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Biochemistry of human milk in early lactation

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Summary: With the analysis of more than 550 human milk samples we showed that triglycerides (accounting for 96–99 % of the total lipids) increased from 2 to 3.5 g/100 ml mainly during the first week postpartum and remained constant thereafter. In contrast, both cholesterol and vitamin E concentrations decreased from 35 to 20 mg/100 ml and from 1.7 to 0.30 mg/100 ml, respectively. The phospholipids remained constant (40 mg/100 ml). Also the fatty acid composition of total lipids changed. Mid chain fatty acids (C10, C12, C14) increased, whereas the long chain polyunsaturated fatty acids decreased. The P/S-ratio of 0.32 remained constant throughout lactation.

The mean protein content of human milk decreased from approximately 2 g/100 ml at day 2 to approximately 1 g/100 ml at day 36 of lactation. The content of each individual amino acid decreased likewise. However, when we expressed the amino acid values in relation to the protein value – that is as g amino acid per g protein – some decreased, some remained constant and some increased indicating a changing protein pattern. From these data we computer-calculated a sharp decrease in IgA content, a moderate decrease for lactoferrin and constant values for casein and a-Lactalbumin.

Throughout the whole period of investigation, non protein nitrogen accounted for about 20 % of total nitrogen. Although the absolute values decreased according to total nitrogen, the sum of free amino acids, as well as the amount of taurine, remained constant.

Lactose increased from about 4 g/100 ml to 6 g/100 ml during the first days of lactation.

The calcium content increased from a mean value of 25 mg/100 ml at day 1 to 32 mg/100 ml at day 5 and remained constant at 30 mg/100 ml up to day 36. Phosphorus content increased from 10 mg/100 ml at day 1 to 17 mg/100 ml at day 8 and then continuously decreased to 13 mg/100 ml at day 36.

The protein-bound part of the calcium remained constant during the period investigation, the fat-associated part increased from 11 % to 26 %.

Protein-bound phosphorus decreased from 45 % in colostral milk to 29 % in transitional and 23 % in mature milk.

Zusammenfassung: Mit der Analyse von mehr als 550 Frauenmilchproben konnten wir zeigen, daß ihr Triglyceridgehalt, der in der Regel 96–99 % der Gesamtlipide ausmacht, innerhalb der ersten Laktationswoche von 2 auf 3,5 g/100 ml ansteigt und dann konstant bleibt. Im Gegensatz dazu nahmen die Gehalte an Vitamin E (1,7 mg/100 ml–0,3 mg/100 ml) und Cholesterin (35 mg/100 ml–20 mg/100 ml) ab. Die Phospholipidkonzentration blieb mit Werten um 40 mg/100 ml weitgehend konstant. Die Fettsäurezusammensetzung der Gesamtlipide änderte sich ebenfalls. Dabei nahmen die prozentualen Anteile der mittelkettigen Fettsäuren (C10, C12, C14) zu, wobei gleichzeitig derjenige der langkettig polyungesättigten Fettsäuren abnahm. Der P/S-Quotient blieb mit 0,32 konstant. Der durchschnittliche Eiweißgehalt nahm von ungefähr 2 g/100 ml am 2. Tag der Laktation auf etwa 1 g/100 ml am 36. Tag ab. Entsprechend nahmen die Gehalte aller Aminosäuren ab. Wurden ihre Gehalte jedoch in Relation zum jeweiligen Eiweißgehalt berechnet – also in g Aminosäure pro g Eiweiß –, nahmen die Gehalte einiger Aminosäuren zu, andere blieben konstant, und wieder andere nahmen ab. Dies kann nur mit einer sich ändernden Eiweißzusammensetzung der Muttermilch erklärt werden. Anhand unserer Daten konnten wir einen steilen Abfall des IgA- und des Laktoferringehaltes innerhalb der ersten Laktationswoche berechnen. Während des gesamten Untersuchungszeitraumes blieb der Anteil des Nicht-Protein-Stickstoffs (NPN) am Gesamtprotein mit 20 % weitgehend konstant. Obwohl die Gesamtmenge an NPN abnahm, blieb die Summe aller freien Aminosäuren und auch der Gehalt an Taurin konstant. Der Laktosegehalt nahm von ungefähr 4 g/100 ml auf 6 g/100 ml zu. Der Calciumgehalt nahm im Mittel von 25 mg/100 ml am 1. Tag auf 32 mg/100 ml am 5. Tag der Laktation zu und blieb dann konstant. Entsprechend nahm Phosphor innerhalb der ersten 5 Tage von 10 mg/100 ml auf 17 mg/100 ml zu, um dann wieder langsam auf Werte von 13 mg/100 ml bis zum 36. Tag abzufallen. Der eiweißgebundene Anteil des Calciums blieb mit 35 % weitgehend konstant, wogegen der fettassoziierte Anteil von 11 auf 26 % zunahm. Eiweißgebundener Phosphor nahm von 45 % in Kolostralmilch über 29 % in transitorischer Milch auf 23 % in reifer Milch ab.

