Comparative Lipid Profiles of Milk Bank Breast Milk and Infant Formulas

Anya Oleynik, Tatiana Eliseeva and Jack Y. Vanderhoek*

Department of Biochemistry and Molecular Medicine, The George Washington University, School of Medicine and Health Sciences, Washington, DC 20037

Abstract: Lipid profiles of seven human breast milk samples obtained from milk banks and four infant formulas were compared in view of the potential food hypersensitivities of certain infants to human milk.The cholesterol (0.15-0.26 mM) content of the human samples was about 50% lower than that found in the infant formulas whereas the triglyceride (TG, 173-386 mM) contents of these products were found to be comparable.The major saturated fatty acid (SFA) and mono-unsaturated fatty acid (MUFA) were 16:0 and 18:1 respectively. The major poly-unsaturated fatty acid (PUFA) was 18:2 with other PUFA members of the C18, C20 and C22 families identified and quantified. Although conjugated linoleic acid (CLA) was not detected in any infant formulas tested, no other major differences in the fatty acid patterns were found. However, the mean (13.6) of the ratio of n-6 PUFAs/n-3 PUFAs in the human milk samples was about 50% higher than that observed in the infant formula samples.Although our results indicate that there are small yet significant differences in cholesterol and CLA content and the ratio of n-6 PUFAs/n-3PUFAs, the lipid composition of milk bank breast milk and infant formulas is quite comparable.

Keywords: cholesterol,CLA, fatty acids, human, milk, PUFA, triglycerides.

INTRODUCTION

Milk has long been an essential component in human nutrition. Newborns totally depend on "mothers' milk" for their sustenance and according to the World Health Organization, breast feeding for the first six months is advantageous to the infant's development and long-term health status [1]. However, under certain circumstances breast-feeding is either impractical or undesirable, e.g for premature infants, special needs infants or the mother's medical condition, and mothers who still want to provide human milk for their infants have turned to human milk banks since wet nurses are no longer in vogue.To prevent disease transmission, human milk banks screen donor milk for various diseases prior to distribution. Other mothers have opted for infant formulas to feed their babies as these commercial products are easily accessible and manufactured to conform to certain standards. Obviously each nutritional option (milk bank or commercial formula) represents different benefits and risks [2, 3].

Both human milk and infant formula contain milk triglycerides as the major source of energy in addition to other essential nutrients for infants. A number of studies have examined the lipid profiles of human milk samples [4-6] and several studies have compared the lipid composition of human milk and infant formulas [7-9]. In most of these studies, milk samples were collected at different post-partum stages and immediately frozen and stored prior to analyses. However, milk banks only use mature breast milk and routinely pasteurize donor milk samples prior to distribution [2]. Since we are not aware of any reports concerning milk lipid analyses from samples obtained from milk banks, we carried out the present study and compared the lipid composition of pasteurized breast milk from human milk banks and several infant formulas in order to determine which milk group might be a better lipid source for infant nutrition.

EXPERIMENTAL SECTION

All infant formulas used in this study were purchased from local grocery stores. The infant formulas purchased were Enfamil LIPIL® infant formula, Nestle Good Start Gentle Plus® formula, Similac Advance Early shield® infant formula and SimilacIsomil Advance® soy formula. The human breast milk samples were obtained either from the Mothers' Milk Bank (Raleigh, NC) or the Breastfeeding Center of Greater Washington (DC). Approval for the use of human subjects was obtained from the Office of Human Research, The George Washington University, and informed consent was obtained from the donors.

Triglyceride (TG) analyses were carried out using the Triglyceride Infinity reagent TR 22321 (Thermo Fisher Scientific, Inc., Atlanta, GA) according to the protocol described in reagent technical bulletin. Cholesterol quantitation was determined using the fluorogenicAmplex Red cholesterol assay kit (A12216) obtained from Invitrogen (Carlsbad, CA) following the procedure described in the kit bulletin. GLC analyses were carried out with a helium carrier gas on a Supelcowax 10 fused silica capillary GLC column # 24081 (60m x 0.25 mm ID, 0.25 μm, Supelco, Inc. Bellefonte, PA).

