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LIPIDS IN HUMAN MILK AND INFANT FORMULAS

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INTRODUCTION

Although the pace of research on lactation, human milk, and infant nutrition has slowed, work on milk lipids has continued because milk fatty acids can be altered by dietary as well as genetic factors. The lipids in human milk have been reviewed by Jensen through mid-1988 (51, 52). We have also compared the lipids in bovine and human milks (54). This review covers the period from mid-1988 through September 1991.

DETERMINATION OF MILK VOLUME

Test Weighing

In order to ascertain the quantities of nutrients conveyed to the infant in breast milk, the amount of milk must be determined. Estimation of 24-h milk intake is desirable, but often unattainable. Because of practical limitations, efforts have been made to lessen the problems. Arthur et al (3) measured milk intake of breast-fed infants by weighing the infant or the mother with a sensitive electronic balance. The method correlated highly ($r = 0.927$) with measurements done by direct test weighing and was validated in the field (43). Neville and colleagues (76, 79) reported that test weighing of the infant gives reliable estimates of breast milk intakes when an integrating electronic balance is employed and when done by motivated mothers in field conditions.

Deuterium Oxide Method

The deuterium oxide elimination method for measuring average daily milk intake has been validated (27). Deuterium oxide was given orally to the infants and isotope dilution was measured in the urine.

Doubly Labelled Water Method

The doubly labelled water method has made it possible to study the energy content of human milk without sampling the product (67, 68). Measured water output is equivalent to water (milk or formula) intake. Twenty-nine infants at 3 months of age consumed 892 ml of formula per day that had a metabolizable energy content of 66 kcal/dl. The calculated energy was 68 kcal/dl. At 6 weeks of age, the metabolizable energy content for formula-fed infants was 60 kcal/dl. The amounts for breast-fed infants were 53 kcal/dl at 6 weeks and 58 kcal/dl at 3 months. These data are lower than the accepted value of 70 kcal/dl (73). The authors suggested that expressed milk, either by hand or pump, contains more fat than the infant actually consumes. This occurs because hind milk always contains more fat than fore milk. Since the fat content of breast milk must be known to determine the quantities of fatty acids consumed by the infant, sampling before and after nursing to determine

the fat content should be done. The fatty acid composition is not affected by amounts of fat expressed.

SAMPLING OF MILK FOR FAT CONTENT

Milk samples that represent the composition of milk consumed over 24 h should be obtained. Since it is often impractical to take samples from every nursing during 24 h, Jackson et al (46) evaluated the accuracy of predicting the 24-h fat content from daytime samples. Samples (0.5 ml) were hand expressed before and after nursing, the fat contents were determined by the Creamatocrit procedure (51), and the amounts were averaged (47). If only two random daytime samples were taken, the 24-h concentration could be predicted with 95% confidence limits of 7.0 g per liter or 21% of average amount. It was preferable to sample all daytime feeds, which reduced the 95% confidence limits to +/− 3.3 g per liter (10%).

An automatic sampling shield was developed and used to obtain milk samples while the baby was nursing (48). The unit did not measure milk volume. A series of small samples (averaged total of 1.3 g) were taken while the baby suckled. The shield was well accepted by the babies, but mean milk intakes were 17% lower than without the shield. Furthermore, it was shown that the fat content increased about 2-fold and was nonlinear at the beginning and end.

DETERMINATION OF LIPID CONTENT

Earlier work is presented in Reference (51). Procedures most widely used are the Creamatocrit in which the height of a packed volume of milk fat globule is measured and calibrated against a method employing solvent extraction followed by gravimetric determination of lipid. Among more recent developments is an automated enzymatic method for measurement of fat in human milk (69). The method, which requires only 25 μl of milk, can be used in commercial sequential analyzers. Glycerol released from milk triacylglycerol (TG) is quantitated after enzymatic treatment. The enzymatic procedure was checked with the dry column procedure and gave similar results on milks with various fat contents (19).

A rapid procedure for quantitation of fat, protein, and lactose in human milk by infrared analysis has been reported (71). The procedure is used in the dairy industry. The equipment is expensive, hence not readily available, and must be recalibrated for human milk. It should be useful for selection of high protein milks by milk banks as was done by Michaelsen et al (71). Clark & Roche (18) have refined the gas-liquid chromatography (GLC) of total lipids in 100 μl of human milk. The amounts obtained were essentially equal (r =

0.99) to the quantities extracted by the dry column method. The fatty acid composition and lipid content of 100 μl of human milk can be determined simultaneously.

Collins et al (19) investigated the extraction, separation, and quantitation of human milk lipids by the dry column method. Milk (1 ml) was applied directly to the column (mainly Celite) and extracted. One milliliter of milk was separated into neutral and polar lipids, but there was some carryover of TG in the polar fraction. The completeness of recovery of TG, cholesterol, phospholipids, and vitamin E was comparable to that attained by the Folch extraction (51).

FACTORS AFFECTING TOTAL LIPID CONTENT

The increase in fat content that occurs during a nursing was confirmed (46, 48). A circadian rhythm was found in the milk from women in northern Thailand (47), but not in milk from women in the United States (65). The lack of an effect was attributed to individuality. The time elapsed between nursings influences the fat content. As the interval lengthened, the fat content of the subsequent nursing decreased (47). The time elapsed from birth or age postpartum influences the fat content. The amount of lipid increased from 3.98% at 12 weeks to 5.50% at 16 weeks; energy increased from 68.5 to 83.0 kcal/dl (25). The volume and nutrient composition of milk was determined during lactogenesis (1–8 days postpartum) and weaning (6 to 15 months postpartum) by Neville et al (78). Fat content and secretion rates (grams per day) increased during lactogenesis while both decreased after the initiation of weaning. Allen et al (2) examined the macronutrients and their daily secretion rates in the first year of lactation. Michaelsen and colleagues (70) observed a slight decrease in the fat content from 0 to 4 months postpartum, followed by an increase up to 17 months. The mean content was 3.9%. In contrast, the fat content in milk from California mothers increased only slightly from 3 months (3.62%) to 12 months (3.72%) while the volume consumed by the infant (grams per day) decreased from 811 to 514 ml (80). Allen et al (2) saw increases in fat content and amounts secreted from days 21 to 180 postpartum.