Key words: human milk, lipids, protein, lactose, minerals, trace elements

Introduction

Human milk is uniquely adapted to the nutritional needs of the infant during the first month of life. Although its composition is variable from mother to mother, from day to day, between different times of the day and even during one single nursing (26–28, 34, 37, 44), it has been proposed that the average composition of human milk can be taken as a basis for the calculation of the infant's requirements. In the absence of other reliable data it remains the best guide available for recommendations on the composition of formula feeds (14).

In recent years much analytical work has been done on human milk and many data have been accumulated with respect to its protein, fat, carbohydrate, mineral, trace element, and vitamin content (4, 23, 36). Nevertheless, there are still a lot of open questions. For example, the exact protein composition is not known. Mostly because of methodological problems, there have been published no convincing data on the exact whey to casein ratio (3, 5, 39–41). Also little is known about the different casein fractions occurring in human milk (20, 40, 41). Further, it became clear in recent years that not only the composition of human milk but also interactions between the different components are of great importance. An example would be the interaction of trace elements with different chelating agents (12, 15, 38) which may be the reason for the good bioavailability of trace elements from mothers' milk.

For many compounds identified in human milk so far, it is not yet clear what their physiological role for the infant may be. Of course drugs, pesticides, heavy metals or other environmental chemicals are thought to be harmful. But what about the high levels of cholesterol or the living cells? Are they of any physiological benefit?

This leads to a point where data on the human milk composition become of limited value to the question of the infant's requirements. Additional information can only be obtained by appropriately designed clinical studies. They, however, can only be done based on reliable data on the levels of the various nutrients found in mothers' milk.

In this paper, data are represented on the changing composition of human milk during the first five weeks of lactation. As in the past many studies have been done on randomly sampled milks, which may have led to somewhat questionable results (25, 48), all our milk samples were obtained according to a standardized method of collection. This is thought to provide profound data for establishing the average composition of mature human milk with respect to its major nutrients.

Materials and Methods

Breast milk samples were obtained from healthy mothers of term infants on the 1st, 3rd, 5th, 15th, 22nd, 29th, and 36th day of lactation. All mothers breast-fed their babies, but on the study days they completely expressed both breast into sterile containers at each feed using an electric breast pump. An aliquot was removed immediately for analysis and the remainder was fed to the infant as required. Samples from each 24 hour period were appropriately pooled and stored at −20 °C until analyzed.

Lipids including triglycerides, phospholipids, cholesterol and fatty acids were analyzed as described previously (26). Vitamin E was determined after hexane extraction using reversed phase HPLC and fluorescence detection (33). Protein and lactose were measured by the Lowry method (42) using an enzyme test kit from Boehringer, Mannheim, respectively. Zinc, copper, magnesium, and calcium were analyzed after ashing the milk samples by atomic absorption spectroscopy, using a model 430 atomic absorption spectrophotometer from Perkin Elmer. Phosphorus was assayed by a vanadium molybdenum method according to Epps (13). Analysis of non-protein-nitrogen including taurine, urea, and free glutamic acid was done as described previously (29). Appropriate statistical analyses were performed according to Glanz (18).

Results and Discussion

Triglycerides (TG), accounting for 96–99 % of the total lipids, increased from 2 to approximately 3.5 g/100 ml mainly during the first week postpartum and remained constant thereafter (Fig. 1). In contrast, both vitamin E and cholesterol (chol) concentrations decreased from 1.7 to 0.3 mg/100 ml and from 35 to 20 mg/100 ml, respectively, whereas the phospholipids (PL) remained rather constant (40 mg/100 ml) all throughout lactation (Fig. 2).

