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PROTEIN AND LIPID COMPOSITION OF HUMAN MILK AND INFANT FORMULAS: COMPARISON AND NUTRITIONAL CONSEQUENCES

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Summary. - *This work results from some research carried out by the authors during the last few years in nutrition field. The data collected allowed to compare protein and lipid composition of infant formulas with human milk and to evaluate the influence of such feedings on plasma aminoacid levels and erythrocyte fatty acids. In spite of different infant formula compositions with respect to human milk, our nutritional studies did not demonstrate physiological differences between breast and bottle-fed infants if formulas provide with an adequate intake of protein and linoleic acid.*

Riassunto (La componente proteica e lipidica del latte umano e dei latti artificiali destinati all'infanzia: confronto e riflessi nutrizionali). - *Questo lavoro è il compendio di una serie di studi da noi condotti nel corso degli anni nel campo dell'alimentazione infantile. I dati finora raccolti ci hanno permesso di confrontare la composizione in proteine e lipidi di diversi latti in polvere con quella del latte materno e di valutare l'influenza dei diversi tipi di alimentazione sui livelli degli aminoacidi plasmatici e sugli acidi grassi eritrocitari. Nonostante i latti adattati presentino alcune differenze rispetto al latte umano, i nostri studi nutrizionali non hanno evidenziato differenze fisiologiche tra i lattanti alimentati al seno e quelli allattati artificialmente quando l'apporto di proteine e di acido linoleico si avvicina a quello del latte materno.*

Introduction

The first four to six months of life constitute the period of most rapid growth and development in an infant's life. All nutrients required during this period can be found in one single food item: human milk. The levels of nutrients and important components such as immunologically active substances cause human milk to be uniquely adapted to infant's needs.

Infant formulas used in bottle feeding must provide the same essential nutrients and at the same time should be adapted to infants limited food tolerance. There is an increased interest in recent years to formulate infant formulas based on cow's milk with a composition as similar as possible to mother's milk. International Committees (ESPGAN, AAP, CEE, LARN, RAD) use the composition of human milk as a reference to provide standards for infant formulas [1-5].

A knowledge of the composition of human milk and of the various formulas is essential to adequate management of infant feeding. Among the main milk components, proteins and lipids have a primary nutritional role.

Proteins provide infants with the essential aminoacids and nitrogen necessary for growth of lean body mass and for protein synthesis.

Fats, in addition to providing about 50% of all calories, are an essential nutrient for the development of the central nervous system and a vehicle for absorption of fat-soluble vitamins.

This work is the result of researches carried out for many years in the nutritional field. The data collected allow us to make a comparison between the protein and lipid composition of infant formulas and human milk, thus evaluating the influence formulas have on plasma aminoacid levels and erythrocyte fatty acids.

Protein

Methods

Nitrogen, non-protein nitrogen and aminoacids were determined in milk proteins.

Aminoacids were analyzed in infant's plasma.

Infant formulas were assayed to evaluate processing effects on constituent proteins by particular analysis.

Determination of protein nitrogen (N x 6.38). - Raw proteins were determined as nitrogen according to Kjeldahl [6]. Non-protein nitrogen was determined according to Kjeldahl's method after protein precipitation [7]. The difference between these two values is represented by protein nitrogen.

Determination of milk aminoacids after acid hydrolysis. - Defatted milk samples containing about 12-15 mg of protein were mixed with 15 ml HCl 6 N [8, 9] and placed in small penicilline-type bottles. The bottles were carefully closed with rubber stoppers and metallic rings, and then introduced into an air oven at 110 °C for 22 h. Then hydrochloric acid was removed by evaporation and the residue dissolved in buffer citrate at pH 2.2. A portion of the clear hydrolysate was analyzed with a Carlo Erba 3A29 aminoanalyzer equipped with ion-exchange column according to Spackman and Moore [10]. Since extensive destruction of cystine and methionine occurs in acid hydrolysis, these substances were determined as cysteic acid and methionine sulfone after oxidation at 0 °C for 16 h with a performic acid solution [11].

