Protein Content of Human Milk, from Colostrum to Mature Milk

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COMPARATIVE ASPECTS OF PROTEIN CONTENT IN MAMMALIAN MILKS

By definition, mammals are those animals that suckle their young and they comprise the highest class of vertebrates. Thus lactation is one important distinguishing characteristic of this class of animals. Mammals are born at markedly different stages of maturity and if it is assumed that their nutritive requirements correspond to their physiological maturity, one may reason ideologically that the milk of a given species is best adapted to the nutritional needs of the young of that same species. The nutritional adequacy of mother's milk for the young depends not only on the composition of the milk, but also on the quantity of milk produced. Linzell's comparative studies (1) of milk production in 22 different species indicate a mean daily yield of 0.126 kg milk/kg body weight and a daily energy output of 140 kcal/kg. The milk yield is closely related to the body size of the lactating mother. In the human, however, there seems to be a great individual variation in the milk yield and it may therefore be more common in humans than in animals for the milk production to be insufficient for optimal growth.

Protein in milk is the source of amino acids needed for protein synthesis and growth of the young. The protein content of milk produced by various species ranges from about 1% in humans to about 20% in rabbits. At present the compositions of milks of about 150 different mammalian species are known. For six species in addition to humans (horse, goat, cow, sheep, and reindeer) the data are extremely comprehensive and detailed (Table 1), due to the fact that the milks of all these animals, particularly the cow and goat, have commonly been used for human consumption.

The protein content of milk has been related to the growth rate of the young. Bernhart (2) suggested that there is a direct correlation between milk protein and the time required to double the weight of the young, based on studies of nine different species: humans, horse, cow, goat, pig, sheep, dog, cat, and rabbit. The data range from humans, with 125 days to double birthweight and 7% of energy intake from protein, to the rabbit, with 7 days to double birthweight and nearly 30% of the energy
TABLE 1. Protein content of milk from some mammals

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein (%)</th>
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<tbody>
<tr>
<td>Humans</td>
<td>1.0</td>
</tr>
<tr>
<td>Horse</td>
<td>2.5</td>
</tr>
<tr>
<td>Goat</td>
<td>2.9</td>
</tr>
<tr>
<td>Cow</td>
<td>3.4</td>
</tr>
<tr>
<td>Sheep</td>
<td>5.5</td>
</tr>
<tr>
<td>Reindeer</td>
<td>11.5</td>
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intake from protein. This generalization, however, does not hold true for the arctic and aquatic mammals, in which milk energy is derived mainly from fat and in which the growth of the young is due to a large extent to deposition of fat.

Figure 1 shows that in most mammals the concentration of lactose in milk is inversely proportional to that of fat and protein (3). It is seen that human milk has the
highest lactose content and the lowest protein content of all the milks shown in the figure. In this chapter I shall discuss the quantitative and some qualitative aspects of the major proteins in human milk during the various stages of lactation.

TOTAL PROTEIN IN HUMAN MILK DURING PHASES OF LACTATION

Colostrum may be defined as "the thin, yellow, milky fluid secreted by the mammary gland a few days before or after parturition." In general, the milk excreted during the first 3–4 days is called colostrum. Transitional milk is that excreted from day 6 to day 15 and mature milk is the milk excreted from day 15 (4). The mean concentrations of total protein during these phases of lactation are shown in Fig. 2. Colostrum has a mean protein content of about 20 g/liter, transitional milk about 15 g/liter, and mature milk between 10 and 11 g/liter (5). After the first month of lactation the total nitrogen content decreases somewhat and then fluctuates, following no particular pattern, in the period between 3 and 6 months' postpartum. The changes in concentration of the major macronutrients of human milk are shown in Fig. 3. As the concentration of the two major energy-containing components, fat and lactose, increases, the concentration of protein decreases (5). This leads to a change in the energy distribution (Table 2). In early lactation during the colostral stage, protein is responsible for 17% of the energy content, but by the third week of lactation it accounts for only 7% of the total energy. Thus one may ask from a teleological point of view whether the infant needs more protein during the first days of life than later.
In order to answer this question one must examine the functions of the different milk proteins.

