonrandomly as breast-fed infants (**Table 2**). Of the 47 infants enrolled as formula fed, 22 were fed the standard formula and 25 were fed the formula with synthesized triacylglycerol. Nineteen of the 22 infants fed the standard formula completed the study; 3 infants were withdrawn because of possible formula intolerance. Seventeen of the 25 infants randomly assigned to the formula with the synthesized triacylglycerol completed the study: 4 infants were withdrawn because of suspected formula intolerance, 2 because of suspected cow milk allergy, and 1 because of a doctor's decision that was unrelated to the formula. Eighteen of the 40 breast-fed infants were withdrawn: 6 because of formula supplementation, 6 because of difficulty in obtaining a blood sample, 4 as a result of the parents' decision not to continue in the study, 1 because the infant's family moved, and 1 because of missed study appointments. There were no significant differences in birth weight, birth length, gestational age, sex distribution, or Apgar scores among the groups of infants enrolled to be fed the formula with synthesized triacylglycerol or standard formula, or as breast-fed infants (Table 2). There was no follow-up with measures of growth or collection of blood samples for infants who were withdrawn from the study.

TABLE 2
Characteristics of infants randomly assigned to be fed a standard formula, formula with synthesized triacylglycerols, or breast-fed to 120 d of age[1]

	Standard formula (n = 22)	Synthesized triacylglycerol formula (n = 25)	Breast-fed (n = 40)
Birth weight (kg)	3.6 ± 0.4[2]	3.5 ± 0.5	3.5 ± 0.3
Birth length (cm)	51.1 ± 2.2	52.0 ± 2.5	51.7 ± 2.0
Gestational age (wk)	39.8 ± 1.2	39.5 ± 1.1	39.7 ± 1.1
Apgar score (5 min)	>9	>7	>7
Sex (M:F)	12:10	14:11	23:17
Ethnicity (white:nonwhite)[3]	16:6	13:12	32:8

[1] There were no significant differences among the groups.
[2] $\bar{x}$ ± SD.
[3] Of the nonwhite infants, there were 3, 6, and 3 Chinese and 2, 1, and 1 East Indian infants in the groups fed the standard formula, the synthesized triacylglycerol formula, and the breast-fed group, respectively. The other infants had parents of different ethnic backgrounds, and there was 1 infant of South American descent.

Growth

There were no significant differences in the weight, length, or head circumference between the infants fed the 2 formulas, or between the infants fed formula and those who were breast-fed at any age (**Table 3**). Similarly, there were no differences in weight gain either for the groups as a whole or when the results for the male and female infants were considered separately (data not shown). The weight gain for the male and female infants fed formula and the breast-fed infants in this study were similar to those reported by Guo et al (19) for normal, healthy, full-term infants.

Plasma lipids and apolipoproteins

The concentrations of cholesterol, triacylglycerol, fatty acids, and apo A-I and B in the plasma of the infants fed the formulas or breast-fed at 30 and 120 d of age are shown in **Table 4.** No significant diet-by-age interactions were found in the statistical analyses (**Table 5**). Infants fed the standard formula had significantly lower apo B concentrations than breast-fed infants and both groups of formula-fed infants had significantly lower plasma total cholesterol, but not triacylglycerol concentrations, than the breast-fed infants. Infants fed the formula with synthesized triacylglycerol also had significantly lower plasma HDL-cholesterol and apo A-I concentrations at both 30 and 120 d of age than infants fed the standard formula or who were breast-fed. Of note, the plasma apo B concentration of infants fed the syn-

TABLE 3
Weight, length, and head circumference of infants fed the standard formula, formula with synthesized triacylglycerols, or breast-fed to 120 d of age[1]