Determination of available lysine. - This method measures both total lysine in acid hydrolysate and the lysine remaining in solution after a separate hydrolysis of the material treated with 1-fluoro-2,4-dinitrobenzene (FDNB) [12]. The difference between these two values is represented by the lysine contained in the protein which has free ε-amino groups and is interpreted as available lysine. After treatment with FDNB in alkaline medium, acid hydrolysis liberates the mono ε-DNP-lysine and also hydrolyzes the NH-X groups of the "unavailable" lysine to form one molecule of free lysine, which is measured by the aminoanalyzer with a 0.1 M citrate buffer at pH 5.98.

Determination of tryptophan. - Food proteins were completely solubilized by partial hydrolysis with papain in the presence of 8 M urea. An aliquot was diluted with 8 M urea and the fluorescence of tryptophan directly measured at an emission wavelength of 348 nm and an excitation wavelength of 288 nm [13].

Determination of protein digestibility. - An *in vitro* method using a multienzyme system for the evaluation of protein digestibility was applied according to Satterlee *et al*. [14].

Determination of lysinoalanine. - A milk sample was hydrolyzed according to the previously described method. The determination of lysinoalanine was carried out by ion-exchange chromatography with aminoanalyzer employing a lithium citrate buffer at pH 4.50 for elution [15].

Determination of free and total hydroxymethylfurfural (HMF). - A milk sample was dissolved in methylalcohol determining HMF spectrophotometrically after thiobarbituric acid reaction [16, 17].

Determination of plasma amino acids. - Blood drawn 4 h after feeding was collected in heparinized tubes. The blood sample was centrifuged, the plasma was deproteinized with a sulfosalicylic acid solution [18, 19] and then frozen at - 20 °C until actual analysis was effected. Amino acid analyses were performed with lithium citrate buffers using an aminoanalyzer [20]. Tryptophan, aspartic acid and asparagine were not calculated because they were lost during the storage and preparation phases [21, 22].

Results

The proteic composition of human milk in three different stages of breast feeding was assayed:

- colostrum (up to 5th day),
- transitional milk (up to 15th day),
- mature milk (after 15th day).

The average protein (g/100 ml) and amino acid (μmoles/100 ml) contents of milk assayed are reported in Table 1 [23, 24]. Milk samples were collected from mothers living in Rome. Figure 1 shows a decrease in concentration of proteins in human milk during the course of lactation. In agreement with results reported in the literature, the protein content of milk decreases progressively: it is considerably higher in the colostrum phase, and decreases to half amount in mature milk [25]. Similarly, the content of each amino acid decreases as the duration of the lactation increases. The amount of non-protein nitrogen, in the form of urea, creatine, alpha-amino nitrogen compounds and other substances, is 15% in colostrum, rising to 21% in transitional milk and to 22% in mature milk (Table 1).

Concomitantly, 14 infant formulas, widely used for full-term infant feeding, were analyzed [26, 27]. At present, commercial formulas can be divided into four main classes: acidified, modified, partially adapted, adapted, the latter being the most suitable because of its similarity to human milk. With respect to the protein components, these formulas are modified by reducing protein concentration and sometimes varying the ratio of whey proteins to casein (80:20 in cow's milk, 40:60 in human milk). Table 2 presents the amount of proteins and aminoacids in 100 ml of milk reconstituted to 13.5%. The same table reports

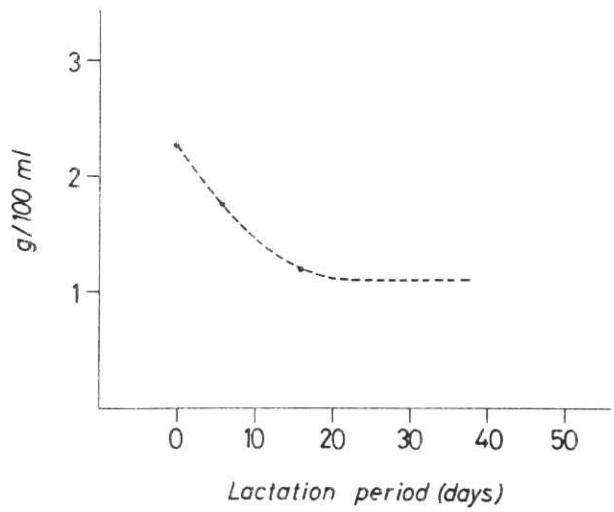

Fig. 1. - Changes in the content of proteins in mother's milk during the course of lactation.

human and cow milk composition to show the changes produced in different classes of infant formulas. From these results it derives that protein and amino acid contents are higher than in human milk: this difference increases as changes decrease; the opposite behaviour occurs only with cystine because its content in whey protein is eight times higher than in casein. To evaluate the nutritional damage of constituent proteins caused by processing, further studies were carried out.