Few controlled investigations are available on changes that occur in the fat content of milk as a result of alterations in diet (51, 52). In general, the amounts of fat, protein, and lactose are little changed by variations in diet. Allen et al (2) reported that undernutrition can reduce the fat content by 25%, but they did not provide supporting data. Hachey et al (31) noted that the fat content of milk from 5 women was 2.5% on a low fat diet (5% fat, 80% carbohydrate) as compared to 3.3% on a high fat diet (40% fat, 45% carbohydrate). Nommsen et al (80) did not observe any relationship between milk fat content and maternal fat intake either in terms of total fat intake or percent

of calorie intake. They did note a significant relation between milk fat content and maternal protein content in the later stages of lactation. Silber et al (88) fed diets high in carbohydrate (5% fat, 15% protein, 80% CHO) to 10 lactating mothers. After 5 days they found relative and absolute increases in the amounts of 10:0, 12:0, and 14:0 and decreases in 18:0, 18:1, and 18:2. The responses were similar in milk from mothers of term and preterm infants.

The effects of several diseases on milk lipids have been examined and reviewed (37). The milk from one diabetic patient contained 2.95% fat; control milks (n = 13) contained 4.53% fat at days 6–7 postpartum (7). However, the fat content in milk from diabetics later in lactation did not differ much from that of the controls (49, 64). The fat content of milk from two patients with cystic fibrosis was normal when the disease was mild but was reduced when it became severe (8, 87).

The milk from a patient with abetalipoproteinemia was low in TG (1.5%) at 2 weeks postpartum but was equivalent to normal concentrations later in lactation (103). Chylomicrons and VLDL, which are the major carriers of dietary fatty acids to the mammary gland, are completely absent in the syndrome.

The effect of the mother's level of adiposity has a positive influence on the level of fat in milk (51, 52). Nommsen et al (80) obtained a positive and significant correlation between milk lipid concentration and maternal ideal body weight at 6, 9, and 12 months postpartum. Volume of milk was not a factor, although Michaelsen et al (70) saw a higher fat content in mothers who produced large amounts of milk.

Parity, 4 or higher, reduced the fat content of milk (51, 52). Corroboration at different levels of parity was provided by Nommsen et al (80) and Michaelsen et al (70). Seasonal effects may be related to parity and region in the populations studied. However, few data are available.

Lipogenesis is the ultimate major factor controlling the fat content in human milk. Unfortunately very little information is available on this aspect. Neville (77) noted that "the regulation of the total fat content of human milk is less well understood, in part because the lipid content of secreted milk is extremely difficult to measure accurately." The fat in milk is derived from fat in the blood or by synthesis in the mammary gland. The sources of fat in the blood are the diet via the gut, the liver, and adipose tissue. Dietary fatty acids are transported to the mammary gland in chylomicrons from the intestine and VLDL from the liver while adipose tissue fatty acids travel bound to albumin. In the mammary gland, fatty acid synthesis is limited to 10:0–14:0 using glucose as the source of carbon. Diet and adipose are sources of 16:0 and longer fatty acids.

Peak amounts of dietary fatty acids appear in milk about 8–10 h after consumption with an exponential decay from 48 to 72 h (32). About 10% of

the total acids, primarily 10:0 to 14:0, are synthesized in the mammary gland, 60% are derived from tissue synthesis and adipose tissue, and 30% come from the diet. In this study (32), the percentages of kcal in the diet were protein, 17; carbohydrate, 56; and fat, 27. Later, Hachey et al (31) determined the effects of low and high fat diets on the synthesis of endogenous fatty acids by the mammary gland. Some of these data are presented above in the section on diet. The relative amounts of dietary fat and carbohydrate influence the amounts of 10:0–14:0 synthesized in the mammary gland. Very little production of 16:0 and 18:0 occurred in the high fat diet, but the amounts were increased 6-fold in the low fat diet. One women had a substantial decrease in body fat after changing from a low to a high fat diet, but no changes were observed in the fat content or amounts of 10:0–14:0 in her milk. In continuations of these studies, Silber et al (88) confirmed the influence of dietary carbohydrate. Emken et al (23) showed that milk TG, phospholipids, and cholesteryl esters were synthesized from the same fatty acid pool, but the influence of dietary fatty acids was greater for TG. Hachey et al (30) reported that on the low-high fat regimens mentioned above, consumption of the low fat diet produced milk with a fat content of 2.2% while consumption of the high fat diet produced milk with a fat content of 3.28%. The amounts of fat produced per day per breast, however, were almost the same: 8.0 g and 7.74 g. Milk fat content was inversely correlated with body water flux, i.e. volume adjusted for fat content.

LIPID CLASSES

Introduction

Information on the general composition of human and bovine milk (54) and several infant formulas is given in Table 1. The data were obtained from the manufacturers of these products and from Reference 96. The lipids in human milk are mostly TGs (98 + %), cholesterol (0.4%), phospholipids (1.3%), and traces of other lipids (51, 52, 54). Small amounts (<5%) of 3-chloropropanediol in the alkyldiacylglycerols and monoalkylglycerols have been detected in bovine and human milk lipids (63). The lipid composition of milk from mothers with diabetes (64) and cystic fibrosis (8) has been reported. Several papers on milk sterols have appeared (10, 19, 25, 56, 65).

Triacylglycerols

Some papers have been published on the structure of TG in human milk. Dotson et al (22) separated and tentatively identified TGs by high performance liquid chromatograph (HPLC). The major TGs were composed of 16:0, 18:1, and 18:2 regardless of variations in dietary fat. The kinds and amounts of major TGs are listed in Table 2. The identifications in Table 2 are

Table 1 General composition (%) of human and bovine milks and some infant formulas

Component	Milk[a]		Formulas[b]				
	Human (36 days)	Bovine	1	2	3	4	5
Protein	1.0	3.4	1.52	1.5	1.5	1.6	1.5
Casein, % protein	40	82	40	82	40	0	40
Fat	3.9	3.3	3.8	3.6	3.6	3.3	3.4
Lactose	6.8	4.8	7.0	7.2	7.2	7.2	7.6
Kilocalories/dl	72	75	68	68	68	65	67

[a] Adapted from Reference 54.