**CHANGES IN THE PATTERN OF MAJOR MILK PROTEIN FRACTIONS DURING LACTATION**

The concentration of the whey protein secretory IgA decreases sharply during the first days of lactation, whereas lactoferrin shows only a moderate decrease. By contrast, casein, α-lactalbumin, and serum albumin concentrations are more or less constant, as shown in Fig. 4. Thus, according to Harzer & Bindels (5), the whey protein/casein ratio of human milk decreases from 80:20 in colostrum to 55:45 in mature milk. This has recently been confirmed by Kunz & Lönneldal (6), who quanti-
PROTEIN CONTENT OF HUMAN MILK

Human milk protein composition during the first 5 weeks of lactation: casein; α-lactalbumin; lactoferrin; lysozyme; SIgA; serum albumin. [From Harzer G, & Bindels JG (5).]

The nutritional availability of human milk proteins for amino acid metabolism and protein synthesis of the infant has recently been discussed by several investigators: Hambraeus et al. (7), Räihä (8), and Butte et al. (9). The functions of many major

Whey proteins, secretory IgA, lactoferrin, and lysozyme are to some extent physiological rather than nutritional. Secretory IgA is the main immunoglobulin of human milk and acts locally in the infant's gut to prevent attachment of microbes to intestinal cells, thereby preventing infection. Lactoferrin is the major iron binding protein of human milk and is believed to prevent microbial proliferation by reducing the availability of iron. Lysozyme attaches to the bacterial cell wall, causing lysis.

These three protective proteins comprise about 30% of the total proteins in mature human milk. Recent studies have shown that these proteins are resistant to low pH and to proteolytic enzymes (10, 11), which explains why it was possible for Davidson & Lönnegal (12) to detect large amounts of both secretory IgA and lactoferrin in the stools of exclusively breast-fed infants. Figures 6 and 7 show that the excretion of these proteins correlates with the concentration in the milk, both of which decrease with the age of the infant. The amount of secretory IgA excreted is high, 60% of intake, in the early weeks of lactation and decreases to 10% of the intake during the later phase of lactation. A similar pattern is found for lactoferrin (Fig. 7).

By calculating the amounts of whey proteins ingested and subsequently excreted it has been estimated that between 3% and 10% of the milk proteins are unavailable to the infants as a nutritional source of amino acids (13, 14). The higher percentage
of excretion is found in early lactation when the milk content of secretory IgA and lactoferrin is greater. The non-protein nitrogen (NPN) fraction of human milk comprises 20–25% of the total nitrogen. Of this, urea forms some 50%, but its concentration fluctuates with the state of lactation. The NPN fraction of human milk also contains some peptides and free amino acids, of which taurine is the most prominent. The utilization of urea nitrogen for de novo synthesis of amino acids and body proteins
TABLE 3. Theoretical estimation of the nutritional value of proteins in human milk

<table>
<thead>
<tr>
<th>Component</th>
<th>g/liter</th>
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<tbody>
<tr>
<td>Total protein (N x 6.38)</td>
<td>11.6</td>
</tr>
<tr>
<td>Non-protein N (25%) (2% utilized for protein synthesis)</td>
<td>2.8</td>
</tr>
<tr>
<td>True protein</td>
<td>8.8</td>
</tr>
<tr>
<td>Non-nutritional proteins (3%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Nutritional proteins</td>
<td>8.5</td>
</tr>
</tbody>
</table>

in the normal newborn infant has been much debated and the data are conflicting (15). Fomon (16) has estimated that an average of 13% of the ingested urea could be available for endogenous synthesis of amino acids in term infants. Most of this synthesis may be carried out by intestinal bacteria (17). The total nutritional contribution of the protective whey proteins and the NPN components in human milk for the normal infant is still not fully understood and needs further elucidation. Thus the minimum nutritionally available protein in mature human milk may be as low as 8.5 g/liter (8), as shown in Table 3. From a nutritional point of view, these results also imply that the whey/casein ratio of the nutritionally available proteins of mature human milk may be 50:50 or perhaps even slightly casein predominant and thus different from that in the total milk proteins.