Table 1. - *Protein (g/100 ml) and amino acid (µmoles/100 ml) content of human milk during course of lactation*

	Colostrum (8)*	Transitional milk (9)*	Mature milk (12)*
Protein	2.3 ± 0.74	1.8 ± 0.30	1.2 ± 0.24
Lysine	1017 ± 249	606 ± 157	571 ± 109
Histidine	390 ± 118	281 ± 146	236 ± 105
Arginine	644 ± 345	331 ± 61	236 ± 54
Aspartic acid	1590 ± 393	908 ± 155	760 ± 171
Threonine	1082 ± 319	567 ± 84	476 ± 110
Serine	1207 ± 360	611 ± 109	504 ± 118
Glutamic acid	2275 ± 496	1327 ± 169	1419 ± 255
Proline	1489 ± 373	1091 ± 166	784 ± 123
Glycine	939 ± 305	440 ± 115	372 ± 87
Alanine	1953 ± 821	574 ± 107	739 ± 278
1/2 Cystine	460 ± 142	111 ± 21	206 ± 52
Valine	1100 ± 245	541 ± 181	550 ± 123
Methionine	161 ± 58	80 ± 30	99 ± 22
Isoleucine	696 ± 106	425 ± 127	430 ± 81
Leucine	1570 ± 332	900 ± 142	853 ± 182
Tyrosine	496 ± 114	279 ± 52	244 ± 49
Phenylalanine	637 ± 135	259 ± 40	326 ± 54

The values are expressed as mean and standard deviation.
* number of samples.

For this purpose it was necessary to determine free and total hydroxymethtylfurfural [16] and lysinoalanine [15] contents to evaluate the Maillard reaction. The results confirm adequate processing taking protein digestibility as an example, and no significant differences between formulas were found. In addition, the average value of 84% is fairly close to that of human milk. From these studies it appears that some remarkable differences in protein composition exist between human milk, cow's milk and formulas. Consequently, it becomes important to know if these differences affect infant organ functioning.

For this purpose, an experimental study on the influence these three diets have on plasma amino acid levels was carried out at our Institute with a National Research Council grant [24]. Protein intake influence was evaluated in infants (10 days - 4 months) divided into 5 different groups based on the type of milk used (Table 3) and its protein content, minimum in human milk (1.2 g/100 ml), intermediate in 3 adapted formulas (1.5, 1.7 and 1.9 g/100 ml), maximum in cow's milk (2.5 g/100 ml).

Table 4 reports the data of free amino acids in infant plasma. The results obtained show that an increase in protein intake causes an increase in the number of amino acids with significative higher concentration than breast-fed infants. For an early recognition of the influence of dietary protein quantity and quality on plasma amino acids we calculated some parameters normally used in nutritional studies (Table 5). From the data analysis, it was possible to observe that only in infants nourished with diluted cow's milk signs of protein hypernutrition manifest themselves in a clear and complete way. The plasma of these babies showed a significant increase in free amino acids compared with the higher protein contribution. On the contrary, no significant variations were found in the behaviour of the various parameters in infants nourished

Table 2. - *Protein (g/100 ml) and amino acid (µmoles/100 ml) content of human milk, formulas and cow's milk*

