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The Stereospecific Triacylglycerol Structures and Fatty Acid Profiles of Human Milk and Infant Formulas

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ABSTRACT

Background: The stereospecific structures of the triacylglycerol molecules in human milk differ from that of cow's milk and vegetable oils, which are the fat sources used in infant formula. In human milk, palmitic acid (16:0) is predominantly esterified in the *sn*2 position, whereas vegetable oils or cow's milk fat contain most of their 16:0 in the outer positions of the triacylglycerol molecules. Furthermore, human milk contains long-chain polyunsaturated fatty acids, which are not present in either cow's milk or vegetable oils.

Methods: By standard lipid analysis procedures, we examined the triacylglycerol structures and fatty acid profiles of fats from 28 infant formulas or formulas for special indications available in the Danish market from 1999 to 2003.

Results: The total fatty acid compositions of the formulas showed a 16:0 content almost similar to human milk, whereas the content in the *sn*2 position was considerably lower. The content of oleic acid was found to be equal to or higher than in human milk in 21 of 28 formulas, whereas the content in the *sn*2 position was higher in all but one formula. Most formulas had linoleic acid levels considerably above that of human milk. Long-chain polyunsaturated fatty acids (arachidonic acid and docosahexaenoic acid) were present in all preterm formulas, but only in 3 of the term formulas.

Conclusion: We found that most of the examined infant formulas, both preterm and term as well as special formulas, had stereospecific structures and fatty acid profiles that differed considerably from that of human milk. ***JPGN 42:293–299, 2006.*** **Key Words:** infant formulas—fat—human milk—triacylglycerol structure—polyunsaturated fatty acid—ARA, arachidonic acid—DHA, docosahexaenoic acid—FAME, fatty acid methyl esters—LA, linoleic acid—LCPUFA, long-chain polyunsaturated fatty acid—LNA, α-linolenic acid—MCFA, medium-chain fatty acid—PUFA, polyunsaturated fatty acid.
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INTRODUCTION

During the last decades, there has been increasing interest in the fatty acid composition of infant formulas (1,2). The interest has focused on the content of polyunsaturated fatty acids (PUFAs), especially the balance between n-3 and n-6 PUFAs and the possible need to add the long-chain PUFAs (LCPUFAs), docosahexaenoic acid (22:6 n-3, DHA) and arachidonic acid (20:4 n-6, ARA). Recently, the importance of the stereospecific triacylglycerol structures has been demonstrated (3,4). The target has been to produce an infant formula with a fat content, composition, and structure close to those found in human milk. Using a blend of different fats, mainly vegetable oils, it has been possible to match the overall fatty acid composition of human milk, but not the triacylglycerol structure.

Fat absorption is higher from human milk than from infant formula, despite the similarities between the fatty acid profiles (5). This may partly be explained by the unique triacylglycerol structure of human milk. Palmitic acid (16:0) is abundant in human milk and is an important source of energy. Most of the 16:0 in human milk is located in the *sn*2 position of the triacylglycerol molecules (6), in contrast to cow's milk and vegetable oils which have 40% (7) and 5% to 20%, respectively, of the 16:0 in the *sn*2 position (8). Therefore, hydrolysis of human milk fat results in 16:0 mainly as *sn*2-monoacylglycerol, which are well absorbed (7,9). In contrast, after hydrolysis, 16:0 from vegetable oils will be present as free fatty acids, which tend to bind calcium and form insoluble calcium soaps in the intestine that may cause constipation.

Long-chain polyunsaturated fatty acids are present in human milk but have not, until now, been added to the majority of standard infant formulas available in the Danish market. Docosahexaenoic acid is important for

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development of the nervous system (2) and ARA is the precursor of eicosanoids (10), which are local mediators involved in, for example, the regulation of the immune function and platelet aggregation. It is presently recommended that the contents of the precursors for these LCPUFAs, linoleic acid (18:2 n-6, LA) and α-linolenic acid (18:3 n-3, LNA), are 1.2–2.9 μg/kJ and more than 119 μg/kJ, respectively, to the infant formulas without LCPUFAs and that the ratio of LA to LNA should be between 5:1 and 15:1 (11). It is generally agreed that LCPUFAs should be added to formulas for preterm infants, whereas no agreement has been reached on whether to add LCPUFAs to formulas for term infants (11–13).