Measure and age	Standard formula	Synthesized triacylglycerol formula	Breast-fed
Weight (kg)			
7 d	3.6 ± 0.4 [21]	3.6 ± 0.5 [25]	3.6 ± 0.3 [34]
30 d	4.5 ± 0.4 [19]	4.6 ± 0.6 [21]	4.5 ± 0.4 [29]
60 d	5.5 ± 0.5 [19]	5.6 ± 0.7 [20]	5.5 ± 0.5 [26]
120 d	7.0 ± 0.6 [19]	7.1 ± 0.6 [19]	6.8 ± 0.6 [25]
Length (cm)			
7 d	51.7 ± 1.9	51.9 ± 1.9	51.9 ± 1.9
30 d	54.6 ± 1.7	54.7 ± 2.3	54.9 ± 1.4
60 d	58.6 ± 2.0	59.3 ± 2.2	59.4 ± 1.6
120 d	64.1 ± 1.7	64.4 ± 2.0	63.9 ± 1.8
Head circumference (cm)			
7 d	36.0 ± 1.2	35.8 ± 1.1	35.8 ± 0.9
30 d	38.1 ± 1.0	38.3 ± 1.2	38.0 ± 0.9
60 d	39.8 ± 1.0	39.9 ± 1.1	39.6 ± 0.8
120 d	42.4 ± 1.2	42.3 ± 1.0	42.0 ± 0.8

[1] $\bar{x}$ ± SD; number of infants for whom weight, length, and head circumference were obtained in brackets. There were no significant differences among the groups.

thesized triacylglycerol formula was significantly higher than that of infants fed the standard formula at both 30 and 120 d of age and was not different from that found in breast-fed infants.

Chylomicron triacylglycerol fatty acids

The composition of the chylomicron total triacylglycerol and the fatty acids at the triacylglycerol 2 position of the infants at 120 d of age are shown in **Table 6**. There were no significant differences in the fatty acid composition of the chylomicron triacylglycerol in the groups of formula-fed or breast-fed infants at 30 (data not shown) compared with 120 d of age. No significant differences in the effects of the diet at 30 and 120 d (diet-by-age interactions) were found (**Table 7**). No significant differences in the fatty acid composition of the chylomicron triacylglycerol were found between the infants fed the 2 formulas, except that the percentages of 18:3n−3 and 22:4n−6 were higher in the chylomicron triacylglycerol of infants fed the synthesized triacylglycerol formula ($P < 0.05$). Infants fed the standard formula and infants fed the formula with synthesized triacylglycerol had significantly higher percentages of 18:2n−6 in their chylomicron triacylglycerol than the breast-fed infants. This difference in 18:2n−6 probably reflects the higher amount of 18:2n−6 in the formulas than in the human milk (Table 1). Both groups of formula-fed infants had significantly lower percentages of 20:4n−6, 22:5n−6, 20:5n−3, and 22:6n−3 in their plasma chylomicron triacylglycerol than the breast-fed infants. Infants fed the standard formula had significantly lower percentages of 18:0 and 22:5n−3, whereas infants fed the synthesized triacylglycerol formula had a significantly lower percentage of 20:3n−6 in chylomicron triacylglycerol than the breast-fed infants. Of note, there were no significant differences in the percentages of 16:0 or 18:1 in the chylomicron triacylglycerol between the formula-fed and the breast-fed infants (Table 6).

The analyses of the fatty acids at the 2 position of the chylomicron triacylglycerol identified some differences between 30 and 120 d of age, but no significant differences in the effects of the diets at the different ages. The percentages of 16:0 and 18:0 were significantly lower, whereas amounts of 22:5n−3 were higher, at 120 compared with 30 d in both groups of infants fed formula (data not shown). The percentage of 20:4n−6 was lower at 120 than at 30 d for all of the infants. At 120 d of age, infants fed the formula with synthesized triacylglycerol had higher 16:0 and 22:6n−3 and lower 18:1 and 18:0 in their chylomicron triacylglycerol 2-position fatty acids than infants fed the standard

TABLE 4
Plasma lipid and apolipoprotein concentrations at 30 and 120 d of age in infants fed standard formula, formula with synthesized triacylglycerols, or breast-fed[1]