	Human milk *	Adapted formulas					Partially adapted formulas				Modified formulas			Acidified formulas		Cow's milk **
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Protein	1.2 ±0.24	1.5	1.7	1.4	1.9	2.0	2.1	1.6	2.1	2.0	2.1	2.6	2.6	2.0	2.0	2.6
Lysine	571 ±109	758	942	813	859	803	1450	803	1191	877	1044	1293	1357	1228	1302	1448
Histidine	236 ±105	234	252	313	313	191	435	296	365	287	418	461	479	548	435	459
Arginine	236 ± 54	265	296	279	325	294	473	263	403	380	449	581	519	434	488	542
Aspartic acid	760 ±171	987	1194	1106	1146	862	1359	974	1197	1258	1177	1379	1329	1359	1197	1516
Threonine	476 ±110	670	843	850	873	555	918	669	771	827	839	827	850	941	480	1089
Serine	504 ±118	810	910	809	1002	822	1195	848	976	1092	1092	1143	1182	1118	1079	1356
Glutamic acid	1419 ±255	2177	2443	1945	2303	2175	2281	1955	2615	3093	3138	3203	3873	3074	2744	3777
Proline	784 ±123	1096	1347	1055	1372	1255	1700	1231	1595	1548	1724	2099	1982	1700	1747	2234
Glycine	372 ± 87	400	460	1205	504	432	593	450	522	611	575	629	593	611	539	71
Alanine	739 ±278	675	854	697	788	546	939	652	803	833	833	894	864	924	788	1002
1/2 Cystine	206 ± 52	154	210	212	223	101	256	134	178	212	123	201	178	201	145	192
Valine	550 ±123	815	931	795	956	807	1175	876	1129	922	1268	1302	1291	1175	1095	1479
Methionine	99 ± 22	223	252	535	741	638	967	597	905	741	658	1131	1152	926	823	463
Isoleucine	430 ± 81	608	734	700	813	638	926	792	875	772	937	937	1039	916	844	1247
Leucine	853 ±182	1175	1391	1132	1400	1070	1791	1194	1554	1400	1688	1760	1863	1698	1688	2024
Tyrosine	244 ± 49	338	351	621	834	905	1224	710	1171	1047	1331	1419	2360	1260	1384	745
Phenylalanine	326 ± 54	452	471	360	466	441	605	458	572	499	695	711	711	662	654	803
Tryptophan				204	302	229	196	221	163	245	196	204	188	147	163	

* Mean and standard deviation of 12 milk samples; ** diluted 3:1.

Table 3. - *Protein (g/100) and amino acid (μmoles/100 ml) content of human milk, formulas and cow's milk*

	Human milk *	Formula 1	Formula 2	Formula 3	Cow's milk**
Protein	1.2 ± 0.24	1.5	1.7	1.9	2.6
Lysine	571 ± 109	758	942	859	1448
Histidine	236 ± 105	234	252	313	459
Arginine	236 ± 54	265	296	325	542
Aspartic acid	760 ± 171	987	1194	1146	1516
Threonine	476 ± 110	670	843	781	1089
Serine	504 ± 118	810	910	1200	1356
Glutamic acid	1419 ± 255	2177	2443	2303	3777
Proline	784 ± 123	1096	1347	1372	2234
Glycine	372 ± 87	400	460	504	71
Alanine	739 ± 278	675	854	788	1002
1/2 Cystine	206 ± 52	154	210	223	192
Valine	550 ± 123	815	931	956	1479
Methionine	99 ± 22	223	252	326	463
Isoleucine	430 ± 81	608	734	813	1247
Leucine	853 ± 182	1175	1391	1400	2024
Tyrosine	244 ± 49	338	351	834	745
Phenylalanine	326 ± 54	452	471	466	803

* Mean and standard deviation of 12 milk samples; ** diluted 3:1.

Table 4. - *Plasma amino acids (μmoles/l)*

	Human milk (16)*	Formula 1 (4)*	Formula 2 (9)*	Formula 3 (8)*	Cow's milk (5)*
Taurine	75 ± 32	91 ± 61	72 ± 37	27 ± 8 ***	68 ± 42
Threonine	104 ± 42	133 ± 65	163 ± 44 ***	134 ± 33	159 ± 37 **
Serine	123 ± 32	122 ± 33	124 ± 36	127 ± 28	153 ± 35
Glutamic acid	95 ± 33	136 ± 67	99 ± 39	75 ± 29	131 ± 63
Glutamine	741 ± 228	772 ± 265	713 ± 81	743 ± 165	896 ± 191
Proline	189 ± 45	168 ± 53	185 ± 42	162 ± 34	311 ± 67 ***
Glycine	189 ± 48	187 ± 58	206 ± 41	184 ± 36	196 ± 45
Alanine	244 ± 83	248 ± 74	323 ± 88 **	264 ± 81	371 ± 154 **
Citrulline	16 ± 6	32 ± 12 ***	22 ± 10	24 ± 10 **	20 ± 7
Valine	148 ± 63	122 ± 23	165 ± 50	201 ± 34 **	328 ± 111 ***
1/2 Cystine	73 ± 21	65 ± 16	89 ± 52	117 ± 40 ***	93 ± 25
Methionine	31 ± 10	31 ± 6	37 ± 8	44 ± 8 ***	51 ± 9 ***
Isoleucine	48 ± 16	43 ± 7	51 ± 22	49 ± 9	98 ± 47 ***
Leucine	92 ± 31	73 ± 12	89 ± 34	92 ± 20	187 ± 90 ***
Tyrosine	78 ± 34	68 ± 13	66 ± 24	78 ± 25	154 ± 70 ***
Phenylalanine	55 ± 15	54 ± 11	66 ± 20	63 ± 21	113 ± 39 ***
Ornithine	84 ± 30	64 ± 23	111 ± 58	101 ± 31	98 ± 35
Lysine	165 ± 52	150 ± 54	158 ± 40	163 ± 33	213 ± 57
Histidine	91 ± 60	84 ± 32	107 ± 49	100 ± 13	92 ± 17
Arginine	74 ± 37	75 ± 18	72 ± 31	81 ± 18	112 ± 62