The aim of this study was to describe the fatty acid compositions and the stereospecific triacylglycerol structures of a selection of formulas in the market in Denmark, to compare these with human milk, when appropriate, and to discuss some of the consequences of these issues. Our focus was on the content and position in the triacylglycerols of 16:0, oleic acid (18:1 n-9), LA, LNA, DHA, and ARA. Furthermore, we studied the structure and profile differences of the fat from formulas with different applications (for premature infants, term infants, or infants with various disorders), with special focus on the presence of medium-chain fatty acids (MCFAs, 8:0 and 10:0).

MATERIALS AND METHODS

Twenty-eight different formulas were analyzed. Five of the formulas were for premature infants, 8 were for term newborn infants (term infant formulas), 4 were follow-on formulas for infants older than 4 months, and 11 were special formulas for infants and young children with different disorders (Table 1). The fatty acid profiles and structures were compared with values from different types of human milk. We analyzed a pooled sample of human milk derived from mothers delivering prematurely before the gestational age of 34 weeks. The mothers emptied both breasts with the help of an electrical pump, and each mother collected the milk in a 24-hour pool. The milk was collected between the second and fourth weeks after delivery. Furthermore, we included in Table 3 sets of values from the literature (6,9,14). We also analyzed cow's milk (standard full fat pasteurized cow's milk bought during the month of February).

The formulas were purchased and analyzed between 1999 and 2003. Seven of the formulas were purchased and analyzed both in 1999 and in 2003 (Allomin-1, Allomin-2, soured Allomin, Baby Milk, Enfalac, Nutramigen, and NAN H.A.). These analyses gave similar results with respect to profile and structure for all except one of the formulas. In 2003, Enfalac had increased proportions of 18:1 n-9, ARA, and DHA and decreased proportions of lauric acid (12:0) and LA compared with that in the 1999 formula. This article presents the results of the most recent analyses.

Formula Fat Analysis

Powdered formulas were dissolved in water (250 mg formula/mL water), whereas liquid formulas, human milk, and cow's milk were extracted from their origin. The fats were extracted by the Folch procedure (15), and fatty acids were dissolved in heptane.

TABLE 1. *List of infant formulas and foods for special medical purpose, the year they were purchased, manufacturer, and type and indications for special medical foods*

Formula	Purchased	Manufacturer
Formula for preterm infants		
Enfalac	2003	Mead Johnson Nutritionals, Lyngby
Pre-Aptamil	2001	Milupa GmbH
Pre-Babymin A	2000	Milupa GmbH
Pre-Babymin B	2000	Milupa GmbH, Friedrichsdorf
		Denmark, Copenhagen
Pre-NAN	2000	Nestlé
Infant formula for term infants		
Allomin	2003	Beauvais, Tåstrup
Allomin Soured	2003	Beauvais
Aptamil	2001	Milupa GmbH
Babymilk	2003	Arla Foods, Viby J
Babymin	1999	Milupa GmbH
HIPP 1	2002	HIPP GmbH and Co Vertrieb KG
		Pfaffenhofen
NAN 1 new	2003	Nestlé
Nidina 1	2002	Nestlé
Follow-on formula (from 4 mo)		
Allomin 2	2003	Beauvais
Allomin rice flour	2003	Beauvais
HIPP 2	2002	HIPP GmbH & Co Vertrieb KG
NAN 2	1999	Nestlé
Foods for special medical purpose		
Generaid Plus	1999	SHS Int (for hepatic disorders, for sole source of nutrition, it is recommended to supplement with a source of LNA)
Monogen	1999	SHS Int (high content of medium-chain triglycerides for use in lipid and lymphatic disorders)
NAN H.A.	2003	Nestlé (hydrolyzed protein for infants at risk for allergy)
Neocate	2003	SHS Int (protein as amino acids, for cow's milk allergy and multiple food intolerance)
Nutramigen	2000	Mead Johnson (hydrolyzed protein, for prevention and treatment of cow's milk allergy)
Nutramigen 2	2003	Mead Johnson (hydrolyzed protein, for prevention and treatment of cow's milk allergy from 4 mo)
Pregestimil	1999	Mead Johnson (hydrolyzed protein for fat malabsorption)
Profylac	1999	Alk (hydrolyzed protein, for prevention and treatment of cow's milk allergy)
Prosobee	2001	Mead Johnson (soy protein, for cow's milk allergy)
Super Soluble Duocal	2001	SHS Int (energy supplement with carbohydrate and fat, for liver disease and catabolic states)
Other foods		
Therapeutic milk	1999	Compact (for "therapeutic feeding" in severely malnourished children in developing countries)