From these data it could be calculated that there is a drastic decrease of both PL/TG and chol/TG ratios. As PL and chol are constituents of the fat globule membrane (49) and TG is the major component of the core one has to assume that the size of milk fat globules increases as lactation progresses. This is further evidenced by the fact that appropriate regression analysis (TG versus chol and TG versus PL) resulted in different regression lines for either colostral or mature human milk (Table 1, 2).

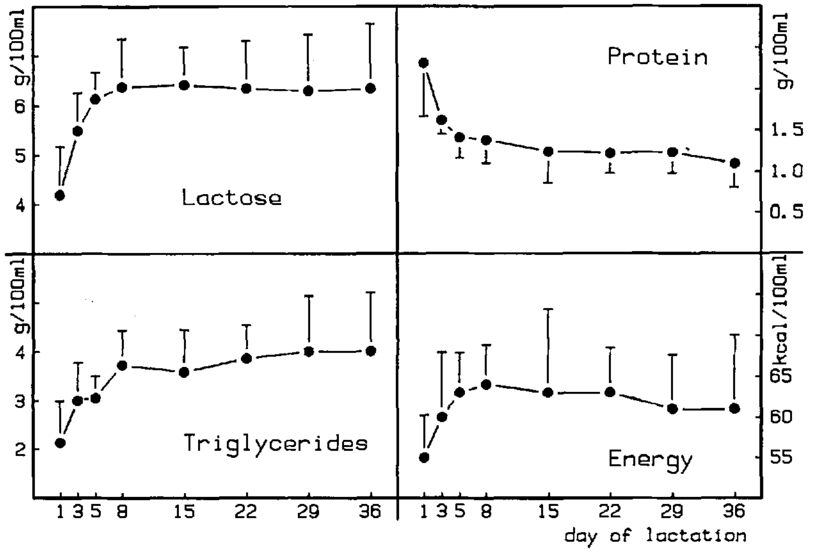

Fig. 1. Lactose (n = 10), total protein (n = 10), triglycerides (n = 15), and energy (n = 10) of human milk in the first five weeks of lactation (means, SD).

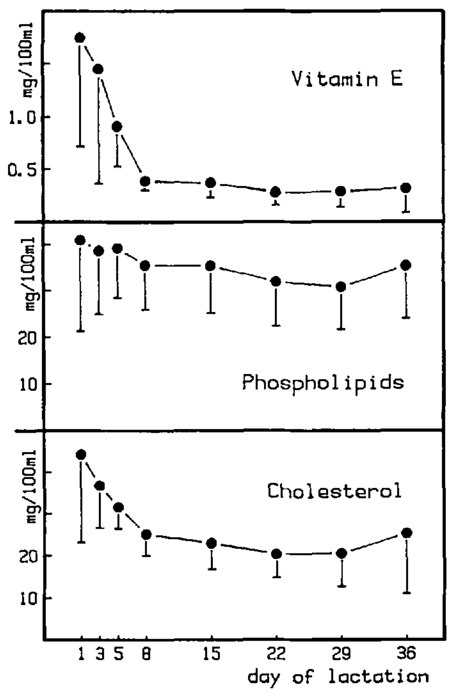

Fig. 2. Vitamin E (alpha, beta + gamma-tocopherol), phospholipids, and cholesterol of human milk in the first five weeks of lactation (n = 9, means, SD).

Table 1. Correlations between TG and PL in human milk. y = TG (mg/100 ml), x = PL (mg/100 ml).

Day of lactation	n	y = a + bx	Correlation coefficient
1	12	y = 185 + 49x	0.66
3	11	y = 379 + 60x	0.65
5	15	y = 391 + 72x	0.62
8	16	y = 476 + 71x	0.89
15	14	y = 528 + 76x	0.67
22	15	y = 595 + 80x	0.89
29	14	y = 416 + 84x	0.80
36	15	y = 437 + 88x	0.92
1– 5 (colostral)	38	y = 218 + 60x	0.61
8–15 (transitional)	30	y = 817 + 65x	0.75
22–36 (mature)	44	y = 476 + 84x	0.86

At any given amount of fat in human milk, the overall oil/water interface is determined by the size of the fat globules: the smaller the globules, the larger the interface. A large surface on which the infant's lipase can act is thought to be a prerequisite for optimal lipid hydrolysis. In this regard the good fat absorption from human milk by the newborn, despite poor pancreatic secretion (19) may at least partially be explained by the small fat globules at the early stages of lactation.