The values are expressed as mean and standard deviation; * number of subjects; ** $p < 0.05$; *** $p < 0.01$.

Table 5. - *Parameters of protein nutritional status*

	Human milk (16)*	Formula 1 (4)*	Formula 2 (9)*	Formula 3 (8)*	Cow's milk (5)*
Total amino acids (TAA)	2713 ± 555	2698 ± 414	2914 ± 328	2828 ± 429	3844 + 562 ***
Total non-essential amino acids (TNEAA)	1883 ± 466	1843 ± 376	1926 ± 171	1779 ± 302	2356 + 350
Total essential amino acids (TEAA)	886 ± 241	805 ± 143	988 ± 244	1042 ± 161	1488 + 420 ***
Total branched chain amino acids	287 ± 106	239 ± 33	305 ± 104	342 ± 58	613 + 245 ***
Glycine/Valine	1.43 ± 0.50	1.52 ± 0.50	1.37 ± 0.58	0.92 ± 0.18 **	0.66 +0.31 ***
TNEAA/TEAA	2.26 ± 0.53	2.41 ± 0.65	2.42 ± 0.35	1.71 ± 0.19 **	1.67 + 0.50 **
TEAA/TAA	0.325 ± 0.05	0.301 ± 0.06	0.336 ± 0.05	0.372 ± 0.03 **	0.384 + 0.07 **

The values are expressed as mean and standard deviation; * number of subjects; ** $p < 0.05$; *** $p < 0.01$.

with the two formulas whose protein content closely resembles that of human milk. An intermediate behaviour was found in infants nourished with the formula containing the highest protein content.

Lipids

Methods

Total lipids and fatty acids were determined in milk fat. In infants red blood cells fatty acids were analyzed.

Determination of total lipids. - The total lipid content was determined by the Rose-Gottlieb method [27] for human milk and by Soxhlet extraction after acid hydrolysis according to the EEC Official Method [28] for infant formulas.

Determination of milk fatty acids. - Methyl esters of fatty acids were prepared from the lipid extract according to the C41/C method of the Norme Grassi e Derivati [29]. We employed methyl alcohol-hydrochloric acid in sealed penicilline-type bottles heated for 3 h. Hydroquinone was used as antioxidant.

Table 6. - *Lipid content (g/100 ml) and fatty acid percentage of human milk and formulas*