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Fatty acid methyl esters (FAMEs) were prepared from total lipids through transesterification catalyzed by KOH in methanol (16). The FAMEs were analyzed by gas-liquid chromatography using a Hewlett-Packard 5890 series II Chromatograph with flame-ionization detection (Hewlett-Packard GmbH, Waldbronn, Germany) and a fused silica capillary column (SP-2380; 60 m; internal diameter, 0.25 mm; Supelco, Bellefonte, Pa). Helium was used as carrier gas. A split ratio of 1:11 was applied in the injector. The column flow was constant at 1.2 mL/min. Initial oven temperature was 70°C for 0.5 minute, and temperature programming was as follows: 15°C/min to 160°C/min, 1.5°C/min to 200°C/min, which was maintained for 15 minutes followed by a rate of 30°C/min to 225°C/min, which was then maintained for 5 minutes. Fatty acid methyl ester peaks in the chromatogram (from octanoic acid [8:0] to DHA [from 4:0 for cow's milk]) were identified from external standards (Nu-Chek-Prep, Inc, Elysian, MN). The chromatogram areas of FAMEs with fewer than 12 carbons were corrected by response factors.

Regiospecific analyses of the formula fats were performed by degradation of the triacylglycerol molecules with allylmagnesium bromide as Grignard reagent (17). The *sn*2-monoacylglycerol fraction was isolated by thin-layer chromatography on boric acid–impregnated thin-layer chromatography plates developed twice (2 × 60 minutes) in chloroform/acetone (90:10 vol/vol), methylated, and analyzed by gas-liquid chromatography. The fatty acid profiles of triacylglycerol and the *sn*2 position (Tables 2–4) are given as the distribution in weight percent of all fatty acids (set to 100%). The relative proportion of specific fatty acids in the *sn*2 position of the triacylglycerol molecule was calculated as $(M/T \times 3)$ 100, where M is the percentage of the fatty acid in the *sn*2 position, and T is the percentage of the fatty acid in

TABLE 2. *The fatty acid profiles and composition in the sn2 position of triacylglycerols of human milk, cow's milk, and infant formulas intended for preterm infants**