The changing fatty acid (FA) pattern of human milk during lactation is presented in Table 3. As can be seen, the quantitatively predominating FA such as C16, C18 and c18:1w9 steadily decreased in progressing lactation, accompanied by an increase of the mid-chain FA C12 and C14. As the latter compounds are exclusively synthesized by the mammary gland (2) and unlike the others are not transferred from the mother's blood to the milk, it seems that the capacity of the glands to synthesize these FA was

Table 2. Correlations between TG and Chol in human milk. y = TG (mg/100 ml), x = Chol (mg/100 ml).

Day of lactation	n	y = a + bx	Correlation coefficient
1	12	y = 852 + 33x	0.57
3	11	y = 631 + 74x	0.76
5	15	y = 705 + 75x	0.67
8	16	y = 1233 + 75x	0.81
15	14	y = 1686 + 86x	0.60
22	15	y = 1754 + 100x	0.63
29	14	y = 1374 + 112x	0.52
36	15	y = 1400 + 118x	0.75
1– 5 (colostral)	38	y = 1653 + 28x	0.35
8–15 (transitional)	30	y = 2291 + 28x	0.54
22–36 (mature)	44	y = 2291 + 48x	0.68

Table 3.

Fatty acid	Day							
	1	3	5	8	15	22	29	36
C10	0.09	0.26	0.38	0.61	0.79	0.88	0.96	0.86
C11	ND	tr	tr	tr	ND	ND	tr	tr
C12	1.13	2.25	2.93	3.87	4.91	5.82	6.16	5.47
C14	4.25	5.34	5.26	5.91	6.73	7.48	7.82	7.20
C14:1tw5	0.07	0.11	0.09	0.11	0.17	0.11	0.12	0.11
C14:1w5	0.35	0.53	0.50	0.57	0.54	0.52	0.51	0.49
C16	26.21	26.01	25.66	24.79	23.27	23.29	22.53	23.10
C16:1tw7	0.59	0.57	0.51	0.45	0.50	0.46	0.50	0.48
C16:1w7	3.46	3.55	4.36	3.92	4.10	3.90	4.05	3.90
C17	0.75	0.79	ND	0.68	tr	tr	ND	ND
C17:1tw7	tr	tr	tr	tr	0.03	tr	0.03	tr
C17:1w7	tr	0.19	0.21	0.22	tr	0.17	0.21	0.16
C18	9.32	8.30	8.44	8.55	8.48	8.61	8.72	8.37
C18:1	38.68	37.76	37.92	36.36	36.82	36.01	34.92	35.02
C18:2w6	9.70	10.30	9.97	10.76	10.14	9.96	10.04	11.78
C18:3w6	tr	0.67	0.81	tr	1.04	0.36	0.91	ND
C20:1w9	1.92	1.37	1.04	0.88	0.70	0.81	0.93	0.85
C18:3w3	0.60	0.70	0.62	0.69	0.70	0.70	0.83	0.71
C20:2w6	0.90	0.65	0.51	0.44	0.33	0.32	0.39	0.35
C22	tr	tr	tr	tr	tr	tr	tr	tr
C20:3w6	0.42	0.35	0.37	0.34	0.38	0.29	0.34	0.30
C22:1w9	0.46	0.20	0.20	0.19	0.91	tr	tr	tr
C20:4w6	0.75	0.55	0.54	0.50	0.43	0.36	0.39	0.39
C22:2w6	0.24	0.17	0.24	0.17	0.06	0.08	0.12	0.12
C24	0.45	0.29	0.19	0.15	0.10	0.10	0.14	0.20
C20:5w3	0.64	0.43	0.18	0.22	0.16	0.14	0.13	0.05
C24:1w9	0.68	0.36	0.31	0.18	0.11	tr	0.17	0.22
C22:4w6	0.25	0.15	0.12	0.09	0.05	tr	0.06	0.05
C22:5w6	0.13	0.10	0.05	0.06	0.03	tr	tr	tr
C22:5w3	0.25	0.13	0.11	0.09	0.06	tr	0.06	0.05
C22:6w3	0.22	0.21	0.29	0.26	0.17	0.15	0.16	0.16

not fully developed at birth. The suckling of the infant affects the mother's prolactin synthesis, causing a further maturation (21).