[b] Adapted from Reference 96, and personal correspondence. 1 = Enfamil, Mead Johnson; 2 = Similac, Ross; 3 = SMA, Wyeth; 4 = Good Start, Carnation, Reconstituted; protein is hydrolyzed whey; contains lactose and maltodextrin; 5 = NAN, Nestle. All are for healthy term infants and the proteins in all except 4 are bovine milk whey and casein.

carbon numbers only and do not indicate position of the fatty acids. For example, 16:0–18:1–18:1 represents 16:0–18:1–18:1, 18:1–18:1–16:0, and 18:1–16:0–18:1. However, we know that most of the 16:0 is located at *sn*-2, so there will be more 18:1–16:0–18:1 (51, 52). The 8 TGs in Table 2 totaled 89.5%, but 19 more were detected. Milk TGs were separated by carbon number with capillary GLC in the search for chloropropanediol diesters (63), and TGs were analyzed in milk from a patient with abetalipoproleinemia (103). The amounts of lower molecular weight TGs were greater than normal in these milks.

Phospholipids

Phospholipids originate from the plasma membrane of the secreting cell. The major classes are (wt%): phosphatidyl choline, 30.0; phosphatidyl ethanolamine, 28.0; and sphingomyelin, 32 (51, 52). Smaller amounts of phosphatidyl serine and inositol, along with cerebrosides and gangliosides, have been found. The gangliosides bind enterotoxins. Milk from cystic fibrosis patients contained more sphingomyelin and less phosphatidylethanolamine than normal milk (8, 36).

van Beusekom et al (98) found a positive relationship between the 6:0–14:0 contents of total milk lipids and total phospholipids. Incorporation of 6:0–14:0 into phospholipids was favored over polyunsaturated fatty acids (PUFAs) in the mammary gland. Diets rich in CHO increased secretion of 6:0–14:0 into phospholipids at the expense of PUFAs.

Sterols

Earlier research has established that the sterol content of human milk ranges from 10 to 20 mg/dl with cholesterol as the major component. Most of the

Table 2 Major triacylglycerols in human milk[a]

TG	Area %	TG	Area %
14:0–14:0–12:0[b]	2.62	16:0–12:0–18:1	4.84
18:2–18:2–18:1	3.27	12:0–16:0–16:0	14.69
18:1–14:0–14:0	4.70	12:0–18:0–18:1	6.21
16:0–14:0–14:0	4.47	18:1–18:1–18:1	3.17
18:2–18:2–16:0	8.84	16:0–16:0–18:1	11.96
18:2–16:0–12:0	6.07	18:0–18:0–18:1	7.38
12:0–18:1–18:1	1.99	18:0–18:0–18:1	3.44

[a] Adapted from Reference 22.
[b] 14:0–14:0–12:0 represents the isomeric TGs 14:0–14:0–12:0, 12:0–14:0–14:0, and 14:0–12:0–14:0.

cholesterol is located in the milk fat globule membrane and the amount is not affected by diet (51, 52). Cholesterol is not present in formulas unless animal fats are added. Kallio et al (56) analyzed milk for cholesterol and its precursors by GLC and mass spectrometry. At 2 months postpartum values (μg/dl) were: cholesterol, 15800; desmosterol, 1509; squalene, 386; lanosterol, 94; methosterol, 48; dimethylsterol, 45; and lathosterol, 45. Other sterols detected in small and unreported amounts were cholestanol, campesterol, and sitosterol. Data are also given for milks at 6 and 9 months postpartum.

Lammi-Keefe et al (65) detected a diurnal pattern in the cholesterol content of milk—ranging from 8.75 mg/dl at 0600 h to 11.2 mg/dl at 2200 h. Clark & Hundrieser (17) found that the mean total cholesterol content of 25 milk samples was 13.5 mg/dl and was significantly correlated with the lipid content. The cholesteryl ester content was about 20%. Collins et al (19) found 10.58 mg/dl total cholesterol in 12 samples. The composition of the esters is given in (17). Emken et al (23) determined that the influence of dietary fatty acids on cholesteryl esters occurred at 8 to 10 h after consumption, the same as for TG and phospholipids. Boersma et al (10) measured cholesterol in milk from women in St. Lucia as follows: 36.0 mg/dl at 0 to 4 days, 19.7 mg/dl at 5 to 9 days, and 19.0 mg/dl at 10 to 30 days postpartum.

FATTY ACIDS

Introduction

Most of the earlier data listed in Table 3 were obtained by GLC instruments equipped with packed columns (94). These columns are incapable of the resolution attainable with wide-bore capillary columns of suitable length (at least 15 m, preferably 30 m), coated with polar stationary phases and utilizing temperature programming. Recently, newer stationary phases have been used to separate *trans* isomers and PUFAs. Details of operation are in the papers

Table 3 Saturated fatty acid (wt%) in human milk lipids

Fatty acid	Reference number[a]							
	94	60	85	10	22	22	86	86
		$n = 15$	$n = 23$	$n = 12$	$n = 30$	CV[b]		±SEM[b]
4:0	0.19	—	—	—	—	—	—	—
6:0	0.15	—	—	0.07	—	—	—	—
8:0	0.46	—	—	0.37	0.02	0.04	0.03	—
10:0	1.03	0.71	0.92	2.39	0.06	0.05	1.4	—
11:0	—	—	—	—	—	—	—	—
12:0	4.40	4.41	6.99	12.32	4.34	0.11	6.2	—
13:0	0.06	0.05	—	—	0.05	0.14	—	—
i-14:0[c]	0.04	—	—	—	—	—	—	—
14:0	6.27	6.73	8.80	11.78	4.65	0.07	7.6	—
a-15:0[c]	0.21	—	—	—	—	—	—	—
15:0	0.43	0.46	—	—	0.34	0.09	—	—
i-16:0	0.17	—	—	—	—	—	—	—
16:0	22.00	21.83	14.10	23.61	19.25	0.03	20.5	0.70
a-17:0	0.23	—	—	—	—	—	—	—
17:0	0.58	0.57	—	—	0.43	0.07	—	—
i-18:0	0.11	—	—	—	—	—	—	—
18:0	8.06	8.15	3.94	5.83	7.97	0.06	9.0	0.46
19:0	—	0.04	—	—	0.10	0.07	—	—
20:0	0.44	0.22	0.47	0.24	0.21	0.10	0.3	0.02
21:0	0.13	0.35	—	—	—	—	—	—
22:0	0.12	0.09	0.30	0.12	0.12	0.06	—	—
23:0	—	—	—	—	0.03	0.02	—	—
24:0	0.25	—	—	0.07	0.22	0.14	0.5	0.01
26:0	—	—	—	—	0.03	0.31	—	—
Total saturates	45.33	43.61	34.90	56.8	37.82	—	38.6	0.72