	Human milk					Standard preterm formula				
	Preterm milk (pooled sample)	Innis et al (9) 3 mo after delivery	Jensen et al (6) mature milk	Martin et al (14) colostrum	Cow's milk	Enfalac	Pre-Aptamil	Pre-Babymin A	Pre-Babymin B	Pre-NAN
Triacylglycerol										
8:0†	0.2	—	—	—	**1.1**	**22.3**	**2.3**	**0.7**	**2.0**	**15.8**
10:0	1.4	—	2.9	0.4	2.8	**12.1**	1.7	1.3	1.5	**10.6**
12:0	5.7	4.1	7.3	4.8	*3.4*	*1.6*	**13.5**	5.2	**11.4**	*0.5*
14:0	6.9	5.5	9.4	8.5	**11.1**	*1.0*	5.6	5.8	4.7	*0.7*
16:0	25.7	21.0	27.0	25.8	**32.3**	*6.1*	*15.9*	**28.6**	*17.1*	*11.2*
16:1	2.8	3.1	3.6	3.2	*2.1*	*0.1*	*0.5*	*1.1*	*0.5*	*0.3*
18:0	8.1	7.1	7.1	5.9	**11.2**	*2.5*	*4.4*	6.8	*4.5*	*3.0*
18:1t	0.7	—	—	—	**2.9**	—	*0.2*	*0.3*	*0.1*	—
18:1 n-9	33.1	40.2	34.2	32.6	*20.7*	*29.0*	**40.4**	33.4	37.3	35.9
18:1 n-7	1.8	—	—	—	*0.6*	*0.7*	*1.0*	*0.9*	*1.0*	*1.0*
18:2 n-6	9.5	13.4	7.9	13.2	*1.8*	**20.5**	11.9	12.5	**16.8**	**16.7**
18:3 n-3	1.1	1.5	Trace	0.7	*0.2*	1.8	1.3	0.9	1.4	2.2
20:4 n-6	0.4	0.5	—	0.6	—	**0.8**	0.4	*0.3*	*0.3*	*0.2*
22:6 n-3	0.4	0.2	—	0.5	—	0.4	0.2	0.2	0.2	0.3
Others	2.3	1.2	—	3.5	**9.8**	1.2	*0.6*	2.1	1.2	1.4
18:2 n-6/18:3 n-3	8.8	8.9	—	18.9	9.9	11.6	9.3	14.3	12.2	7.6
*sn*2 position										
8:0	0.2	—	—	—	**0.8**	**21.3**	0.2	*0.1*	*0.1*	**13.6**
10:0	0.9	—	1.6	0.4	**3.3**	**12.9**	*0.9*	1.1	0.7	**11.7**
12:0	6.1	2.5	6.9	3.8	4.9	2.7	**24.0**	6.4	**19.7**	*0.8*
14:0	12.0	6.2	15.4	11.1	**18.0**	*0.8*	*3.6*	9.1	*2.9*	*0.9*
16:0	57.1	54.2	57.1	53.5	*37.8*	*1.2*	*3.3*	*19.4*	*4.7*	*3.7*
16:1	2.9	3.5	1.6	3.2	2.6	*0.1*	*0.3*	*1.1*	*0.3*	*0.3*
18:0	1.6	2.9	4.9	1.7	**5.5**	*0.3*	*0.6*	2.7	*0.7*	*0.7*
18:1t	—	—	—	—	**2.3**	—	**0.1**	**0.2**	**0.1**	—
18:1 n-9	9.5	17.1	8.1	13.8	15.3	**31.8**	**45.5**	**39.1**	**44.0**	**41.2**
18:1 n-7	0.7	—	—	—	*0.4*	*0.3*	*0.5*	*0.6*	*0.6*	*0.2*
18:2 n-6	5.4	8.1	3.7	8.4	*1.8*	**25.5**	**18.7**	**17.7**	**24.3**	**23.0**
18:3 n-3	0.5	0.9	—	0.4	0.4	**1.5**	**2.1**	0.8	**1.6**	**3.1**
20:4 n-6	0.3	0.7	—	0.7	—	0.7	*0.1*	*0.1*	*0.1*	—
22:6 n-3	0.6	0.4	—	0.7	—	0.6	—	—	—	*0.3*
Others	1.7	0.9	—	3.4	**6.9**	*0.3*	*0.2*	1.5	*0.3*	*0.4*

Values in **bold** font are higher than the range of human milk values, and values in *italic* are lower.

*Data represent the average of 2 determinations for triacylglycerols and 3 to 4 determinations of the *sn*2 position and are expressed in weight percent of total fatty acids in triacylglycerol or the *sn*2 position, respectively.

†Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule is also indicated.

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triacylglycerol (18). Data for the fatty acid profiles represent the mean of 2 determinations, whereas the analyses of the *sn*2 position represent the mean of 3 or 4 determinations. The SEM was less than 3% for fatty acids in triacylglycerol and less than 5% for the *sn*2-monoacylglycerols.

RESULTS

The fatty acid profiles and the *sn*2–fatty acids of the analyzed infant formulas were compared with those of a range of human milk values and cow's milk (Tables 2–4). Values market in bold font are higher than the range found in human milk, and values market in italic are lower. In general, the contents of 18:1 n-9, LA, and LNA were higher in the infant formulas than in human milk both in total fatty acids and in the *sn*2 position. The content of MCFAs was more than 20-fold higher than in human milk in 6 of the formulas: 2 preterm, 2 for use in malabsorption, and 2 for use as energy supplement. Docosahexaenoic acid was detected in 7 of the 28 formulas and in the *sn*2 position of 2, whereas ARA was detected in 8 formulas and in the *sn*2 position of 6.

The ratios of LA to LNA in all of the formulas and cow's milk were within the interval recommended for standard infant formula of 5:1 to 15:1 (Tables 2–4), except Pregestimil, Super Soluble Duocal, Generaid Plus, Neocate, and Therapeutic milk (all special formulas), which all had ratios greater than 15:1. The proportions of LA in preterm infant formulas were about twice that in human milk, whereas that of LNA was similar to or twice that in human milk, resulting in LA/LNA ratios that, in most of the formulas, were higher than in human milk.