The problem of the infant's requirement for essential fatty acids has been discussed in many papers over the past several years (6–9, 51). It is well accepted that the infant has an urgent need for linoleic and linolenic acids, in order to synthesize long-chain polyunsaturated fatty acids (PUFA), which are important for cell membrane and prostaglandin synthesis (11, 21). However, there are still some doubts whether the newborn is capable of synthesizing these FA in sufficient amounts (6, 22). Our results may imply that a certain amount of dietary long-chain PUFA are necessary to meet the infant's requirements. This assumption is supported by the fact that long-chain PUFA (chain length > C18) as part of the total polyunsaturates were about 30 % in colostrum declining to 15 % in mature milk (Table 3). The P/S-ratio (0.32) remained at a constant level

Table 4. Regression analysis of vitamin E and other lipids in human milk. y = vitamin E (mg/100 ml); x = triglycerides or cholesterol or phospholipids (mg/100 ml), respectively. Colostral milk: n = 25; mature milk: n = 43.

Correlation between	y = a + bx	Correlation coefficent (r)
Cholesterol and vitamin E		
colostral	y = −0.58 + 0.05608x*	0.75
mature	y = +0.08 + 0.01425x*	0.71
Triglycerides and vitamine E		
colostral	y = +1.04 + 0.00017x	0.17
mature	y = +0.13 + 0.00008x	0.45
Phospholipids and vitamin E		
colostral	y = +0.39 + 0.02849x	0.40
mature	y = +0.12 + 0.00857x	0.51

* regression coefficient (slope) significant $p < 0.0001$.

throughout lactation, because the decrease of long-chain PUFA was compensated for by an increase of linoleic acid. This may reflect the increasing capability of the infant to synthesize long-chain PUFA from linoleic acid.

Regression analysis performed with the different lipid fractions of human milk shown in Table 1 are in accordance with the theory that PL and chol form the membrane of secreted fat gloubles and therefore derive

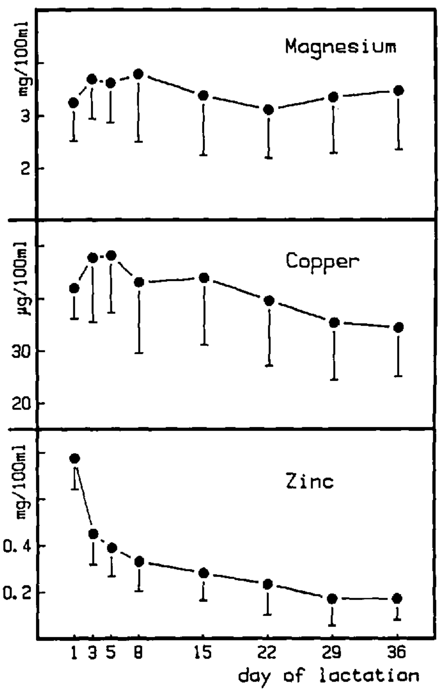

Fig. 3. Magnesium, copper, and zinc of human milk in the first five weeks of lactation, (means, SD) n = 10.

from the apical membrane of secretory cells (10, 46, 47). They may, however, also indicate that at least some chol is secreted by a membrane independent pathway (30). In addition, regression analysis performed with vitamin E and the different lipids shown in Table 4 gave rise to the assumption that the secretion of chol and vitamin E are closely interrelated. Since no or only weak correlations could be found between vitamin E and either TG or PL, we speculated that vitamin E is only partially secreted as constituent of the apical membrane of secretory cells. Good correlations between vitamin E and chol led us to assume that the membrane independently secreted chol is the carrier for some part of the vitamin E (31).