[a] Normalized data from 15 papers (94). Mature milks from German donors (60), Gambian mothers (85), St. Lucian mothers (10), mothers from Illinois (22) and Florida (86).
[b] CV, coefficient of variation; SEM, standard error of mean.
[c] i is iso a, anteiso.

quoted below. In addition, we urge investigators to determine the fat content of milk and formulas, so that the actual amounts of fatty acids conveyed to the infant can be calculated, and to present their data as weight percent (grams per 100 g fatty acid) and as weight of fatty acid per deciliter of milk (51, 52).

Fatty acids are converted to methyl esters prior to analysis by GLC to increase volatility and efficiency of separation. Methanolysis of the extracted fat is usually done by reactions with sodium hydroxide-methanol or boron-trifluoride methanol. Lepage & Roy (66) observed slightly better recovery of human milk fatty acids with a direct transesterification procedure they developed. However, Bitman & Wood (9) did not find any differences in 35 milk fatty acid profiles when they compared direct transesterification with

methanolysis by boron trifluoride-methanol. Many investigators add antioxidants to the solvents to prevent possible loss of PUFA by oxidation (99).

When analyzing human milk lipids for fatty acids, we recommend that investigators (*a*) use columns that will resolve *trans* isomers and PUFA, (*b*) determine and report the total lipid content, and (*c*) if possible, obtain information on maternal diets. Analysts may want to investigate the bracketing procedure used by van der Steege and co-workers (99). Internal standards were employed to quantitate the fatty acids. Data on within series and series-to-series precision and biological variation are given in this study.

Classes of Fatty Acids

Normalized older values from 15 studies are listed in Tables 3–5 (94). Most of these data were obtained by GLC analyses with packed columns and in some cases with prior separation of the fatty acid classes. The resolution and level of detection were not as good as observed in the later studies using capillary columns and electronic integrators (10, 22, 60, 85, 86, 98, 99). The data from an earlier study on Floridian milks (86) are given for comparison and also to exemplify the remarkable absence of a large data base on the fatty acid profiles of human milk obtained with the latest methods.

A few other recent publications report on changes due to age postpartum

Table 4 Monounsaturated fatty acid (wt%) in human milk lipids

Fatty acid	Reference number[a]							
	94	60	85	10	22	22	86	86
		n = 15	n = 23	n = 12	n = 30	CV		±SEM
13 : 1	—	—	—	—	0.03	0.10	—	—
c- 14 : 1n5[b]	0.41	0.29	0.23	0.29	0.31	0.20	—	—
t- 14 : n5[b]	0.07	0.19	—	—	—	—	—	—
15 : 1	0.11	—	—	—	0.09	0.09	—	—
c- 16 : 1n7	3.29	2.68	0.66	3.55	2.58	0.07	—	—
t- 16 : 1n7	0.36	0.46	—	—	—	—	—	—
17 : 1	0.37	0.32	—	—	0.33	0.07	—	—
c- 18 : 1n7	—	—	—	3.64	—	—	—	—
c- 18 : 1n9	31.30	34.31[b]	47.0	22.63	33.23	0.04	37.6	0.75
t- 18 : 1n9	2.67	3.12	—	—	4.72	0.07	—	—
20 : 1n11	—	—	—	—	0.17	0.20	—	—
20 : 1n9	0.67	—	0.83	0.42	0.38	0.11	0.9	0.7
21 : 1n9	—	—	—	—	0.01	0.29	—	—
22 : 1n9	0.08	0.08	0.22	—	0.07	0.05	0.1	0.02
24 : 1n9	0.12	—	0.05	0.04	0.03	0.07	—	—
Total monoenes	39.45	42.38	48.80	30.6	41.95	—	38.5	—

[a] See Table 3.
[b] c is *cis*; t, *trans*.

Table 5 Polyunsaturated fatty acid (wt%) in human milk lipids

Fatty acid	Reference number[a]							
	94	60	85	10	22	22	86	86
n6 series		n = 65	n = 23	n = 12	n = 30	CV	—	±SEM
18:2cc	10.85	10.76	13.0	9.57	15.55	0.05	15.8	0.61
18:2tt	0.46	0.14	—	—	—	—	—	—
18:2ct	0.69	0.14	—	—	—	—	—	—
18:2tc	—	0.07	—	—	—	—	—	—
18:3	0.25	0.16	—	0.09	0.18	0.08	—	
19:2		—	—	—	0.51	0.05	—	—
20:2	0.27	0.34	0.83	0.31	0.38	0.04	0.4	0.03
20:3	0.32	0.26	0.21	0.42[b]	0.46	0.05	0.4	0.03
20:4	0.46	0.36	0.31	0.58	0.53	0.06	0.6	0.03
22:2	0.11	0.11	—	—	0.05	0.06	—	—
22:4	0.09	0.08	0.08	0.15	0.06	0.07	0.2	0.02
22:5	0.09	—	0.30	0.07	—	—	0.1	0.02
Total n6	13.59	12.26	14.7	11.2	17.72	—	17.4	0.62
n3 series								
18:3	1.03	0.81	0.84	0.62	1.11	0.08	0.8	0.09
20:3	—	0.06	—		0.03	0.13		
20:4	0.09	—	—	—		—	—	—
20:5	0.12	0.04	—	0.07	0.07	0.12	0.1	0.03
22:3	—	—	—	—	0.13	0.05	—	—
22:5	0.19	0.17	0.20	0.16	—	—	0.1	0.01
22:6	0.25	0.22	0.39	0.56	0.16	0.08	0.1	0.01
Total n3	1.68	1.38	1.6	1.4	1.5		1.1	0.09
Total PUFA	15.27	13.64	16.3	15.6	19.22	—	17.4	0.62
Total n6/n3	8.10	9.23	9.19	8.0	11.8	—	18.8	1.2

[a] See Table 3.
[b] 0.05% of 20:3n3 not listed.