	Human milk *	Adapted formulas			Partially adapted formulas				Modified formulas			Acidified formulas	
		1	2	3	4	5	6	7	8	9	10	11	12
Lipids	3.8	3.5	3.8	3.8	2.6	3.3	2.8	3.0	3.0	1.5	2.0	2.0	2.4
4:0 butyric acid		2.0	1.6	-	1.5	0.4	1.9	1.3	0.6	1.6	1.9	2.4	1.3
6:0 caproic acid	0.1 ± 0.0	1.9	1.2	0.4	1.1	0.5	1.5	1.2	0.6	1.4	1.6	2.2	1.0
8:0 caprylic acid	0.3 ± 0.1	1.2	1.1	5.2	0.7	0.9	0.9	1.4	0.8	0.8	1.0	1.3	0.7
10:0 capric acid	1.8 ± 0.6	2.8	1.7	4.0	1.5	1.1	2.1	2.2	1.3	2.3	2.1	3.5	1.7
12:0 lauric acid	6.7 ± 2.1	3.3	4.0	27.0	1.6	4.4	2.6	5.9	4.0	3.0	2.6	4.2	2.2
14:0 myristic acid	6.5 ± 1.7	9.2	6.3	11.3	5.8	4.5	7.8	8.1	5.0	7.6	7.8	10.6	5.8
14:1 myristoleic acid	0.3 ± 0.1	0.5	0.1	-	0.3	0.1	0.2	0.4	0.1	0.4	0.5	0.6	0.2
15:0 pentadecanoic	0.3 ± 0.1	0.8	0.5	-	0.7	0.1	0.8	0.6	0.2	0.8	0.6	0.7	0.4
16:0 palmitic acid	20.4 ± 1.3	24.2	29.6	10.4	18.5	34.0	21.2	25.1	32.3	23.6	23.0	28.0	28.5
16:1 palmitoleic acid	3.1 ± 0.9	0.5	0.3	-	0.4	0.1	0.6	0.5	0.2	0.7	0.8	0.7	0.2
17:0 eptadecanoic acid	0.5 ± 0.1	0.5	0.3	-	0.4	0.1	0.5	0.4	0.2	0.4	0.4	0.5	0.3
18:0 stearic acid	6.5 ± 1.2	8.8	7.3	2.6	8.1	4.9	8.0	6.6	5.3	7.1	7.2	9.0	6.8
18:1 oleic acid	36.5 ± 4.6	28.0	31.6	15.7	27.8	33.4	37.6	32.6	35.2	36.4	35.4	24.3	31.8
18:2 linoleic acid	12.1 ± 3.9	13.1	11.9	21.6	27.7	11.3	10.7	11.3	11.5	10.2	12.9	8.9	15.9
18:3 linolenic acid	1.0 ± 0.5	1.0	0.8	0.5	1.5	0.8	0.8	0.6	0.8	0.4	0.5	0.3	1.1
20:0 arachidic acid	0.3 ± 0.1	0.4	0.4	0.4	0.8	0.4	0.9	0.5	0.5	0.4	0.2	0.3	0.7
20:1 gadoleic acid	0.7 ± 0.2	0.5	0.4	0.3	0.5	0.4	0.8	0.5	0.5	0.5	0.4	0.1	0.4

* Mean and standard deviation of milk samples.

Table 7. - *Lipid components of human milk, formulas and cow's milk*

	Human milk *	Adapted formulas						Cow's milk **
		1	2	3	4	5	6	
Lipids (g/100ml)	3.8	3.1	3.6	3.6	3.6	3.2	2.5	2.7
Fatty acids (% w/w):								
4:0 butyric acid	-	1.3	-	2.0	-	-	-	3.6
6:0 caproic acid	0.1 ± 0.0	1.4	0.2	1.7	0.5	1.1	0.2	2.3
8:0 caprylic acid	0.3 ± 0.1	3.8	1.1	1.0	6.0	0.2	2.7	1.3
10.0 capric acid	1.8 ± 0.6	3.4	0.8	1.9	4.1	1.4	1.9	2.7
12:0 lauric acid	6.7 ± 2.1	1.7	5.1	2.0	27.6	1.7	13.0	3.3
14:0 myristic acid	6.5 ± 1.7	5.2	2.8	6.1	10.4	5.7	5.4	10.7
14:1 myristoleic acid	0.3 ± 0.1	0.8	0.1	0.9	-	1.1	-	1.4
15:0 pentadecanoic acid	0.3 ± 0.1	0.7	0.1	0.8	-	0.7	-	1.2
16:0 palmitic acid	20.4 ± 1.3	18.2	32.6	24.8	9.5	28.5	28.2	27.6
16:1 palmitoleic acid	3.1 ± 0.9	1.6	0.3	1.8	0.2	1.7	0.1	2.6
17:0 eptadecanoic acid	0.5 ± 0.1	0.7	0.2	0.7	0.1	0.7	0.1	0.9
18:0 stearic acid	6.5 ± 1.2	7.6	5.4	8.5	2.2	7.4	4.1	10.1
18:1 oleic acid	36.5 ± 4.6	31.4	33.4	30.3	14.1	31.0	26.9	26.0
18:2 linoleic acid	12.1 ± 3.9	15.6	16.5	13.0	23.0	16.3	16.6	2.5
18:3 linolenic acid	1.0 ± 0.5	-	0.4	0.9	0.2	0.8	-	1.4
20:0 arachidic acid	0.3 ± 0.1	1.1	0.5	0.4	0.2	0.4	0.3	0.6
20:1 gadoleic acid	0.7 ± 0.2	-	0.2	1.1	0.2	1.1	0.4	0.3
Unsaturated fatty acids (% w/w)	54	51	51	48	37	52	44	34
Linoleic acid content (kcal/100 kcal)	6	7	8	6	11	7	6	1

* Mean and standard deviation of milk samples; ** diluted milk 3:1.