From Figure 3 it can be seen that different trace elements change differently during lactation. Magnesium for example remained rather constant throughout the period of investigation (approx. 3.5 mg/100 ml). Copper steadily decreased from about 50 µg/100 ml in the first days to 35 µg/100 ml at day 36. It seems that the copper content may further decrease in prolonged lactation. The decrease of zinc concentration was very rapid during the first 8 days (0.8 mg/100 ml to 0.35 mg/100 ml) and then steadily reached mature levels of about 0.2 mg/100 ml at day 36.

Figure 4 shows the calcium (Ca) and phosphorus (P) concentrations. Contrary to the levels of the investigated trace elements, they increased

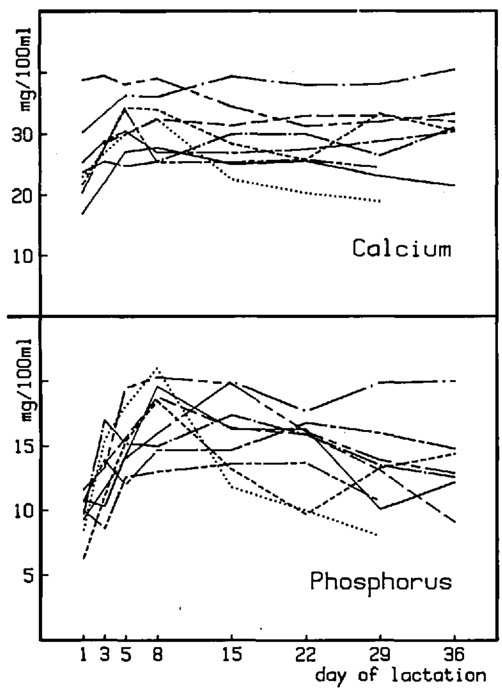

Fig. 4. Calcium and phosphorus of human milk in the first five weeks of lactation. Courses of 9 individual mothers.

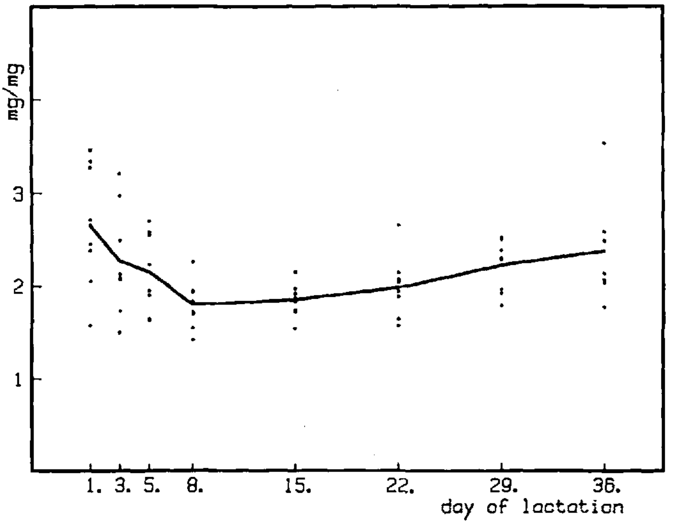

Fig. 5. Calcium to phosphorus ratio of human milk during the first five weeks of lactation (– mean; individual data points).

during the early stages of lactation and remained rather constant from day 8 on. The presentation of the individual values and courses clearly demonstrates the high variability in Ca and P contents of milks from different mothers and from different stages of lactation. The Ca-P ratio is close to 2, independent of the maturity of the milks (Fig. 5). The protein bound part of Ca (35 %) remained constant during the investigation period, whereas the fat associated part increased from 11 to 26 % indicating either a change in fat globule membrane composition, an increase in its Ca-binding capacity, a different degree of saturation or a non fat related Ca-association within the water phase of the separated cream. Further research is needed in this field.

Protein bound P decreased from 45 % of total P in colostral milk to 29 % in transitional and 23 % in mature milk. As in human milk only IgA and lactoferrin decrease with time (Fig. 7), it seems that the decrease of protein bound P is associated with the decrease of these two proteins. In this respect, preliminary data from our laboratory have shown that mainly lactoferrin has a rather high binding capacity for inorganic phosphate ions, indicating a new not yet described feature of lactoferrin.