TABLE 3. *The fatty acid profiles and composition in the sn2 position in triacylglycerols of infant formula fat**

	Standard infant formula for term infants								Follow-on formula (from 4 mo)			
	Allomin	Aptamil	Baby milk (liquid)	Babymin	HIPP-1	NAN-1 new	Nidina	Soured Allomin	Allomin-2	Allomin + rice flour	HIPP-2	NAN-2
Triacylglycerol												
8:0†	**0.4**	**1.2**	**1.0**	**2.1**	—	**1.6**	**2.1**	**0.5**	—	**0.5**	—	**1.2**
10:0	0.9	1.4	1.7	1.7	*0.1*	1.1	1.2	1.0	—	1.1	*0.1*	0.9
12:0	*1.3*	6.0	*3.6*	**13.6**	*0.2*	**8.5**	**8.5**	*1.4*	*0.2*	*1.4*	*0.2*	7.0
14:0	*4.4*	5.8	7.0	5.7	*0.9*	*3.9*	*4.0*	*4.7*	*0.7*	*4.7*	*0.9*	*3.3*
16:0	**29.1**	26.6	**31.7**	*15.9*	**29.7**	24.7	*24.0*	**28.9**	**28.6**	**30.8**	**31.4**	*22.3*
16:1	*0.7*	*1.1*	*1.1*	*0.5*	*0.2*	*0.2*	*0.2*	*0.7*	*0.2*	*0.8*	*0.2*	*0.2*
18:0	6.8	6.3	7.7	*4.4*	*3.8*	*3.3*	*3.2*	7.2	*4.1*	6.3	*3.8*	*3.7*
18:1t	**1.5**	—	**1.2**	—	—	—	—	**1.6**	—	—	—	—
18:1 n-9	33.6	34.7	*29.5*	40.2	**41.0**	**36.6**	**37.1**	**34.7**	**41.7**	**34.2**	40.2	37.2
18:1 n-7	*1.7*	*1.2*	*0.9*	*1.0*	*1.3*	*1.1*	*1.1*	*1.6*	1.2	*1.0*	*1.2*	**1.9**
18:2 n-6	**15.6**	11.2	10.0	11.9	**18.9**	**16.2**	**15.5**	**13.5**	**19.5**	12.7	**18.6**	**19.2**
18:3 n-3	1.8	1.7	1.2	1.3	1.9	2.0	1.6	1.5	1.9	1.4	1.7	2.4
20:4 n-6	—	*0.3*	*0.1*	0.4	—	—	—	—	—	—	—	—
22:6 n-3	—	0.2	—	0.2	—	—	—	—	—	—	—	—
Others	2.3	2.2	3.3	*1.1*	2.1	*0.8*	1.5	2.7	1.9	**5.1**	1.8	*0.8*
18:2 n-6/18:3 n-3	8.5	6.5	8.5	9.2	10.0	8.1	9.5	9.1	10.1	9.0	11.2	8.1
*sn*2 position												
8:0	**0.5**	**0.3**	0.2	0.2	—	**0.4**	0.2	—	—	*0.1*	—	—
10:0	0.7	0.9	1.6	0.8	—	0.5	0.6	0.5	—	0.6	—	—
12:0	*1.4*	**9.4**	5.8	**23.6**	*0.2*	**13.9**	**14.3**	*1.6*	0.1	*1.1*	*0.2*	4.4
14:0	6.9	7.2	11.2	*3.6*	*0.7*	*2.1*	*2.4*	7.1	*0.6*	*3.9*	*0.7*	*1.7*
16:0	*20.7*	*16.6*	*26.3*	*3.4*	*10.3*	*5.3*	*5.6*	*18.4*	*9.3*	*14.4*	*12.3*	*6.0*
16:1	*0.8*	*1.1*	*1.4*	*0.3*	*0.1*	*0.1*	*0.1*	*0.9*	—	*0.5*	*0.1*	—
18:0	3.6	2.6	3.4	*0.6*	*0.6*	*0.7*	*0.5*	3.0	1.8	2.1	*0.8*	*0.9*
18:1t	**1.3**	—	**1.0**	—	—	—	—	**1.3**	—	—	—	—
18:1 n-9	**39.1**	**40.1**	**31.2**	**46.0**	**54.2**	**49.1**	**48.0**	**44.2**	**55.3**	**48.2**	**53.2**	**52.1**
18:1 n-7	*0.6*	*0.6*	*0.5*	*0.5*	*0.6*	*0.6*	*0.5*	—	*0.6*	*0.6*	*0.6*	**0.8**
18:2 n-6	**21.1**	**17.4**	**13.9**	**18.6**	**29.0**	**24.5**	**23.9**	**19.7**	**27.0**	**22.5**	**27.4**	**30.0**
18:3 n-3	**2.6**	**2.3**	**1.5**	**2.1**	**3.1**	**2.9**	**2.6**	**2.1**	**2.7**	**2.3**	**2.7**	**4.0**
20:4 n-6	—	*0.1*	—	*0.1*	—	—	—	—	—	—	—	—
22:6 n-3	—	—	—	—	—	—	—	—	—	—	—	—
Others	*0.7*	1.4	1.9	*0.3*	1.2	—	1.3	1.2	2.6	**3.8**	1.9	—