*Address correspondence to this author at the Department of Biochemistry and Molecular Medicine, The George Washington University, School of Medicine and Health Sciences, Washington, DC 20037; Tel: 202-994-2929; Fax: 202-994-8974; E-mail: jyvdh@gwu.edu

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The column was attached to a Shimadzu GC 14A chromatograph connected to a Shimadzu CR8A Chromatopac data processor (Shimadzu Scientific Instruments, Inc., Columbia, MD). In order to determine the fatty acid content of the milk samples, the milk lipids were first extracted using the Folch extraction method [10] and then transmethylated using the sodium methoxide approach [11]. The fatty acid methyl esters (FAMEs)were analyzed using injector and flame ionization detector temperatures set at 250° C. Two different column temperature programs were run for each sample: program 1 started at 100° C for 1 min, then 100° to 210° C (10 min), 210° C (60 min), 210° to 240° C (5 min) and 240° C for 5 min; program 2 started at 65° C, then 65° to 195° C (10 min), 195° C (20 min), 195° to 240° C (3 min) and 240° C for 75 min [11]. A known amount of 13:0 methyl ester was used as an internal standard for all GLC analyses as separate experiments had shown the absence of this FAME in all original milk samples. GLC reference FAME standard mixtures # 461, 538, 606 and some individual FAMEs were obtained from Nu-Chek Prep, Inc. (Elysian, MN) to confirm the identities of the FAME components in the milk samples. Each sample was analyzed at least twice to ensure reproducibility.

RESULTS

As shown in Table **1**, the cholesterol content of seven human breast milk samples obtained from milk banks ranged from 0.155 to 0.265 mM (mean 0.219 mM) whereas the cholesterol content of the infant formulas was about 50% higher ($p<0.05$) and ranged from 0.250-0.385mM (mean 0.318 mM). The TG concentrations of the seven human donors varied from 173 to 386 mM(mean 292 mM) which was comparable to the TG content range of the infant formulas, 228-297 mM (mean 274 mM).

The fatty acid profiles of human breast milk samples from seven donors and four different baby formulas are presented in Tables **2** and **3**. In general, a number of qualitative similarities between these groups were observed. For example, the major saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) are palmitic acid and octadecenoic acids respectively. In human breast milk, the palmitic acid content ranged from 18.4-28.0 mole % (mean 22.8%) and in infant formula from 8.81-24.4 %. The octadecenoic acid content in human breast milk ranged from 35.4-50.7% (median 40.5 %) and in baby formulas from 37.0-46.1%. The major poly-unsaturated fatty acid (PUFA) present in all these samples is linoleic acid with lesser amounts of 18:3(n-3). In the human donors, the linoleic acid content ranged from 14.2-22.1 % (mean 18.0 %) and in infant formulas, the range was from 17.6-23.7%. The PUFA family with the highest mole % was the C18 and included 18:2 (n-6), 18:3 (n-6), 18:3 (n-3) and conjugated linoleic acid (CLA). In human breast milk samples, the total C18 PUFA mole % ranged from 16.1-24.4 % (mean 19.6%) and in infant formula, from 19.3-25.9% (Table **4**). For all milk samples, the relative mole % content for the three PUFA families was in the order of C18>>C20>C22 (Table **4**). No CLA isomers were detected in any of the infant formulas.

When the ratios of (SFA+MUFA)/PUFA were determined, the human milk samples [ratios ranged from 2.78-4.64 (mean 3.78)] were comparable to those calculated for the infant formula samples [ratios varied from 2.72-3.84 (mean 3.15), Table **4**]. The ratio of n-6 PUFA/n-3 PUFA is generally utilized to determine the balance between essential fatty acids. As shown in Table **4**, this ratio varied from 7.63-20.1 for seven human donors and from 9.00-9.64 for infant formula samples. The mean (13.6) of this n-6/n-3 ratio for human donors was significantly higher ($p<0.05$) than the corresponding mean (9.35) observed for the formula samples.