One of the methods used to estimate protein requirements of infants is based on measurements of protein intake from breast milk in healthy breast-fed infants maintaining satisfactory growth (18). The true nutritionally available protein content of human milk must be considered when assessing protein requirements of infants. On the basis of the facts presented it is obvious that the requirement may be considerably less than recommended previously.

AMINO ACID CONTENT OF NUTRITIONALLY AVAILABLE PROTEINS OF HUMAN MILK

Harzer & Bindels (5) have studied the amino acid profile of the major human milk proteins. Figure 8 shows the amino acid concentrations in the various human milk proteins, secretory IgA, lactoferrin, α-lactalbumin, and casein. In comparison to the other milk proteins, secretory IgA is rich in threonine and has more valine than α-lactalbumin. Casein is rich in tyrosine and has a low content of tryptophan and cystine. Due to the change in the nutritional availability of secretory IgA, the threonine and valine available in vivo may be less than what would be predicted on the basis of the amino acid profile of the hydrolyzed total protein of human milk.

The quantities of amino acids ingested by healthy breast-fed infants have been estimated on the basis of milk consumption and the amino acid concentrations of human milk samples (19). Since not all human milk proteins are fully hydrolyzed it is clear that these estimations have been too high and qualitatively misleading. Harzer & Bindels (5) have measured the nutritionally available amino acid composition of
human milk at 2, 8, and 36 days of lactation (Fig. 9). Their data show that although the amino acid content of human milk decreases considerably from day 2 to day 36 of lactation, the pattern of nutritionally available amino acids remains essentially the same.

Thus it is difficult to design infant formulas on the basis of the amino acid composition of human milk. It is essential to study the composition of the nutritionally available protein amino acids in vivo. By simply changing the quantity or the whey/casein ratio of bovine milk protein it will not be possible to achieve plasma amino acid profiles identical to those found in breast-fed infants (Fig. 10) (20). If the purpose of formula design is to produce human milk substitutes that will produce plasma amino acid profiles similar to those found in infants fed human milk, the protein composition must be modified by increasing the bovine α-lactalbumin fraction (see the chapter by Heine) or human milk proteins must be produced by the transgenic technique in large quantities to be used for formula production.

PROTEIN CONTENT OF PRETERM BREAST MILK

Atkinson et al. 1978 (21) were the first to observe a higher total nitrogen content in milk from mothers having given birth to preterm infants when compared to that
FIG. 9. Comparison of the nutritionally available amino acid concentrations with that of total protein of human milk at days 2, 8, and 36 of lactation. [From Harzer G, & Bindels JG New aspects of nutrition in pregnancy, infancy and prematurity. Amsterdam: Elsevier Scientific Publishers, 1987; 83–94.]

FIG. 10. Plasma amino acid profiles of infants fed either human milk (circle) or formulas with various concentrations of protein. [From Picone TA, et al. (20).]

of mothers of term infants (Fig. 11). Subsequent studies (22–24) revealed that it was the protein nitrogen that was increased, whereas the NPN fraction was similar to that of term milk. One study (25), however, using a different method to determine the milk protein concentration, found no difference in the protein content between preterm and term breast milk.

Most of the increased concentration of protein in preterm milk may be due to higher concentrations of the protective proteins of breast milk. Considerably higher concentrations of lactoferrin, secretory IgA, and lysozyme have been documented in preterm than in term milk (26,27). Lemons et al. (24) have suggested that the higher protein content of preterm milk may represent a prolonged colostral phase in premature mothers who are establishing lactation by artificial means during periods of stress.

It may thus be that some of the protein which makes up the higher concentration of preterm milk is not nutritionally available to the infant. This is supported by the fact that plasma threonine concentration is not higher in infants receiving preterm milk containing more total milk proteins, especially more of the threonine-rich secretory IgA, than pooled banked human milk (Fig. 12) (28).