Values in **bold** font are higher than the range of values for human milk (Table 2), and values in *italic* are lower.

*Data represent the average of 2 determinations for triacylglycerols and 3 to 4 determinations of the *sn*2 position and are expressed in weight percent of total fatty acids in triacylglycerol or the *sn*2 position, respectively.

†Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule is also indicated.

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TABLE 4. *The fatty acid profiles and composition in the sn2 position of triacylglycerols of foods for special medical purpose**

	Foods for special medical purpose										Other foods
	Generaid plus	Monogen	NAN H.A.	Neocate	Nutramigen	Nutramigen 2	Pregestimil	Profylac	Prosobee	Super soluble duocal	Therapeutic milk
Triacylglycerol											
8:0†	**23.5**	**51.5**	**1.2**	**2.2**	**1.4**	**1.5**	**35.4**	—	**1.4**	**23.3**	**3.9**
10:0	**5.6**	**33.7**	**1.0**	**1.7**	**1.1**	**1.1**	**16.4**	—	**1.2**	**5.8**	2.8
12:0	**13.6**	*0.5*	**7.8**	**13.0**	**9.1**	**8.8**	*0.1*	*0.3*	**9.5**	**13.8**	**22.2**
14:0	*5.4*	*0.6*	*3.8*	*5.2*	*4.2*	*3.9*	—	*0.8*	*4.3*	5.5	**9.5**
16:0	*8.3*	*2.7*	24.3	*8.3*	22.6	22.9	*4.4*	**27.7**	22.2	*7.9*	25.6
16:1	*0.1*	—	*0.2*	—	*0.1*	*0.1*	—	*0.2*	*0.1*	*0.2*	—
18:0	*2.7*	*2.0*	*4.0*	*4.1*	*3.7*	*3.8*	*1.4*	*3.3*	*4.1*	*2.4*	**8.4**
18:1t	*0.3*	—	—	—	—	—	—	*0.4*	—	—	**1.3**
18:1 n-9	*13.7*	*2.6*	35.8	**46.6**	37.9	36.5	*20.3*	**48.2**	36.2	*13.8*	*20.0*
18:1 n-7	*0.3*	—	1.8	*0.6*	*1.4*	*0.8*	*0.4*	**2.2**	*0.8*	*0.3*	—
18:2 n-6	**25.2**	*5.3*	**17.1**	**16.3**	**17.1**	**18.3**	**20.1**	**14.5**	**17.7**	**26.0**	*5.2*
18:3 n-3	0.7	1.0	2.0	1.0	1.2	1.4	1.0	1.7	1.8	*0.5*	*0.3*
20:4 n-6	—	—	—	—	—	—	—	—	—	—	—
22:6 n-3	—	—	—	—	—	—	—	—	—	—	—
Others	*0.4*	—	*1.0*	*0.9*	*0.1*	*1.0*	*0.5*	*0.7*	*0.7*	*0.6*	*0.7*
18:2 n-6/ 18:3 n-3	**36.4**	5.5	8.4	**16.8**	14.5	13.5	**20.3**	8.7	9.9	**54.8**	**17.8**
*sn*2 Position											
8:0	**20.4**	**52.0**	—	0.2	*0.1*	0.2	**36.1**	—	**0.4**	**22.7**	—
10:0	**5.6**	**39.0**	—	0.9	0.4	0.4	**19.6**	—	0.6	**5.9**	1.0
12:0	25.7	0.9	**5.8**	**23.7**	**14.6**	**14.6**	*0.1*	—	**15.8**	**23.5**	**35.5**
14:0	3.3	1.1	*1.8*	*3.2*	*2.4*	*2.4*	—	*0.2*	*2.1*	*2.9*	*5.1*
16:0	2.3	2.1	*5.7*	*3.0*	*6.2*	*6.1*	*0.4*	*5.0*	*4.9*	*1.4*	*10.1*
16:1	0.1	—	*0.2*	—	—	—	—	*0.1*	*0.1*	—	—
18:0	1.7	1.9	*0.6*	2.6	*1.4*	*1.4*	*0.1*	*0.7*	*0.5*	*1.2*	**6.7**
18:1t	0.3	—	—	—	—	—	—	**0.6**	—	—	**1.7**
18:1 n-9	12.7	1.1	**52.3**	**46.9**	**50.8**	**50.6**	**19.8**	**63.6**	**49.2**	**13.0**	**30.1**
18:1 n-7	0.1	—	**1.3**	—	*0.4*	*0.4*	*0.2*	**1.3**	*0.4*	*0.1*	—
18:2 n-6	27.4	1.6	**28.6**	**18.7**	**22.5**	**22.5**	**22.8**	**26.0**	**25.0**	**28.9**	**9.4**
18:3 n-3	0.4	0.3	**3.7**	**0.8**	**1.0**	**1.0**	**0.8**	**2.5**	**1.1**	0.4	0.5
20:4 n-6	—	—	—	—	—	—	—	—	—	—	—
22:6 n-3	—	—	—	—	—	—	—	—	—	—	—
Others	—	—	*0.1*	—	—	*0.3*	*0.1*	—	—	—	—