Table 1. Cholesterol and TG Levels in Milk Samples from Human Breast and Infant Formulas. Cholesterol and TG Analyses were Carried Out as Described in Materials and Methods. Data are mean ± SD (n) where n is the number of analyses Carried Out

Source	Cholesterol (mM)	Triglycerides (mM)
Human		
Subject 1	0.252 ± 0.037 (2)	364 ± 80 (4)
Subject 2	0.235 ± 0.035 (2)	273 ± 72 (4)
Subject 3	0.215 ±0.025 (2)	278 ± 66 (4)
Subject 4	0.192 ±0.038 (2)	173 ± 10 (4)
Subject 5	0.265 ±0.045 (2)	386 ± 73 (4)
Subject 6	0.217 ±0.052 (2)	275 ± 37 (4)
Subject 7	0.155 ±0.005 (2)	
Infant Formula		
Enfamil	0.250 ± 0.050 (2)	278 ± 13 (4)
Goodstart	0.337 ± 0.023 (2)	291 ± 13 (4)
Similac	0.300 ± 0.050 (2)	228 ± 44 (4)
Simlc/Isomil	0.385 ± 0.045 (2)	297 ± 15 (4)

Table 2. Fatty Acid Composition of Lipids Extracted from Human Breast Milk. After Extraction, Lipids were Trans-esterified to Fatty Acid Methyl Esters and Analyzed by Capillary GLC as Described in Materials and Methods. Data are Expressed as Mole % and are the Average of at Least two Separate Determinations

Donor	1	2	3	4	5	6	7
FA	mole %	mole %	mole %	mole %	mole %	mole %	mole %
10:0	0.760	0.715	0.965	0.581	0.904	0.395	1.560
12:0	3.241	3.860	4.607	2.519	5.122	4.228	5.318
14:0	4.423	6.020	5.490	3.070	5.425	6.024	5.055
14:1	0.168	0.3012	0.165	0.097	0.056	0.127	0.158
15:0	0.309	0.482	0.265	0.236	0.268	0.325	0.240
16:0	28.002	23.474	22.025	18.379	21.327	25.621	20.800
16:1	2.166	3.078	2.239	1.270	1.985	1.121	1.856
17:0	0.313	0.408	0.291	0.256	0.268	0.342	0.341
17:1	0.219	0.276	0.252	0.154	0.185	0.181	0.196
18:0	1.122	0.990	2.510	1.040	3.604	1.068	1.069
18:1	38.766	39.844	40.691	50.692	35.449	41.945	36.289
20:0	0.384	0.199	0.345	0.281	0.307	0.270	0.235
20:1	0.432	0.323	0.419	0.446	0.391	0.465	0.348
22:1	0.062	0.042	0.058	0.052	0.075	0.088	0.055
23:0	0.014	0.013	0.018	0.012	0.018	0.010	0.014
24:1	0.055	0.024	0.019	0.023	0.067	0.056	0.024
Σ SFA+MUFA	80.436	80.049	80.359	79.109	75.451	82.265	73.558
18:2 (n-6)	16.850	17.122	16.637	18.565	20.794	14.234	22.140
18:3 (n-6)	0.104	0.124	0.241	0.133	0.160	0.134	0.233
18:3 (n-3)	1.150	1.069	0.811	0.773	1.259	1.636	1.847
CLA	0.189	0.345	0.189	0.163	0.180	0.122	0.147
20:2 (n-6)	0.311	0.248	0.276	0.234	0.407	0.216	0.280
20:3 (n-6)	0.317	0.352	0.517	0.269	0.447	0.341	0.377
20:4 (n-6)	0.219	0.276	0.442	0.329	0.375	0.364	0.587
20:3 (n-3)	0.029	0.036	0.025	0.021	0.058	0.037	0.041
20:5 (n-3) / 22:0	0.100	0.101	0.152	0.147	0.175	0.137	0.191
22:2 (n-6)	0.032	0.026	0.028	0.026	0.061	0.024	0.031
22:4 (n-6)	0.055	0.063	0.090	0.057	0.083	0.076	0.097
22:5(n-6)	0.019	0.022	0.032	0.023	0.034	0.034	0.020
22:5 (n-3)/ 24:0	0.082	0.109	0.125	0.095	0.222	0.198	0.205
22:6 (n-3)	0.108	0.054	0.081	0.063	0.294	0.181	0.249
Σ PUFA	19. 565	19.947	19.646	20.898	24.549	17.734	26.445