SUMMARY

1. Although total protein concentration is high in colostrum and transitional human milk, this is due primarily to an increased concentration of the protective proteins secretory IgA, lactoferrin, and lysozyme, which may not be fully absorbed and are thus partly unavailable to the infant as a protein or amino acid source.

2. The nutritionally available true protein content of mature human milk may be as low as 8.5 g/liter and the whey/casein ratio of those proteins is about 50:50 and thus not whey-predominant. The amino acid profile of the nutritionally available proteins of human milk does not change much during lactation. Because of the variability in digestion and absorption of human milk proteins, the amino acid composition differs in the hydrolyzed and absorbed protein. Plasma amino acid profiles in infants must thus be determined when improvements of formulations are tested. The discrepancy between threonine concentration of human milk and in the plasma in infants fed human milk is a typical example of this.

3. For a period of some weeks postnatally the breast milk produced by mothers of preterm infants has a higher total nitrogen content than the milk of mothers of term infants. This may reflect a prolonged colostral phase since the increased nitrogen content is due mainly to an increased concentration of secretory IgA and lactoferrin. The nutritional advantage of preterm milk needs to be studied more thoroughly.

REFERENCES


DISCUSSION FOLLOWING THE PRESENTATION OF DR. RÄIHÄ

Dr. Guesry: When you say that the entire 15 to 18 g per liter of protein that infant formula manufacturers put in the formula is available, it is a very nice compliment to the infant milk producers but we couldn't accept it as true. Bo Lönnérdal yesterday showed that on average only 75–80% was available, depending on the processing. We always take this margin of unavailable protein into account because it is probably less dangerous to incorporate a little bit more than a little bit less. Second, I should like to thank you for presenting Atkinson's data showing the protein content of the milk of mothers of preterm babies compared with mothers of term babies. Various people have advocated the use of preterm milk for feeding premature babies, but it should be borne in mind from Atkinson's data that by 10 days of age preterm milk has the same protein content as that of normal term milk. At this age a baby born weighing 1 kg or 1.2 kg is still very small and certainly needs a higher protein intake than that needed by a term baby.

Dr. Raihå: I agree that the preterm infant of less than 1500 g needs more protein than can be provided by his mother's milk.

Dr. Cooper: There are very limited data on the amino acid profiles in fetal life. You suggested that in the preterm baby fed human milk the amino acid profile should be the model for preterm formulas. What I am suggesting is that we should perhaps be looking more at the normal situation in the fetus, let's say at 26–28 weeks, rather than at the preterm baby fed human milk.

Dr. Raihå: This is a much debated question. We do not have and probably never will have a gold standard for the plasma amino acid profile in the preterm baby. However, some years ago we studied preterm babies fed on various amounts of exclusively human milk protein obtained by supplementing human milk with altered filtrated human milk protein. When very low birthweight infants achieve an intake of about 3.5–3.6 g/kg/d of this protein, their growth rate is maximal. We used the plasma amino acid levels obtained at this intake as a baseline and compared them with the profiles of formula-fed infants, and with fetal blood amino acid profiles, umbilical cord amino acid profiles, and amino acid profiles from normal breast-fed term infants. We found that the closest match was with the breast-fed term infant who was growing normally. So my view is that the gold standard for the preterm infant should be the amino acid profile of the breast-fed term infant. I don't think we should use the fetal values because the fetus in utero is physiologically quite different from the preterm baby.

Dr. Marini: The problem of reference amino acid values is the same for total parenteral nutrition. Some solutions for TPN use mature human milk as the reference composition. As you demonstrated, this is by no means the same as the plasma amino acid profile in the infant fed human milk. Cord blood or fetal blood obtained at about 32–24 weeks of gestation would probably be a better reference standard.