Values in **bold** font are higher than for human milk, and values in *italic* are lower.

*Data represent the average of 2 determinations for triacylglycerols and 3 to 4 determinations of the *sn*2 position and are expressed in weight percent of total fatty acids in triacylglycerol or the *sn*2 position, respectively.

†Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule is also indicated.

DISCUSSION

Most infant formula fats are based on mixtures of vegetable oils or a blend of cow's milk fat and vegetable oils that, to some extent, mimic the fatty acid profile of human milk, as observed in the present study. However, the stereospecific structure in the formulas differed considerably from that in human milk. Most (approximately 75%) of the 16:0 in human milk was located in the *sn*2 position, whereas most of the 16:0 in the formulas was located in the outer positions of the triacylglycerols (75%–97%). Comparison of infant formulas and human milk showed that 18:1 n-9 replaced 16:0 in the *sn*2 position, as in vegetable oils (eg, rapeseed and palm oils). This indicates that the largest part of the fat used in the formulas was of vegetable origin, except for Baby Milk, which was primarily based on cow's milk fat.

The human milk values we have used for comparison show a quite constant fatty acid pattern, despite the types of human milk being quite different (preterm milk, colostrums, and mature milk). As expected, the largest variation was in the content of DHA which is highly dependent on the mothers intake of DHA (19).

Constipation is more often observed in formula-fed infants than in breast-fed infants (20). The structure differences between infant formula fats and human milk are one of the major reasons for the formation of insoluble calcium soaps in the intestine. Betapol (Loders Croklaan, Wormerveer, The Netherlands) is a synthetic fat produced to mimic human milk fat both in total fatty acid profile and in structure (45% of the 16:0 in the *sn*2 position). Studies with Betapol and other structured human milk substitutes showed that fats rich in *sn*2-16:0 compared with fats with a lower proportion of the 16:0 in the *sn*2 position improved absorption of 16:0 and

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calcium and reduced the excretion of calcium soaps in the feces of preterm infants (3), term infants (4), and rats (21). These studies support a beneficial physiological effect of the specific structured triacylglycerols on fat and calcium absorption. However, problems with calcium soap formation can also be solved by substituting 16:0 with other fatty acids, for example, 18:1 n-9, as in many of the infant formulas, or with MCFAs.