Table 3. Fatty Acid Composition of Lipids Extracted from Several Infant Formula Samples. See Legend to Table 2 for Explanation. [1]Not Detected

Formula	Goodstart	Enfamil	SmlcBaby	SmlcIso
FA	**mole %**	**mole%**	**mole%**	**mole%**
10:0	0.463	0.890	0.647	0.743
12:0	8.482	7.827	11.340	11.147
14:0	4.569	4.423	5.693	5.167
15:0	nd[1]	0.114	nd	nd
16:0	24.361	24.344	8.809	8.478
16:1	0.201	0.173	0.105	0.107
17:0	0.096	0.093	nd	0.079
18:0	0.590	0.528	0.725	0.599
18:1	37.014	40.379	45.786	46.114
20:0	0.339	0.333	0.306	0.329
20:1	0.178	0.193	0.214	0.231
22:1	0.018	0.008	0.013	0.043
23:0	0.029	0.033	0.027	0.028
24:1	0.015	nd	0.059	0.062
Σ SFA+MUFA	76.356	79.338	73.723	73.127
18:2 (n-6)	20.392	17.617	23.098	23.688
18:3 (n-6)	0.089	0.07	0.115	0.030
18:3 (n-3)	1.948	1.606	2.173	2.189
CLA	nd	nd	nd	nd
20:2 (n-6)	0.022	0.009	0.010	0.013
20:3 (n-6)	0.054	0.055	0.043	0.031
20:4 (n-6)	0.582	0.626	0.340	0.335
20:3(n-3)	nd	0.005	nd	0.003
22:0/20:5	0.166	0.256	0.239	0.252
22:2 (n-6)	0.035	0.029	0.024	0.025
22:4 (n-6)	nd	nd	nd	0.005
22:5(n-6)	nd	nd	nd	nd
22:5(n-3)/24:0	0.135	0.168	0.149	0.169
22:6 (n-3)	0.221	0.222	0.085	0.105
Σ PUFA	23.644	20.663	26.275	26.845

Table 4. Comparison of Fatty Acid ratios from Human Milk and Infant Formula Sources.[2]These Calculations are Based on the Assumption that the GLC Peaks Representing Mixtures of 20:5 (n-3) + 22:0 and of 22:5 (n-3) + 24:0 Consist of Equal Amounts of Each Component

a. Human Milk

Donor	1	2	3	4	5	6	7
FA	mole %	mole %	mole %	mole %	mole %	mole %	mole %
Σ C18 PUFA	18.293	18.660	17.878	19.634	22.393	16.126	24.367
Σ C20 PUFA	0.926	0.963	1.336	0.927	1.375	1.027	1.381
Σ C22 PUFA	0.255	0.220	0.294	0.217	0.583	0.414	0.595
ratio $(SFA+MUFA)^2/PUFA$	4.11	4.01	4.09	3.79	3.07	4.64	2.78
Σ n-6 PUFA	17.907	18.233	18.263	19.636	22.361	15.423	23.765
Σ n-3 PUFA	1.378	1.264	1.056	0.978	1.809	2.022	2.335
$Ratio^2$n6/n3	12.99	14.42	17.29	20.08	12.36	7.628	10.18