Dr. Rassin: Amino acid patterns in fetal blood or cord blood can't be reproduced by feeding formulas or breast milk because they reflect in part the more aerobic metabolism of the baby and several features of the profile are just not matchable. For example, cord blood has a much higher glutamate/glutamine ratio than you would ever see in a formula-fed or breast-fed baby. To feed a formula that would reproduce this ratio would be impossible because you would have to give far too much glutamate. Also, the lysine composition of fetal and cord blood is approximately 4 to 5 times higher than you would ever see in a formula-fed or breast-fed baby. We would have to make some very strange changes to formulas to reproduce those profiles, which reflect the transport mechanism of the placenta. For several
amino acids the placenta produces maternal-to-fetal gradients of three- to fourfold, while for others there is almost no gradient, for example cystine. We are not going to produce these effects with any kind of parenteral feeding regimen without causing possible toxicity in the infant. So I think to suggest that cord blood or fetal blood is a profile to match is just not appropriate.

Dr. Marini: Battaglia carried out several studies in the normal human fetus and in the fetus with intrauterine growth retardation. He found that serine was quite high in the human fetus. Do you have an explanation?

Dr. Rassin: Each individual amino acid has a transport mechanism in the placenta and these transports probably determine the fetal amino acid concentrations.

Dr. Marini: But why should the fetus have more serine? Does it depend on muscle metabolism?

Dr. Rassin: I don’t think we are in a position to answer why there are particular concentrations of individual amino acids. We just know that under certain circumstances and with particular transport mechanisms you develop certain concentrations of these compounds. We have tried very hard to determine the implications of this, but we are a long way from finding the answers.

Dr. Pettifor: The issue then is why do we use any amino acid profile as a gold standard for formula-fed or breast-fed infants. If you believe that we can’t use fetal amino acid profiles as the gold standard, why should we use postnatal amino acid profiles either?

Dr. Raiha: I can only say that it is because we have done studies on them. I still think that even for the preterm infant who is orally fed the ideal protein is human milk protein because it is of the same species. When we feed very low birthweight babies with adequate amounts of human milk protein, in other words mother’s own milk supplemented with ultrafiltrated human milk protein, such that we achieve a growth rate that is actually higher than the intrauterine one, we find that the blood amino acid profiles are close to what you find in breast-fed term babies. That is the only reason for thinking that it should be the norm.

Dr. Kashyap: In most studies in preterm infants, the aim has been to try to keep the amino acid concentrations at least in the safe range. In order to do this, people have come up with standards. Perhaps the aim should be to ensure that the concentrations are less than what the baby is exposed to in utero, on the assumption that they will then be in the safe range.

Dr. Pandit: There is evidence that a good deal of urea is secreted in breast milk. Can this be used for protein building?

Dr. Raiha: From studies done so far it seems that under normal conditions, when the baby is getting an adequate supply of nutritional protein, urea is not used extensively for protein synthesis. Under pathological conditions, if the baby is starved or when he has an infection, the situation may be different.

Dr. Heine: It is really interesting that human milk contains such a high amount of urea and the question is for what purpose this high urea concentration is excreted in the milk. Urea is normally an end product which presents a metabolic load for the baby. We carried out some investigations a few years ago in which we fed $^{15}$Nurea together with human milk. We found variable utilization of the urea nitrogen for protein synthesis in the baby. The lowest utilization was 16% of the administered urea and the highest was 60% (1). When Fomon repeated these investigations some years later he found that only 13% of the urea nitrogen was used in healthy, well-growing babies. We believe that the variable utilization has something to do with catch-up growth and with a low supply of protein in the baby’s diet. It is quite clear, however, that urea is utilized for protein synthesis, since the $^{15}$N labeling was identifiable in the plasma proteins.
Dr. Pandit: Does this mean that we should consider this urea as a part of the utilizable protein in the theoretical figure that was presented?

Dr. Heine: This is in part true.

Dr. Fazzolari: Could you tell me if the diet of the mother influences the quality and quantity of human milk protein?

Dr. Räihä: As far as we know, breast milk protein composition is very resistant to change. The fat content is most likely to be affected by maternal diet, and after this the volume of the milk. The quality of the protein will probably not be much affected, at least from studies published so far.