Gas-chromatographic analysis was carried out by packed column (3 m x 2 mm ID) on SP2330 10% Chromosorb W AW 100/120 mesh, with nitrogen 20 ml/min as carrier gas, injector temperature of 230 °C, detector temperature (FID) 240 °C, oven temperature programmed from 70 °C with starting isotherm of 8 min to 200 °C at a heating rate of 4 °C/min.

Weight percent calculations were performed using response factors obtained from specific concentrations standards.

Determination of erythrocyte fatty acids - The blood was collected in heparinized test tubes and the erythrocytes were separated from plasma by centrifugation at 3000 r.p.m. for 10 min. After washing the erythrocytes with saline solution at pH 7.4 [30] lipids were extracted with chloroform-methyl alcohol according to the Dodge and Phillips method [31]. This extract was employed to prepare the methyl esters according to Angelico *et al.* [32] using butylated hydroxytoluene (BHT) as antioxidant. The methyl esters were isolated by thin layer chromatogra-

Table 8. - *Lipid content (g/100 ml) and fatty acid percentage of assayed milks*

	Human milk *	Adapted formula	Special formula	Cow's milk **
Lipids	3.8	3.6	3.6	2.7
4:0 butyric acid	-	1.0	-	3.6
6:0 caproic acid	0.1 ± 0.0	1.0	0.5	2.3
8:0 caprylic acid	0.3 ± 0.1	1.1	6.0	1.3
10:0 capric acid	1.8 ± 0.6	1.4	4.1	2.7
12:0 lauric acid	6.7 ± 2.1	3.6	27.6	3.3
14:0 myristic acid	6.5 ± 1.7	4.5	10.4	10.7
14:1 myristoleic acid	0.3 ± 0.1	0.5	-	1.4
15:0 pentadecanoic acid	0.3 ± 0.1	0.5	-	1.2
16:0 palmitic acid	20.4 ± 1.3	29.2	9.5	27.6
16:1 palmitoleic acid	3.1 ± 0.9	1.1	0.2	2.6
17:0 eptadecanoic acid	0.5 ± 0.1	0.5	0.1	0.9
18:0 stearic acid	6.5 ± 1.2	7.0	2.2	10.1
18:1 oleic acid	36.5 ± 4.6	31.9	14.1	26.0
18:2 linoleic acid	12.1 ± 3.9	14.8	23.0	2.5
18:3 linolenic acid	1.0 ± 0.5	0.7	0.2	1.4
20:0 arachidic acid	0.3 ± 0.1	0.5	0.2	0.6
20:1 gadoleic acid	0.7 ± 0.2	0.7	0.2	0.3

* Mean and standard deviation of milk samples; ** diluted milk 3:1.