b. Infant Formula

Formula	Goodstart	Enfamil	SmlcBaby	SmlcIso
FA	mole %	mole%	mole%	mole%
Σ C18 PUFA	22.429	19.293	25.386	25.907
Σ C20 PUFA	0.741	0.823	0.512	0.508
Σ C22 PUFA	0.324	0.335	0.183	0.220
ratio $(SFA+MUFA^2/PUFA$	3.23	3.84	2.81	2.72
Σ n-6 PUFA	21.174	18.406	23.629	24.127
Σ n-3 PUFA	2.320	2.045	2.451	2.508
$Ratio^2$n6/n3	9.127	9.000	9.641	9.620

DISCUSSION

This study compared the lipid profiles of seven milk samples obtained from American milk banks with four types of infant formulas with regard to cholesterol and triglyceride levels and fatty acid composition. The TG content of the human milk and infant formulas were comparable but the cholesterol content of the latter was about 50% higher than that found for the human samples. Our human cholesterol milk data is about half that reported by Huisman *et al*. [8] which may be a reflection of the different diets consumed by American and Dutch women and/or a change in eating habits between these groups over the last 17 years.

The fatty acid profiles of our human milk samples were quite similar to those reported for European women [8, 9, 12] and it seems unlikely that the typical pasteurization process used by milk banks [2] has any influence on the lipid profile of breast milk samples. In addition, the fatty acid composition of the American infant formulas tested in this study were comparable to those reported for Dutch, German and Spanish commercial infant formulas [8, 9, 13]. In contrast to our human PUFA profiles, no CLA [a family of isomers with beneficial effects [14, 15] was detected in our infant formula products. However, since many dairy and meat products contain CLA [16], once the infant is weaned from infant formula, these known CLA-containing foods are

common dietary components. A similar concern for the absence of 22:4 (n-6), an essential precursor to very long-chain (n-6) PUFAs needed for normal spermatogenesis and fertility [17], in any of the infant formulas may be forestalled in view of the presence of 20:4 (n-6), the precursor to 22:4 (n-6).A number of reports have shown that both 20:4 (n-6) and 22:6 (n-3) levels in human milk correlate positively with infant growth and development [18, 19]. Our data indicate that the 20:4 (n-6) and 22:6 (n-3) mole % ranges for the human samples (0.22-0.59 and 0.054-0.29 respectively) are quite comparable to those found for the infant formulas (0.34-0.63 and 0.085-0.22 respectively).

n-6 PUFAs and n-3 PUFAs are precursors to different oxidized metabolites (e.g. eicosanoids) that tend to have opposing biological effects in such diverse activities as cellular aggregation, immunological and inflammatory processes [20]. Consequently, the n-6 PUFA/n-3 PUFA ratio (n6/n3) has usually been used as a marker to indicate which of these metabolites are expected to be dominant [21]. Our studies indicate that this n6/n3 ratio for the human milk (mean 13.6) was significantly (about 50%) higher than that found for the infant formulas.

CONCLUSION

Despite the fact that the World Health Organization recommends that mothers breastfeed their infants for the first 6 months, certain circumstances may make the mother's own milk unavailable. Alternative choices are breast milk from milk banks or commercial formula though each option represents different benefits and risks [2, 3]. Our small study indicates that there are small, yet significant, differences in the lipid composition (i.e. cholesterol and CLA contents and certain fatty acid profiles) of milk bank breast milk and infant formulas but that these differences are not sufficient to prioritize either nutritional source.

ABBREVIATIONS

CLA	=	Conjugated linoleic acid
FAME	=	Fatty acid methyl ester
MUFA	=	Mono-unsaturated fatty acid
PUFA	=	Poly-unsaturated fatty acid
SFA	=	Saturated fatty acid
TG	=	Triglyceride

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