Dr. Fazzolari: The reason for my question is that it has been shown that undernourished lactating mothers have very low taurine in their plasma amino acid profile.

Dr. Rassin: Please bear in mind that the taurine is not found in protein. It is much more responsive to diet than is protein. There are several studies showing that vegetarians will get almost no taurine in their diet and have low taurine in their milk. You can’t really use that as a marker of protein response.

Dr. Guesry: Philippe Hennart did a study in eastern Zaire 3–4 years ago in severely malnourished mothers in which he analyzed their milk. As Niels Räihä has just said, it was mainly the volume of milk that was severely decreased (2). The malnourished mother could not produce more than about 250–400 ml of milk per day, but the protein quality was quite well preserved. It is also true that the fat was modified.

I should like to comment on Dr. Räihä’s recommendation that infant food manufacturers use transgenic cows to produce so-called human milk. He rightly says that this would be quite expensive, not only because of the technology involved in creating such transgenic animals, but also because purification and separation of the human protein from the other proteins produced by the cow will cost a lot of money. However, it is not the price that is most likely to prevent us from doing it; it is more likely to be the law, since it is currently forbidden to give milk from a transgenic animal. It may also prove difficult to get the mother to accept the use of such milk. Public opinion is not yet ready to use transgenic milk for infant feeding. Progress in this will be made initially by the drug industry. Almost every diabetic now uses insulin produced by genetic engineering and it is accepted because it is life-saving and because it is a drug.

Dr. Räihä: This is what I said really. I personally think that it would be much wiser to use all that money to teach mothers to breast feed, but of course in some countries this is difficult. I can just say that in Sweden and Finland, almost 100% of mothers are breast feeding, at least when they go home from the maternity ward, and even at 6 months more than 40% are still breast feeding. I can also say that in Sweden and Finland there is not a single preterm baby who has not been fed exclusively on human milk. We would consider it unethical to give formula.

Dr. Marini: How do you manage if you have a very sick mother? Banked milk is not the same as milk coming from the baby’s own mother. For example, if you don’t use fresh human milk it is quite impossible to protect from infection.

Dr. Househam: One of the most important causes of low birthweight in South Africa, as in many developing countries, is fetal growth retardation. What information is available on the composition of the breast milk in such cases?

Dr. Lönnnerdal: It seems unlikely that there would be any specific adaptation of the mother’s milk that would benefit the infant in such cases, but what happened during gestation may of course affect lactation. Poor nutrition may have been one factor, of course, and another could be infection. We have done a study in Lima, Peru, looking at women who are
sick during lactation, the reason being that infection is so common in developing countries that its effects on lactation need to be examined. We found there was a higher protein content in the milk of these women, probably due to the increase in the acute-phase reactants that we see both in plasma and in some cases in breast milk. Perhaps the reasons for fetal growth retardation may still be present during lactation and therefore affect the milk composition.

Dr. Pettifor: What are the factors that influence the changing concentrations of IgA, lysozyme, and lactoferrin in breast milk?

Dr. Marini: When you look at concentrations there are big differences between colostrum and mature milk, but when you look at quantities the difference is not so great. The amount of colostrum is probably around 300–350 ml/d. During mature lactation, the mother can produce almost a liter of milk a day, so the quantity of IgA is not very much reduced in terms of the amount consumed by the baby.

Dr. Lönnerdal: Although the volume consideration is important, there is more secretory IgA early on, even taking volume into account, than there is later. We need to consider milk protein gene expression. We know too little about this at present. We know in general that hormonal factors and nutritional factors will affect milk protein gene expression but we know little about how nutrition and stress and all the other factors that can affect the hormonal profile will affect milk protein gene expression.

Dr. Räähä: I still think that we have missed an important issue in this whole context and that is the question of the current protein content in standard formulas for normal babies. Nobody has discussed this. It is my personal feeling that most formulas in most countries have too much protein.

REFERENCES