High proportions of MCFAs were found in special formulas for infants with malabsorption, lymphatic disorders, renal disease, or liver disease (Pregestimil, Monogen, Super Soluble Duocal, and Generaid Plus) and for preterm infants (Pre-NAN, Enfalac). These were added primarily instead of 16:0 as a readily available energy source. Infants with malabsorption can easier degrade, absorb, and transport MCFAs than fatty acids of longer chain length. Medium-chain fatty acids are absorbed mainly to the portal vein and transported directly to the liver for β-oxidation (22). Formation of insoluble calcium soaps in the intestine is low to nonexisting, when MCFAs replace 16:0, because of the higher absorption (23,24). The nutritional value of special formulas with a high content of MCFAs may be increased by addition of LCPUFAs, possibly even further if the fat is structured (3). Long-chain polyunsaturated fatty acid absorption has been shown to be improved in rats with malabsorption, if LCPUFAs are supplied in fat with MCFAs in the outer positions and LCPUFAs in the *sn*2 position (25,26), and so have fat utilization and nitrogen retention in piglets (27).

The LA and LNA contents of infant formulas are believed to cover the need of n-6 and n-3 LCPUFAs in term infants through elongation and desaturation to ARA and DHA. According to the current Nordic recommendations for infants aged 6 to 11 months, the dietary intake of n-6 and n-3 PUFAs should supply at least 4.0% and 1.0% of the energy intake, respectively (28). Because the fat content of breast milk and formulas constitutes approximately 50% of the energy, this is equivalent to an n-6 and n-3 PUFA content of approximately 8 and 2 wt%, respectively. Only 4 of the formulas in our study were found to have LNA content of around 2 wt%. The ESPGHAN Committee on Nutrition (29) and the Scientific Committee on Food (15) recommend that the ratio of LA to LNA in infant formulas should be 5:15. This recommendation was made in the absence of data on functional or clinical outcomes (30). Lowering the ratio of LA to LNA from 10:1 to 5:1 in formula fats resulted in modest increase in plasma DHA, but with no detectable effect on visual acuity or growth rate (30). Most of the formulas were found to have a high LA content well above that in human milk. A few of the formulas, mostly special formulas intended for infants with special needs, had a very high content of LA and ratios of LA to LNA between 17:1 and 55:1. Recent studies indicate that DHA formation is inhibited by a high supply of LA (31,32). Therefore, there may be a need to decrease the LA content or supply LCPUFAs, to meet the n-3 LCPUFA needs in infants. However, the ratio of LA to LNA in infant formulas has improved since we analyzed infant formulas in the Danish market in the mid-1990s (33).

Only 7 of the 28 infant formulas contained DHA, and only 8 of them had ARA. In human milk, 55% of DHA was found to be specifically located in the *sn*2 position of the triacylglycerol. Of the 7 infant formulas that contained DHA, only Pre-NAN and Enfalac had high levels in the *sn*2 position (34%–50%). Preterm infants have significantly lower ARA and DHA status than full-term infants because they do not receive the intrauterine supply of these fatty acids during late pregnancy (34). Dietary deficiency of ARA and DHA has therefore become an issue for the nutrition of preterm infants. Our results showed that the contents of these fatty acids in the formulas were similar to or lower than in human milk in 4 of 5 formulas for ARA and in all the preterm formulas for DHA. However, compared with other countries, Danish mothers have a high n-3 LCPUFA intake and, thus, high levels of DHA in their milk (2).

The ratio of LA to LNA and the presence of LCPUFAs in some of the formulas demonstrate that an improvement of the fatty acid profiles of the infant formulas has taken place over the last decades, although the content of LNA is still low and that of LA is high compared with current recommendations and compared with the content in human milk from Danish mothers. In the present study, the structure analyses of the formulas showed that there was a discrepancy between the locations of the fatty acids in formula fats compared with that in human milk. In contrast to human milk, 16:0 in infant formulas was located primarily in the outer positions of the triacylglycerol molecules, which may increase the risk of constipation. Only few formulas contained LCPUFAs, most of which were located in the outer position of the triacylglycerol molecules and thus at potential risk of slow and low absorption.

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