(10), diet and parity of rural Gambian mothers (85), dietary n3 fatty acids in milk from Inuit and Canadian women (44), effects of vegetarian and non-vegetarian diets (93), and fatty acids in milk from mothers in Tanzania, Curacao, and Surinam (72).

Diabetes increased the quantities of 10:0–14:0 in milk (49). Linoleic acid and its derivatives were low in milks from patients with cystic fibrosis, but other PUFAs were elevated (8). The contents of long-chain fatty acids in the milk from the women with abetalipoproteinemia were much lower than normal (103).

Human milk lipids contain more fatty acids than those listed in Tables 3–5, a total of 185 at the last count (54). Among these is 9,11–18:2ct, a conjugated fatty acid that is anticarcinogenic and a potent antioxidant (29, 54). We found

an average content of 0.186% in 20 samples (55). The quantities found are dependent on the amounts of dietary bovine milk lipids and oxidized food lipids consumed. This compound is an example of an important nonnutritive role of milk lipids.

Human milk contains prostaglandins in very small quantities (51, 52). The prostaglandin content in milk from mothers of term babies was low and comparable to that of milk from mothers of preterm babies (75). Prostaglandins E2 and F2 were stable in human milk and gastric fluid. The compounds may protect and maintain the integrity of the intestinal epithelial cells in the developing infant (5).

MILK FAT GLOBULE EMULSION AND MEMBRANES

The Emulsion

Lipids in human milk are dispersed as emulsified globules composed mostly of nonpolar TG, cholesteryl esters, and other lipids in the core (51, 52, 54). Bipolar substances, proteins, phospholipids, and cholesterol from the plasma membrane of the secreting cell envelop the globule as it is extruded, forming an emulsion-stabilizing membrane. The globules have an average diameter of 4 μm, with a surface area of 1.4 m/g of fat and numbering about 1.1×10^{10} ml. There are almost no data on factors affecting the size and numbers of globules.

The Globule Membrane

The membranes are derived from apical plasma membrane of mammary epithelial cells (11, 51, 52, 54, 57). A hypothetical model (57) includes an inner layer of phospholipids with fatty acids oriented into the TG core (cholesterol will also be here). A second layer contains phospholipids, proteins, and glycolipids. Human globule membranes are coated with an array of glycoprotein filaments whose functions are unknown but may enhance digestion and release of TG fatty acids by binding lipases. The filaments are removed by heating the milk. Some of the globules have a cytoplasmic crescent (42, 84). Although quantitatively negligible, the crescents could contain trace elements, enzymes, hormones, and growth factors of importance to the infant.

FAT-SOLUBLE VITAMINS

Introduction

Since the fat-soluble vitamins A, D, E, and K are required for growth and development, the belief that human milk is the ideal food for infants has focused attention on their amounts and availability (51, 52 74a). Analyses of

these vitamins, once arduous, have been eased by HPLC and other developments. Some of the analyses can be done quickly and reliably.

Retinoids and Carotenoids

The quantities of vitamin A (retinol) range from 40 to 70 μg/dl and of carotenoids from 20 to 40 μg/dl in well-nourished women in Europe and the United States (74a). Most of the retinol is esterified and the main carotenoid is beta-carotene. The bioavailability of carotenoids varies, so retinol equivalents (RE) are used. One RE is defined as 1 μg of all-*trans* retinol, 6 μg of all *trans* beta-carotene, or 12 μg of other provitamin A carotenoids. Vitamin A deficiency, which can lead to xerophthalmia, is rare in the US but is a major problem elsewhere affecting mostly children. The current RDA for infants 0 to 0.5 yr is 375 μg RE (74a).

Recent data are presented in Table 6 (58, 82, 83). All of these analyses were done by HPLC. Patton et al (83) observed a marked (10-fold) decrease in carotenoids and retinoids as lactation progresses. Patton et al (83) reported a drop in carotenoid contents 3 to 126 h after parturition. Lycopene, alpha-carotene, and lutein were detected in mature milks (58), and beta-cryptoxanthin and possibly zeaxanthin in colostrum (83).

Vitamin D

Earlier papers on vitamin D in human milk revealed a content of 0.63 to 1.25 μg per liter (51, 52, 74a). The Recommended Dietary Allowance (RDA) for infants 0.0–0.5 months postpartum is 7.5 μg and for their mothers is 10 μg. Since the content in human milk does not provide 7.5 μg, infants not exposed to sunlight may be at risk for vitamin D deficiency. Lack of the vitamin causes inadequate bone mineralization. However, Atkinson et al (4) found 80 IU per liter (2 μg) for preterm milk and 60 IU (1.5 μg) for term milk (Table 6). These quantities are higher than those quoted above. On the other hand, the quantities determined in Finnish fore milks ranged from 0.35 to 3.1 μg per liter depending on the season (1). The amounts were greater and the seasonal differences not so pronounced in hind milks. Supplementation of mothers with 25 or 50 μg of vitamin D significantly increased the amounts in February and April. Theoretically, the calculated antirachitic activity of milk in winter should have been increased by supplementation (50 μg) to levels of unsupplemented mothers in September, but responses were variable.

In metabolic studies, a maternal vegetarian diet was associated with increased serum 1,25-dihydroxyvitamin D during lactation (92), and unsupplemented breast-fed infants (n = 22) in Madison, Wisconsin, had no evidence of vitamin D deficiency during the first 6 months of life (28). The vitamin-D supplemented term infant fed human milk or cow milk or soy-based formula regulates mineral metabolism normally (41). The RDAs for

Table 6 Recent data on fat-soluble vitamins in human milk

Vitamin and milk type			Reference
Retinoids and carotenoids μg/dl			
Mature milk			
Retinol	52, 57		58, 82
Beta-carotene	23		51, 52
Colostrum			
Carotenoids, parity 1	114 ± 1.32		83
parity 2–3	218 ± 1.96	66 beta-carotene	83
D μg per liter[a]			
Preterm milk	2.0		4
Term milk	1.5		4
Winter foremilk	0.35		1
Summer foremilk	3.1		1
E mg/dl			
Preterm milk, 3 days	1.45		39[b]
Preterm milk, 36 days	0.29		39[b]
Term milk 3 days	1.14		39[b]
Term milk 36 days	0.28		39[b]
Mature milk	0.34		19
Colostrum, 0–4 days	2.2		10[c]
Transitional milk, 5–9 days	1.4		10
Mature milk, 10–30 days	0.8		10
K μg per liter			
Colostrum, 30–81 h	3.39[d]	Range: 2.3 to 7.6	12–14
Mature milk, 6 months	2.87[d]	Range: 2.1 to 9.28	12–14