Sodium and potassium changed as shown in Figure 6, the mean potassium concentration of mature human milk being approx. 60 mg/100 ml and that of sodium being approx. 30 mg/100 ml. The mean protein content of human milk decreased from approx. 2 g/100 ml at day 2 to approx. 1 g/100 ml at day 36 of lactation, as can be seen from Figure 1. The content of each individual amino acid decreased likewise, as has been expected. However, when we expressed the amino acid values in relation to the protein value – that is as g amino acid per g protein – only threonine, serine, glycine, alanine, cystine, valine, and arginine decreased, while

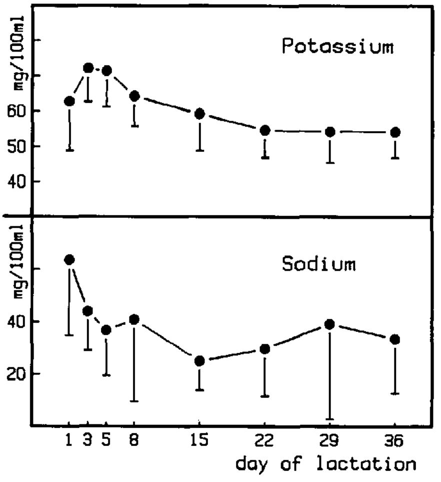

Fig. 6. Potassium and sodium of human milk during the first five weeks of lactation, (means, SD) n = 10.

asparagine, methionine, tyrosine, phenylalanine, and tryptophan remained constant and glutamic acid, proline, isoleucine, leucine, histidine, and lysine increased (3) (Table 5).

This can only be explained by a changing protein composition as lactation progresses. We could show this change in the human milk protein

Table 5. Amino acid composition of human milk, (means) n = 10.

	Day 2		Day 8		Day 36	
	mg/100 ml	mg/g protein	mg/100 ml	mg/g protein	mg/100 ml	mg/g protein
Asp	218	107	153	112	121	109
Thr	144	71	80	58	60	54
Ser	148	73	83	61	64	58
Glu	332	163	252	184	215	194
Pro	177	87	134	98	113	102
Gly	79	39	43	31	34	31
Ala	106	52	65	47	49	44
Cys	53	26	28	20	19	17
Val	139	68	87	64	66	59
Met	25	12	19	14	15	14
Ile	97	48	80	58	65	59
Leu	220	108	155	113	124	112
Tyr	119	58	78	57	64	58
Phe	110	54	73	53	60	54
His	71	35	52	38	44	40
Lys	155	76	114	83	91	82
Arg	123	60	68	50	49	44
Trp	55	27	35	26	30	27
Total protein	2040		1380		1100	

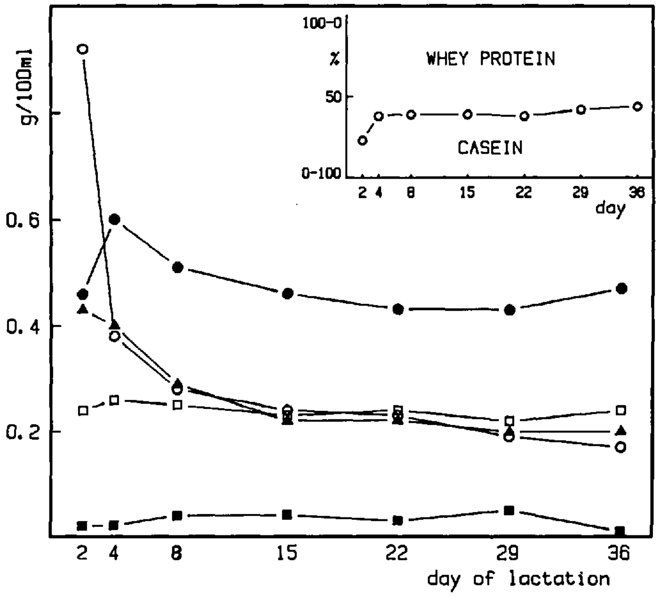

Fig. 7. Changing pattern of human milk protein composition during the first five weeks of lactation (calculated according to 43). – ● – casein, – ■ – α-Lactalbumin, – ▲ – lactoferrin, – ○ – sIgA, – □ – serum albumin.

composition by computer calculation (32) based on the amino acid composition of the isolated proteins casein, IgA, lactoferrin, α-lactalbumin, serum albumin. As can be seen from Figure 7, we found a sharp decrease in the IgA content, a moderate decrease for lactoferrin and constant values for casein and α-lactalbumin as well as for serum albumin. Interestingly these data indicate a time dependent change in whey protein to casein ratio in human milk from about 80/20 in early lactation to 55/45 in mature milk. Preliminary, experimental data from our laboratory, using an appropriate HPLC method for the separation and quantification of all major proteins are in good accordance with the calculated results.