	Standard formula		Synthesized triacylglycerol formula		Breast-fed	
	30 d	120 d	30 d	120 d	30 d	120 d
Total cholesterol (mmol/L)	2.9 ± 0.1 [11][2]	3.0 ± 0.2 [11][2]	2.8 ± 0.1 [14][2]	3.0 ± 0.1 [15][2]	3.5 ± 0.2 [13]	4.1 ± 0.4 [10][3]
HDL cholesterol (mmol/L)	1.7 ± 0.1 [11]	1.6 ± 0.1 [12][3]	1.5 ± 0.1 [13][2,4]	1.2 ± 0.1 [12][2,3,4]	1.7 ± 0.1 [14]	1.4 ± 0.1 [10][3]
Triacylglycerol (mmol/L)	1.0 ± 0.1 [17]	1.4 ± 0.2 [17]	1.3 ± 0.2 [15]	1.4 ± 0.2 [15]	1.1 ± 0.2 [20]	1.3 ± 0.2 [19]
Nonesterified fatty acids (mmol/L)	0.2 ± 0.0 [15]	0.4 ± 0.0 [15][3]	0.3 ± 0.0 [17]	0.5 ± 0.1 [13][3]	0.3 ± 0.0 [15]	0.5 ± 0.1 [16][3]
Apolipoprotein A-I (mg/L)	136.4 ± 5.2 [10]	126.5 ± 3.8 [14][3]	118.4 ± 3.5 [14][2,4]	100.1 ± 5.6 [14][2,3,4]	136.4 ± 3.8 [13]	129.7 ± 6.1 [11][3]
Apolipoprotein B (mg/L)	32.1 ± 2.7 [10][2]	43.7 ± 3.7 [11][2,3]	45.1 ± 3.1 [10][3]	58.8 ± 5.2 [15][3,4]	48.2 ± 3.0 [14]	55.9 ± 4.6 [9][3]

[1] $\bar{x}$ ± SEM; number of infants for whom samples were analyzed in brackets; 0.0 indicates SEM values ≥0.01 but <0.05. There were no significant diet-by-age interactions.
[2] Significantly different from breast-fed infants, $P < 0.05$.
[3] Within the same feeding group, values are significantly different from 30 d, $P < 0.05$.
[4] Values for infants fed formula with synthesized triacylglycerol are significantly different from values for infants fed formula, $P < 0.05$.

TABLE 5
Association between plasma lipid and apolipoprotein concentrations and diet over time: main effect *P* values from two-way ANOVA

	Standard × synthesized triacylglycerol formula			Formula-fed × breast-fed		
	Diet	Age	Diet × age	Diet	Age	Diet × age
Total cholesterol	NS	NS	NS	<0.001	0.02	NS
HDL cholesterol	<0.001	<0.001	NS	<0.001	<0.001	NS
Triacylglycerol	NS	NS	NS	NS	NS	NS
Nonesterified fatty acids	NS	<0.001	NS	NS	<0.001	NS
Apolipoprotein A-I	<0.001	0.004	NS	<0.001	0.012	NS
Apolipoprotein B	0.002	0.005	NS	0.009	<0.001	NS

formula (Table 6). The higher percentage of 16:0 and lower percentage of 18:1 at the 2 position of the chylomicron triacylglycerol in infants fed the synthesized triacylglycerol formula reflect the differences in 16:0 and 18:1 in the triacylglycerol 2 position of the synthesized triacylglycerol and standard formulas. When compared with the breast-fed infants, infants fed the standard formula or the formula with synthesized triacylglycerol had a significantly higher percentage of 18:2n−6 but a lower percentage of 16:0, 18:0, 20:3n−6, 20:4n−6, 22:5n−3, and 22:6n−3 in their chylomicron triacylglycerol 2-position fatty acids. Of note, there was no difference in the percentage of 18:1 in the chylomicron triacylglycerol 2 position between the breast-fed infants and infants fed the formula with synthesized triacylglycerol. Infants fed the standard formula, in contrast, had a significantly higher percentage of 18:1 in the chylomicron triacylglycerol 2 position than the breast-fed infants.