[a] As activity of 1 μg of cholecalciferol, which is 40 IU.
[b] As alpha-tocopherol equivalents.
[c] Alpha-tocopherol.
[d] Difference between colostrum and mature milks is not significant.

maternal and infantile uptakes of vitamin D seem to be more than adequate (74a), but the large differences between the low amounts in breast milk and the RDAs suggest that breast-fed infants are at risk for vitamin D deficiency and should be supplemented. Preterm infants and babies who receive prolonged breast-feeding and insufficient exposure to sunlight are at higher risk and need greater supplementation.

Vitamin E

Vitamin E refers to a group of tocopherols, alpha, beta, gamma, and delta, that differ in biopotency (74a). Natural α-tocopherol is prefixed by RRR- (formerly *d*-). One milligram of this isomer is the RRR-tocopherol equivalent

(TE). The other forms have reduced biopotency. The compounds are antioxidants that slow peroxidation of PUFA in membrane phospholipids by trapping free radicals. Cellular damage and neurological symptoms can be prevented by adequate levels of tocopherols in the diet. Deficiencies can occur in premature, very low birth weight infants and in subjects who do not absorb fat properly. Infants may need 0.4 mg of tocopherol per gram of dietary PUFA, although a fixed ratio has not been established.

The concentrations of alpha-tocopherol in milk range from 3.0 to 4.5 mg per liter. These analyses were done by HPLC. The alpha, beta, gamma, and delta isomers have been resolved. The RDA (mg TE) is 3 for infants 0 to 0.5 yr and 12 for lactating women 0 to 0.54 yr (74a). Premature infants may need an oral supplementation of 17 mg per day (74a).

In a recent study, the vitamin E contents (medians and ranges) of milks at day 3 and 36 postpartum (mg TE)/dl were 1.45 (0.64–6.4) and 0.29 (0.19–0.86) preterm and 1.14 (0.63–4.21) and 0.28 (0.19–0.86) term (39). Collins et al (19) detected 0.34 mg of alpha-tocopherol per deciliter in mature fresh milk and 0.33 mg in milk stored for 2 weeks at −70°C. Total lipids were extracted by the dry column method (19). The data from (39) indicate that the needs of the preterm infant may not be met by breast-feeding. Haug et al (39) observed a decrease in the ratios of alpha- to gamma-tocopherol of 10:1 to 4:1 during the first 2 weeks of lactation. The ratio remained constant for 36 weeks. They did not detect delta-tocopherol nor was an increase in milk vitamin E seen when the mothers were given 50 mg per day for a week. Collins et al (19) noted high correlations between alpha-tocopherol and TG or cholesterol but not between alpha-tocopherol and phospholipid. Boersma et al (10) found 0.8 mg/dl of alpha-tocopherol in mature milks from women in St. Lucia. The postpartum decrease was 22 mg TE/dl at 0 to 4 days and 14 at 8 days postpartum. Alpha-tocopherol in human milk (mg/dl) was 0.6 in Curacao, 0.5 in Dominica, and 0.5 in Belize.

Vitamin K

HPLC has shown that human milk contains about 2 μg per liter of vitamin K (51, 52, 74a). The vitamin K denotes a group of compounds containing the 2-methyl-1,4-naphthoquinone moiety. Phylloquinone is the plant form of the vitamin, has a phytyl group at position 3, and is the most prevalent homolog in milk. The RDA for infants, 0 to 0.5 yr, is 5 μg per day, well above the amount in breast milk (74a). Newborn term infants in the US, are given 0.5 to 1 mg of vitamin K by intramuscular injection; preterm infants receive at least 1 mg. The RDA for lactating mothers is 65 μg per day. The vitamin is required for the biosynthesis of prothrombin and other blood clotting factors (74a). In vitamin K deficiency, abnormal proteins are formed. These can be detected in serum by a sensitive assay. Exclusively breast-fed infants are at

risk for hemorrhagic disease of the newborn, hence the supplementation above. Synthesis of vitamin K (menaquinones) by microorganisms is minimal, and in newborns and preterm infants liver stores are low (12, 74a).

Most of the recent analyses of vitamin K in milk have been done by Canfield and associates (12–14). These data are shown in Table 6. Canfield & Hopkinson (12) reviewed the older data. The HPLC assay was described (13). With this method Canfield et al (13) found 2.94 ± 1.94 μg per liter in pooled milk and 3.15 ± 2.87 in individual milks. Later, Canfield et al (14) reported the quantities in colostrum (30–81 h) and milk in Table 6. Postpartum differences were not significant. The amounts of vitamin K in milk were not predicted by dietary intakes of vegetables or fat. The vitamin was located in the fat core of the globule and thus was not associated with the membrane. Canfield et al stated that the amounts of vitamin K are inadequate to meet the recommended intakes of infants <6 months of age. Human milk does not contain adequate vitamin K to prevent hemorrhagic disorders in neonates.

INFANT FORMULAS

The composition of the lipids in formulas is uniform because of legislation and the desire to imitate human milk (38, 91, 96). The formulas contain 3.3 to 3.8% fat (Table 1) and the total lipid is composed of 98 + % TG, 0.03 to 0.1% sterols, and about 0.2% phospholipids. The sterols are phytosterols unless animal fats, usually butter or destearinated tallow, are added. The sterols and phospholipids will include those that were in the oils and lecithin added as an emulsifier. All analyzed formulas contained phosphatidylcholine, and many contained sphingomyelin (105). The fat globules in these formulas are uniformly 0.3 μm in diameter, which results in a surface area 48.6 m^2 per deciliter based on a 3.6% fat content. This is about ten times more globule surface area than in human milk (51, 52).