Only very recently it has been pointed out that apart from the immunological and/or bacteriostatical relevance of IgA and lactoferrin, their presence in human milk also has nutritional implications. The fact that both lactoferrin and IgA were found in the faeces of breast-fed infants (45, 52), makes it reasonable to assume that they are resistant to proteolytic digestion in the newborn's GI tract. This led Hambraeus et al. (24) and Räihä (50) to conclude that the amount of nutritionally available protein in human milk could be as low as 0.7 g/100 ml. Since infant formulae usually have protein contents between 1.5 and 2.0 g/100 ml and all of the protein can be considered digestible, they suggested a re-evaluation of current recommendations on protein requirements during infancy.

On the basis of our data on the human milk protein composition, we therefore calculated the amino acid composition of that part of the protein fraction that is thought to be totally digested. This led to the interesting

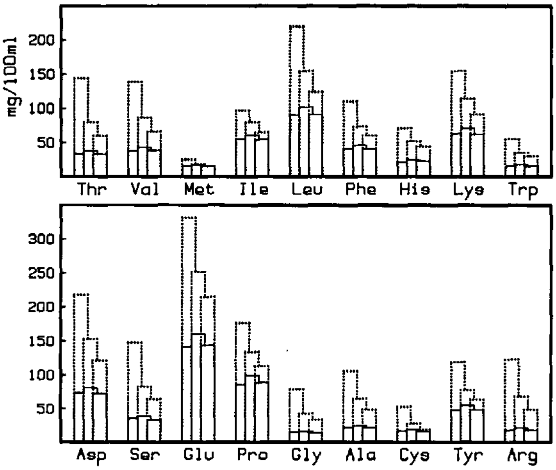

Fig. 8. Comparison of the nutritionally available amino acid composition (———) with that of total protein (– – –) of human milk at days 1, 8, and 36 of lactation.

result, as shown in Figure 8, that the amount of the nutritionally available protein fraction and, in addition, its amino acid composition remain rather constant throughout the lactational period. Looking at the differences obtained between the total of individual amino acids and the respective available proportion it becomes evident that for some amino acids the nutritionally available fraction is only half of the total, whereas with others no or only minor changes could be observed.

We therefore concluded that a re-evaluation of current recommendations on protein requirements during infancy should not only be done with respect to total protein intake, but also with respect to the amino acid composition (3).

Throughout the whole period of investigation non-protein-nitrogen accounted for about 20 % of total nitrogen, the major fractions being urea (approx. 32 %), peptides (8 %), free amino acids (9 %), and glucosamine (40 %). Within the free amino acid fraction glutamic acid increased from 5.2 mg/100 ml to about 14.7 mg/100 ml during the first five weeks of lactation and was found to be the major component within this fraction in mature milk, followed by taurine which remained constant with values of about 6 mg/100 ml. Taurine is important as a conjugating agent for bile acids and because of its involvement in central nervous functions (1, 53, 54). Infants receiving no dietary taurine (formula feeding) tend to save taurine by replacing it with glycine for bile acid conjugation (1). This occurs despite the less favourable emulsifying and free fatty acid releasing properties of the glycine conjugates (35, 44). In breast-fed infants the presence of the more acidic taurine bile acid conjugates is hypothesized to contribute to the relatively acidic intestinal milieu favouring bifidus colonization. Whether the function of taurine as a bile acid conjugator is as important as pointed out above or taurine is of relevance to the infant primarily as a growth modulator is presently under discussion (17).

In view of the low capabilities of infants, especially prematures, to synthesize taurine from methionine, it seems evident that this "amino acid" is essential at least to the newborn, who therefore depends on an exogenous supply. Therefore most modern infant formulae are supplemented with taurine, a step forward to bring infant formulae as close as possible to the composition of human milk.

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