Chylomicron phospholipid fatty acids

The fatty acid composition of the chylomicron phospholipids in the infants in all 3 feeding groups is shown in **Table 8**. There were no significant differences in the fatty acid composition of the chylomicron phospholipids between the infants fed the standard formula and those fed the formula with synthesized triacylglycerol. Both groups of formula-fed infants had significantly lower concentrations of 18:0, 20:3n−6, 20:4n−6, 20:5n−3, 22:5n−3, and 22:6n−3, and higher percentages of 18:1 and 18:2n−6 in their chylomicron phospholipids than did the breast-fed infants (Table 8). A significant interaction between diet and age was found for 18:2n−6, 20:4n−6, 20:5n−3, 22:5n−3, and 22:6n−3 (Table 7). Thus, the percentages of 18:2n−6 were significantly higher and those of 20:4n−6, 20:5n−3, 22:5n−3, and 22:6n−3 were significantly lower in the chylomicron phospholipids at 120 than at 30 d of age in the infants fed formula but not in infants who were breast-fed (data not shown). The percentage of 20:5n−3 increased between 30 and 120 d of age in the breast-fed infants. The percentages of 18:1 in the chylomicron phospholipid decreased from 30 to 120 d of age in all of the infants, irrespective of whether they were breast-fed or fed formula.

Chylomicron cholesterol ester fatty acids

There were no significant differences in the fatty acid composition of the chylomicron cholesterol esters at 30 compared with 120 d of age in the groups of infants fed formula (Table 7). In contrast, the percentage of 18:2n−6 increased and of 18:1 decreased in the chylomicron cholesterol esters of the breast-fed infants between 30 and 120 d of age. There were no significant differences in the chylomicron cholesterol ester fatty acids between infants fed the standard formula and those fed the formula with synthesized triacylglycerol (Table 8). The infants who were fed formula had significantly lower percentages of 20:3n−6, 20:4n−6, 20:5n−3, and 22:6n−3, and higher 18:2n−6 and 18:3n−3 in their cholesterol esters than the breast-fed infants.

DISCUSSION

The results of this study show that infants fed formula with 16:0 enriched at the triacylglycerol 2 position have higher amounts of 16:0 at the 2 position of plasma chylomicron triacylglycerol than infants fed formula with a similar total amount of 16:0, but with <5% 16:0 in the triacylglycerol 2 position. The results also confirm and extend the findings of a previous study that reported relatively high amounts (≈27% of total fatty acids) of 16:0 in the 2 position of plasma triacylglycerol of breast-fed infants (12). The study reported here found ≈40% of the 16:0 in the chylomicron triacylglycerol in the 2 position in the breast-fed infants. The findings in the study reported here of lower HDL cholesterol and apo A-I, but higher apo B in infants fed the synthesized triacylglycerol formula than in infants fed a standard formula are the first data to suggest that dietary triacylglycerol fatty acid distribution may influence lipoprotein metabolism in young infants.

The human milk analyzed in the study reported here had ≈56% 16:0 in the triacylglycerol 2 position. The plasma chylomicron triacylglycerol of the breast-fed infants, on the other hand, had ≈28% 16:0 in the triacylglycerol 2 position. These results suggest that ≈50% of the 2-monoacylglycerols with 16:0 liberated by endogenous lipase hydrolysis of the human milk triacylglycerol were absorbed and conserved through triacylglycerol reassembly. The enzyme bile salt–stimulated lipase may hydrolyze some of the human milk triacylglycerol to glycerol and fatty acids in breast-fed infants (13). However, a similar proportion of the dietary triacylglycerol 2-position 16:0 was conserved by the infants fed formula with synthesized triacylglycerol: 15.8 ± 0.4% 16:0 in the infant plasma chylomicron triacylglycerol 2 position compared with 29% 16:0 in the formula triacylglycerol 2 position. Studies in rats using in situ isolated intestine have suggested that ≈70% of triacylglycerol synthesis in the enterocyte in the fed state proceeds via the 2-monoacylglycerol pathway, with the remaining triacylglycerol synthesis occurring via the de novo 3-glycerophosphate pathway (3, 20). A recent study of adult men given a diet with 31% of energy from synthesized triacylglycerol, similar to the synthesized triacylglycerol used in the study reported here, also recovered ≈70% of the dietary 2 position 16:0 in the 2 position of chylomicron triacylglycerol (21). Thus, absorption of 2-monoacylglycerol from milk with 56% 16:0 at the 2 position should, theoretically, result in ≈39% 16:0 in the chylomicron triacylglycerol 2 position in the fed state.