The fatty acids in formulas represent the profiles in the added fats and oils. These are given as classes of fatty acids in Table 7. Additional information for European formulas is in Reference 59. The formula must contain at least 2.7 en% 18:2n6, and no formula can have less than 0.3 g per 100 kcal of this acid (91). Most formulas contain 18:3n3, but none contain any but trace amounts of 20:4:n6, 20:5n3, and 22:6n3 (59, 86, 91). Since 20:4n6, 20:5n3, and 22:6n3 in as yet unknown quantities (in addition to 18:2n6 and 18:3n3) appear to be needed for optimal growth and development, efforts are underway to determine the effects of adding these acids to formulas (91). Carroll (15) recommended that n6 PUFA should not exceed 20% of total fatty acids; 18:3n3 no more than 3%; and 20:5n3 + 22:6n3, no more than 1%. Total n3 acids should not exceed en% in standard formulas. Uauy (97) also made recommendations to be discussed later. If partially hydrogenated oils or butter

Table 7 Fatty acid class composition (%) of formulas[a]

Fatty acid class	Formula[b]				
	Enfamil	Similac	SMA	Good Start	NAN
Saturates	58.2	45.7	44.2	44.6	50.1
Monounsaturates	14.2	17.2	41.3	33.2	36.3
Polyunsaturates	27.6	37.1	14.5	22.2	13.6
P/S ratio	0.47	0.81	0.33	0.50	0.27

[a] Compiled by authors from personal communications.
[b] Enfamil, Mead Johnson—coconut and soy oils; Similac, Ross—coconut and soy oils; SMA, Wyeth-Ayerst—oleo, high oleic, coconut, and soy oils; Good Start, Carnation—palm olein, high oleic, and coconut oils; NAN, Nestle—butter and corn oils.

are used in formulas, they will contain varying amounts of *trans* fatty acids. There are no reports of these in US formulas, but European products contained 0.2 to 4.6% (59). Carroll (15) recommended that *trans* fatty acids not be used, but if they are the quantity should be no more than 6% of total acids or 3 en%. The gangliosides, specifically GM1, in human and bovine milks and in infant formula bind enterotoxins and are part of the host defense systems (62).

NUTRITIONAL ASPECTS

Introduction

The components in milk are an interrelated system in which compartmentation is one of the factors controlling the flow of nutrients and metabolites to the breast-fed infant (53). These compartments and their interactions help control the temporal sequence of events leading to intestinal absorption of nutrients. The other factors controlling nutrient flow are the amount of milk, the surface area and topography of the fat globules, activity of relevant enzymes, and the status of the absorptive cells in the small intestine. A description of the physiological basis of infant feeding (104) and a discussion of lipids as an energy source for infants are available (21).

Absorption of Dietary Lipids

Few articles have been published in this area since the last reviews (51, 52). Milk and formula TGs are hydrolyzed in sequence, first by gastric lipase in the stomach (34, 35) and then by pancreatic colipase (90) and milk bile salt-stimulated lipase (BSSL) (40) in the small intestine. Formulas and bovine

milk do not contain BSSL, which is synthesized in the human mammary gland and apparently enhances absorption of milk lipids. The enzyme is destroyed by heating at 56°C for 30 min and is nonspecific, also hydrolyzing retinyl and cholesteryl esters. Some of the fatty acids released and monoacylglycerols (MGs) formed during lipolysis of dietary fats in the stomach and small intestine are highly microbicidal, constituting one of the infant's many host defense systems (34, 40).

Milk fat globules must first be altered by gastric lipase before the core TGs can be digested by pancreatic colipase in the small intestine (6). Predigestion by gastric lipase compensates for the relatively low concentrations of pancreatic colipase and bile salts in infants as compared to children and adults (6). Most of the activity designated as lingual in earlier papers is of gastric origin in the human.

Calcium soaps of fatty acids are highly insoluble in water and may be excreted. Milk contains ionized calcium, and insoluble soaps can be formed in the gut. Absorption of calcium and fatty acids may be reduced in the small intestine. Jandacek (50) observed solubilization of calcium soaps of long-chain fatty acids by liquid fatty acids. The solubilities of Ca-16:0, Ca-12:0, and Ca-18:1 were 15.6, 22.8, and 53.3 wt% in 18:1 at 40°C. The solubility of Ca-18:1 in a bile salt micellar system was enhanced by 18:1. Solubilization may explain the high bioavailability of some Ca soaps.

The belief that the preponderance of 16:0 in the *sn*-2 position of milk TG is responsible for the almost complete absorption of human milk fat is based on two studies (26, 95). It was assumed that much of the 16:0 is present as the 2 MG after digestion. However, the experimental subjects in either study did not resemble the milk-infant dyad. Mixtures of oils were fed to rats by Tomarelli et al (95). Rats, unlike humans, do not have much gastric as compared to lingual lipase (35). Native and randomized lards were given to infants (26), but as a formula and presumably homogenized into the mixture, resulting in a large increase in globular surface area. In native lard, most of the *sn*-2 position contains 16:0, similar to the fatty acid in milk TG. The BSSL of human milk was not present in either investigation. Also the composition and topography of the globule membrane differs from that of milk. The complete digestion of milk TG (6) by the lipolytic sequence described above eliminates the relationship between TG structure and absorption because 2-monopalmitoylglycerol is not present in human milk, since all acylglycerols are digested.

Formulas containing different amounts of 16:0 at the *sn*-2 position of TGs did not affect absorption in preterm infants (100, 101). When preterm infants (26.5 to 37.5 weeks) were fed Almiron AB or a modified lard Almiron, neither the fat absorption coefficients, 69.6 and 58.6%, nor the energy absorption coefficients, 80.3 and 75.4%, were significantly different (100). Ninety three percent of the 16:0 was present at the *sn*-2 position of the TGs in

the formula containing lard. A similar study (101) analyzed fecal, plasma, and erythrocyte lipids. The carbon numbers of the formula fecal TGs were also determined. Most (97–98%) of the fecal lipids from the infants fed either formula were free fatty acids (FFAs), whose compositions closely resembled those of the formula. The long-chain fatty acid profiles of plasma and erythrocyte membranes were also correlated with the formula fatty acids. The authors suggested that the fecal FFAs were the result of rapid transit of formula TG through the small intestine, reduced lipase activity in the small intestine, and lipolysis (microbial and residual pancreatic lipases?) and absence of absorptive cells in the colon. No differences in overall absorption of fat were observed, but TGs with 16:0 esterified at *sn*-2, similar to those of human milk, resulted in higher absorption of fatty acids. Arachidonic acid (20:4n6) and 20 other carbon unsaturates were preferentially absorbed, whereas some 16:0, 18:2n6, and 18:3n3 were not absorbed (101).