Table 9. - *Erythrocyte fatty acids (g/100 g)*

	Human milk (11)*	Adapted formula (8)*	Special formula (4)*	Cow's milk (85)*
16:0 palmitic acid	29.35 ± 3.35	29.85 ± 2.06	30.43 ± 1.91	30.68 ± 2.19
16:1 palmitoleic acid	0.40 ± 0.10	0.39 ± 0.11	0.33 ± 0.06	0.60 ± 0.14 ***
18:0 stearic acid	17.22 ± 3.00	15.70 ± 2.29	14.73 ± 0.15	13.86 ± 1.68 **
18:1 oleic acid	14.44 ± 1.74	12.64 ± 1.07 **	12.80 ± 1.68	15.56 ± 1.88
18:2n6 linoleic acid	6.20 ± 1.72	7.31 ± 1.30	9.27 ± 0.85 **	5.52 ± 0.85
18:3n6 γ-linolenic acid	< 0.10	< 0.10	< 0.10	< 0.10
20:0 arachidic acid	0.62 ± 0.19	0.56 ± 0.11	0.53 ± 0.06	0.54 ± 0.11
18:3n3 α-linolenic acid	< 0.10	< 0.10	< 0.10	< 0.10
20:1 gadoleic acid	0.34 ± 0.05	0.28 ± 0.14	0.27 ± 0.06	0.50 ± 0.07 ***
20:3n9 eicosatrienoic acid	0.25 ± 0.11	0.17 ± 0.05	0.20 ± 0.00	0.24 ± 0.08
20:3n6 eicosatrienoic acid	1.36 ± 0.48	1.28 ± 0.32	1.13 ± 0.32	1.12 ± 0.37
22:0 behenic acid	1.05 ± 0.30	1.15 ± 0.13	1.10 ± 0.09	1.04 ± 0.18
20:4n6 arachidonic acid	9.59 ± 3.36	9.90 ± 1.81	9.13 ± 1.25	7.58 ± 0.93
24:0 lignoceric acid	2.25 ± 0.59	2.43 ± 0.49	2.10 ± 0.44	2.04 ± 0.43
22:4n6 docosatetraenoic acid	2.02 ± 0.59	2.13 ± 0.37	2.23 ± 0.15	1.98 ± 0.47
24:1 nervonic acid	2.48 ± 0.67	2.29 ± 0.62	2.10 ± 0.50	2.84 ± 0.58
22:5n6 eicosapentaenoic acid	0.75 ± 0.26	0.69 ± 0.11	0.80 ± 0.00	0.60 ± 0.23
22:5n3 eicosapentaenoic acid	0.56 ± 0.29	0.45 ± 0.20	0.27 ± 0.21	0.46 ± 0.24
22:6n3 docosahexaenoic acid	2.37 ± 1.07	2.23 ± 0.54	2.30 ± 0.79	1.88 ± 0.52

The values are expressed as mean and standard deviation; * number of subjects; ** $p < 0.05$; *** $p < 0.0.1$.

phy to eliminate possible interferences [31, 33]. The gas-chromatographic analysis was carried out on SP 2330 fused silica capillary column (30 m x 0.25 mm ID), with helium 0.75 ml/min as carrier gas, injector temperature of 230 °C, detector temperature (FID) 240 °C, oven temperature from 100 °C with starting isotherm of 5 min to 200 °C at a heating rate of 2 °C/min and a split ratio of 1:40.

Results

In our studies carried out some years ago, on different infant formulas [27, 34, 35] deficiencies in lipid fraction with respect to human milk were often found.

Table 6 reports lipid content (g/100 ml) and percentage of fatty acids of human milk and infant formulas, reconstituted to 13.5 %. Human milk samples were collected from mothers living in Rome and the values averaged.

Assayed formulas are included in the previously described classes characterized by different composition changes. As regards lipid component, these formulas are modified by partial or total substitution of milk fat with vegetable oils.

Table 6 shows that most formulas provide a much lower content of lipids than human milk; the same goes for unsaturated fatty acid percentage. Finally, linoleic acid-originating calories are extremly variable and in some samples lower than the minimum value of human milk (3%).

At present, adapted formulas are the most widely used and a recent study has proven that lipid content resembles the human milk level, as indicated in Table 7; likewise, linoleic acid-originating calories are standardized and their values are equal or higher than human milk. Unsaturated fatty acids on the contrary are still low in spite of changes produced.

The comprehensive analysis of the data reported in Table 7, including also cow's milk composition, emphasizes the different fatty acid concentration of one sample (no. 4), although it belongs to adapted formulas.

Since differences in lipid composition exist between human milk, cow's milk and formulas, it is important to know the eventual effects it has on erythrocyte fatty acids [36-39].

Lipid intake influence was evaluated in infants (10 days - 4 months) divided into 4 different groups based on the type of milk used: human milk, adapted formulas, special formulas and cow's milk (Table 8).

Table 9 summarizes erythrocyte fatty acid percentages. Although dietary fatty acids are different, particularly significant differences between bottle-fed and breast-fed infants are not apparent.

Conclusion

Breast-feeding is superior to any form of infant feeding and therefore it is unnecessary to further elaborate this point.

When breast feeding is not possible it is advisable to use adapted formulas, as they are the most suitable substitutes [40]. In fact, our nutritional studies did not find physiological differences between breast and bottle-fed infants if formulas providing an adequate intake of proteins and linoleic acid were used.

Cow's milk is recommended only for older infants during the mixed feeding period.

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