TABLE 6
Major fatty acids of chylomicron triacylglycerols and triacylglycerol position-2 fatty acids at 120 d for infants fed a standard formula, formula with synthesized triacylglycerol, or breast-fed[1]

	Total triacylglycerol			Triacylglycerol position 2		
Fatty acid	Standard formula (n = 10)	Synthesized triacylglycerol formula (n = 11)	Breast-fed (n = 9)	Standard formula (n = 8)	Synthesized triacylglycerol formula (n = 12)	Breast-fed (n = 11)
		% by wt			*% by wt*	
16:0	23.2 ± 0.4	23.7 ± 0.7	23.3 ± 0.9	8.3 ± 0.4[2]	15.8 ± 0.4[2,3]	28.0 ± 0.8
18:0	5.8 ± 0.7[2]	6.8 ± 0.8	7.1 ± 0.5	1.6 ± 0.8[2]	1.2 ± 0.3[2,3]	1.9 ± 0.6
18:1	41.0 ± 1.4	38.3 ± 1.0	44.4 ± 1.5	52.6 ± 2.8[2]	44.7 ± 0.8[3]	46.5 ± 2.3
18:2n−6	23.2 ± 1.3[2]	23.4 ± 0.7[2]	15.2 ± 0.9	33.0 ± 1.9[2]	33.0 ± 0.4[2]	15.3 ± 1.9
20:2n−6	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
20:3n−6	0.2 ± 0.0	0.2 ± 0.0[2]	0.4 ± 0.1	0.1 ± 0.0[2]	0.1 ± 0.0[2]	0.5 ± 0.1
20:4n−6	0.5 ± 0.1[2]	0.8 ± 0.1[2]	1.1 ± 0.2	0.4 ± 0.1[2]	0.6 ± 0.1[2]	1.1 ± 0.1
22:4n−6	Trace	Trace[2,3]	0.1 ± 0.0	Trace	Trace	Trace
22:5n−6	Trace[2]	Trace[2]	0.2 ± 0.0	Trace	Trace	Trace
18:3n−3	1.4 ± 0.1	1.9 ± 0.1[2,3]	0.9 ± 0.1	1.4 ± 0.2	1.6 ± 0.1[2]	1.3 ± 0.1
20:5n−3	Trace[2]	Trace[2]	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
22:5n−3	0.1 ± 0.0[2]	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0[2]	0.2 ± 0.0[2]	0.3 ± 0.1
22:6n−3	0.1 ± 0.0[2]	Trace[2]	0.5 ± 0.1	0.1± 0.0[2]	0.1 ± 0.0[2,3]	0.3 ± 0.1

[1] $\bar{x}$ ± SEM; 0.0 indicates SEM values ≥0.01 but <0.05. Trace, amounts >0 but <0.05.
[2] Significantly different from breast-fed infants, $P < 0.05$.
[3] Values for infants fed formula with synthesized triacylglycerol are significantly different from those fed standard formula, $P < 0.05$.

One explanation for the results of this study, which show 28% 16:0 in the chylomicron triacylglycerol 2 position in the breast-fed infants, is that the 2-monoacylglycerol pathway accounts for ≈50% of enterocyte triacylglycerol synthesis in young infants. Alternatively, the triacylglycerol-rich lipoproteins collected from the infants at 2–3 h postfeeding may have some triacylglycerols of hepatic origin.

The results of the study reported here show higher amounts of apo B, but not cholesterol, in infants fed a formula with synthesized triacylglycerol than in infants fed a standard formula. Both groups of infants fed formula, however, had lower plasma cholesterol concentrations than the breast-fed infants. These results suggest that although the formula triacylglycerol fatty acid distribution may influence apo B metabolism, important differences in cholesterol metabolism remained between the breast-fed and formula-fed infants. Some of these differences may result from the absence in formula of cholesterol, long-chain n–6 and n–3 fatty acids, or some other bioactive components of human milk. The reason for the higher plasma apo B in infants fed the formula with synthesized triacylglycerol than in infants fed the standard formula is not known. Studies in rats, however, have shown that the uptake of plasma chylomicron remnants is slowed with saturated fatty acids in the triacylglycerol 2 position (7). Infants fed the formula with synthesized triacylglycerol or the standard formula had 15.8% and 8.3% 16:0 in the chylomicron triacylglycerol 2 position, respectively. Whether delayed removal of chylomicron remnants can explain the higher apo B, without a change in plasma total cholesterol, in infants fed the synthesized triacylglycerol formula compared with the standard formula may be worth considering.