While all of the lipases described above hydrolyze medium-chain triglycerides (MCT, 8:0 and 10:0) more rapidly than long-chain fatty acid TG (34, 35, 94), absorption rates were equivalent (37). Substantial quantities of 8:0 and lesser amounts of 10:0 were apparently absorbed through the stomach wall. The authors (37) doubted that large amounts of MCT (40–50% of total fat) would improve fat absorption in preterm infants. They recommended a maximum of 10–15%. They noted that MCT acids act primarily as sources of energy and are not used in membranes, etc.

The Host Defense Effects of Lipids

PRODUCTS OF LIPOLYSIS Protozoa, bacteria, and viruses are destroyed in vitro by lipolysis products: primarily 12:0, 18:2, and their MGs (40, 51, 52). In vivo, these compounds would quickly become available in the stomach and intestines of infants and reduce the colonization of microorganisms at these sites. Recent data (45) revealed inactivation of several enveloped viruses including HIV by human milk stored at 4°C for several days. Inactivation was caused by FFA released by serum lipoprotein lipase, which apparently leaks into milk. Dissolution of viruses also occurred when they were exposed to stomach contents after feeding of milk. The FFA were also produced by gastric lipase.

Enterotoxins from *Vibrio cholerae* and *Escherichia coli* are inhibited by a monosialoganglioside GM1 found in human and bovine milks and in a formula at levels of 12, 1.2, and <1.0 μg per liter respectively (51, 52). There are no new data, but a brief review is available (62).

Requirements for Polyunsaturated Fatty Acids

The requirements of humans for 18:2n6, the original essential fatty acid (EFA), are well established (51, 52, 74). The minimum amount for infants is set at 3 en% (240 mg/dl) by the American Academy of Pediatrics (20).

Deficiency of EFA was established by abnormal triene-tetrane (20:3n9/20:4n6) ratios in plasma lipids or erythrocyte phospholipids. An abnormal ratio is greater than 0.4. Uauy (97) recommended 500 to 700 mg of 18:2n6 per kilogram of infant weight per day, with a maximum of 12 en% (1.5 kg per day). Recent data on EFA deficiency in premature infants resulted in a recommendation that the average amount of 18:2n6 required to achieve normality was 1.19 g/kg per day (24). In this study, 67% of the premature infants had low levels of plasma 18:2n6. The quantities recommended above are higher than those suggested by Uauy (97) in order to overcome the deficiency in premature infants. Human milk and infant formulas contain more than enough 18:2n6 for the infant's needs. The 18:2n6 content of milk ranges from 8 to 16% or higher depending on the maternal diet. The amounts of 18:3n3 in milk range from 0.3 to 1.1% and are responsive to maternal diet. Milk fat contains about 2% of the elongation-desaturation products of 18:2n6 and 18:3n3 and may also be essential.

The necessity for inclusion of n3 PUFA in the human diet has been established (74, 89). The requirements are based on inference from the fatty acid profiles in milk (16) of neonates for these fatty acids and the acids in infant tissues, both postmortem (16) and in erythrocyte phospholipids (89, 91). Uauy made the following suggestion. (*a*) Total n6 and n3 acids should be set at 4–5 en% of total energy for infants, with a maximum of 12 en%. These energy levels represent 600 to 800 mg/kg per day. (*b*) The intake of 18:2n6 should be 500 to 700 mg/kg per day and the total supply of n3 fatty acids 70 to 150 mg/kg daily (97). Since the activity of neonatal desaturases and elongases may be suboptimal, half of the fatty acids should be provided as LCPUFA, 20 and 22C derivatives. (*c*) Formulas for preterm infants should provide 35–75 mg of LCPUFA/kg per day as DHA or EPA + DHA. Ideally, the ratio of n-6 to n-3 PUFA should be maintained within a range of 5/1 to 15/1. Clandinin et al (16) have suggested the following physiological intakes of PUFA (percent of total acids): 1% of 20 and 22:n6, 0.7% of 20 and 22:n3, 12% of 18:2n6, and 0.9% of 18:3n3.

Trans Fatty Acids

The presence of *trans* fatty acids in milk continues to attract attention (51, 52), yet only recently have the contents been regularly reported. Koletzko et al (60; Tables 4, 5) found 4.4% (by weight) *trans* fatty acid with seven isomers. All of these acids are derived primarily from partially hydrogenated oils. Some are derived from bovine milk lipids. The acids were detected in the plasma lipids of 30 mother-infant pairs (61). The amounts of all fatty acids that are present are given in this study, e.g. maternal plasma contained 7.75% n6 LCPUFA and 2.71% n3 LCPUFA; the values for cord blood were 15.62 and 3.82%.

SUMMARY AND CONCLUSIONS

About 50 metabolically important fatty acids can be identified in human milk. The extent of absorption of milk fatty acids varies considerably from infant to infant, particularly in pre-term infants, and requires more study. Human milk provides sufficient vitamins A and E for the term infant, but supplementation with vitamins D and K may be necessary. More research is needed on the amounts of the fat-soluble vitamins in human milk, the efficiency of transfer from mother to infant, the reasons for variation in different women, and the consequences to breast-fed infants of inadequate intake of vitamins D and K.

Breast milk contains the PUFA needed by term infants who are able to synthesize the long-chain PUFA soon after birth. Pre-term infants fed formulae need supplementation with n3 and n6 long-chain PUFA, since formulas currently do not contain these acids. More work is needed to determine the requirements for n3 and n6 fatty acids, expressed as weights per kilogram.

A larger data base using improved analytical procedures to study the nature and content of lipids in human milk is needed. The impact of maternal genetics and diet on fatty acids in milk should be studied, as well as the effect of maternal diet on eicosanoids secreted by the mammary gland. Information on the structure and function of the milk fat globule and its membrane is needed. Little is known about the effect of milk banking on milk lipids. The reader of this review will no doubt find other gaps in our knowledge of the lipid composition and nutritional value of milk that require additional investigation.

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