The explanation for the lower plasma apo A-I and HDL cholesterol in infants fed the formula with synthesized triacylglycerol than in infants fed the standard formula in the study reported here is not known. Studies in men have reported a significant decrease in HDL cholesterol and apo A-I coincident with the peak in HDL triacylglycerol and chylomicron remnants (22). In the current study, infants fed the formula with synthesized triacylglycerol had significantly higher plasma apo B concentrations than infants fed the standard formula, but plasma triacylglycerol concentrations were not different and no significant relations between the plasma triacylglycerol and HDL cholesterol were found (data not shown). Differences in the number of remnant particles, chylomicron remnant metabolism, or effects of the dietary triacylglycerol fatty acid distribution on intestinal HDL synthesis may be causally related to the effects of the formula triacylglycerol fatty acid distribution on apo A-I and HDL cholesterol in formula-fed infants. Future studies might address this by more specific measures of the lipoprotein, apolipoprotein, and lipid concentrations and turnover.

The lower HDL-cholesterol concentrations in the infants fed the formula with synthesized triacylglycerol in the study reported

TABLE 7
Association between plasma chylomicron lipid fatty acids and diet over time: main effect P values from two-way ANOVA

	Standard × synthesized triacylglycerol formula			Formula-fed × breast-fed		
Fatty acid location	Diet	Age	Diet × age	Diet	Age	Diet × age
Triacylglycerol	<0.001	NS	NS	<0.001	NS	NS
Triacylglycerol, position 2	<0.001	0.005	NS	<0.001	0.012	<0.001
Phospholipid	NS	<0.001	NS	<0.001	<0.001	0.005
Cholesterol ester	NS	NS	NS	<0.001	0.036	NS

TABLE 8
Major fatty acids of chylomicron phospholipids and cholesterol esters of infants fed a standard formula, formula with synthesized triacylglycerols, or breast-fed to 120 d of age[1]

	Phospholipids			Cholesterol esters		
Fatty acids	Standard formula (n = 9)	Synthesized triacylglycerol formula (n = 10)	Breast-fed (n = 13)	Standard formula (n = 9)	Synthesized triacylglycerol formula (n = 12)	Breast-fed (n = 10)
		% by wt			*% by wt*	
16:0	27.7 ± 1.1	25.4 ± 0.4	25.8 ± 0.8	18.6 ± 1.6	18.0 ± 0.9	20.6 ± 1.0
18:0	22.8 ± 0.9	23.4 ± 1.0	26.9 ± 0.8[2]	8.4 ± 0.6	9.6 ± 1.2	9.1 ± 1.1
18:1	11.9 ± 0.5	11.5 ± 0.2	10.2 ± 0.3[2]	23.9 ± 0.9	21.3 ± 0.8	21.7 ± 1.1
18:2n−6	26.4 ± 0.8	27.1 ± 0.5	17.3 ± 0.6[2]	36.0 ± 2.2	38.1 ± 1.2	33.0 ± 2.1[2]
20:2n−6	0.6 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
20:3n−6	1.5 ± 0.2	1.7 ± 0.2	2.1 ± 0.3[2]	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.1[2]
20:4n−6	5.3 ± 0.3	5.3 ± 0.5	9.8 ± 0.5[2]	2.6 ± 0.3	2.5 ± 0.2	4.9 ± 0.6[2]
22:4n−6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	Trace	Trace	0.1 ± 0.0
22:5n−6	0.2 ± 0.3	0.2 ± 0.0	0.3 ± 0.1	Trace	Trace	Trace
18:3n−3	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.9 ± 0.0	0.5 ± 0.1[2]
20:5n−3	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1[2]	Trace	Trace[3]	0.2 ± 0.2[2]
22:5n−3	0.3 ± 0.1	0.4 ± 0.1	0.8 ± 0.1[2]	0.1 ± 0.1	Trace	0.1 ± 0.0
22:6n−3	1.1 ± 0.1	1.5 ± 0.2	3.9 ± 0.2[2]	0.1 ± 0.1	0.1 ± 0.0	0.4 ± 0.1[2]

[1] $\bar{x}$ ± SEM; 0.0 indicates SEM values ≥0.01 but <0.05. Trace, amounts >0 but <0.05.
[2] Significantly different from both formula-fed groups, $P < 0.05$.

here could also involve changes in lecithin-cholesterol acyltransferase activity (23). Reduced concentrations of apo A-I are associated with lower lecithin-cholesterol acyltransferase activity, decreased cholesterol esterification, and uptake of cholesterol in HDL (24). Furthermore, in vitro studies have reported lower lecithin-cholesterol acyltransferase activity for phospholipids with 16:0 rather than 18:2n−6 at the 2 position (25). In the current study, infants fed the formula with synthesized triacylglycerol had higher concentrations of 16:0 in the 2 position of HDL phospholipid than infants fed the standard formula (22.3 ± 2.1% and 17.2 ± 1.2% 16:0, respectively). Whether these differences in HDL phospholipid fatty acids are sufficient to influence lecithin-cholesterol acyltransferase activity in vivo is not known.

In contrast with the results of the study with infants reported here, studies in men fed a diet with 28% of energy from triacylglycerol with 54% or 6% 16:0 in the 2 position found no differences in HDL-cholesterol concentrations in fasting plasma (26). This discrepancy in results could be due to the substantially lower proportion of dietary energy from synthesized triacylglycerol consumed by the men (26) than by infants fed the formula with synthesized triacylglycerol (≈48% of energy from fat), or by analysis of plasma taken from the infants 2–3 h postprandial rather than after fasting.

Some information has been reported to suggest that the type of dietary saturated fatty acids can influence plasma n–6 and n–3 fatty acids in infants (27) and piglets (28). This suggests that the amount or pathway of absorption may influence fatty acid metabolism with secondary effects on the clearance, oxidation, or subsequent acylation or desaturation of dietary 18:2n−6 and 18:3n−3. The triacylglycerol fatty acid distribution in the formula fed to young infants had no effect on the concentrations of 20:4n−6 or 22:6n−3, but was associated with differences in 18:3n−3 in plasma chylomicron lipids in the studies reported here. In contrast, studies in piglets have found lower 20:4n−6, 22:6n−3, and 18:3n−3 in chylomicron triacylglycerol as a result of feeding formula with ≈32% 16:0 at the 2 position of the triacylglycerol (28), whereas adults fed a diet with 40% of energy from triacylglycerol with 54% 16:0 at the 2 position had higher 20:4n−6 in plasma triacylglycerol but lower 20:4n−6 in cholesterol esters (29) than when palm olein was used as the source of dietary 16:0. Consistent with the findings of the studies in the formula-fed infants reported here, plasma triacylglycerol concentrations of 18:3n−3 were higher when triacylglycerol enriched in 16:0 at the 2 position was fed (29). The discrepancies among the results of studies with adults, infants, and piglets may involve the differences in age, species, and the analysis of blood collected in the fed and fasted state, and of total plasma chylomicron triacylglycerol. Furthermore, more specific studies of the interaction between dietary saturated fatty acids and the metabolism of unsaturated fatty acids may be worthwhile.

In summary, these studies showed that ≈50% of the 16:0 at the 2 position of human milk triacylglycerol and formula with synthesized triacylglycerol is transferred to the chylomicron triacylglycerol 2 position of breast-fed and formula-fed infants, respectively. Furthermore, these studies showed that the effect of dietary triacylglycerol fatty acid distribution extends beyond facilitating the absorption of long-chain saturated fatty acids (11, 30, 31) to affecting plasma lipoprotein lipid and apolipoprotein concentrations and possibly metabolism. Further studies are needed to consider in more detail the implications of human milk and infant formula triacylglycerol patterns to lipid metabolism and tissue fatty acid delivery in young infants.

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