Nitrogen Balance and Plasma Amino Acids in the Evaluation of Protein Sources for Extremely Low Birthweight Infants


The nutritional problems of preterm babies have become particularly relevant in the last decade because of the increased survival of extremely low birthweight (ELBW) infants and the numerous studies underlining the importance of early feeding on short- and long-term development (1). It is well known that the nutritional requirements of extremely premature infants are greater than those of other, larger premature and full-term neonates and that nutritional support should be initiated early in the neonatal period, combining the use of parenteral and enteral feeding (2). The reduction of gut motility, enzyme function, and intestinal nutrient absorption in these very small babies has led to the use of total parenteral nutrition as a prolonged exclusive feeding process during the first weeks of life. However, “minimal enteral feeding” has been shown to enhance gut motility and accelerate establishment of full enteral feeding (3,4). There is now some evidence that early enteral nutrition may be well tolerated without adverse effects in ELBW infants, but precise guidelines on early enteral support need to be established.

Enteral feeding is generally initiated with the baby’s own mother’s milk, with or without fortification with energy, minerals, and proteins, or with a preterm formula. More recently, to improve gastric emptying and gastrointestinal digestibility and to prevent atopic diseases, the use of partially hydrolyzed protein formulas has been suggested (5). There are insufficient published data for accurate evaluation of the influence of protein quality on nitrogen absorption, nitrogen utilization, and plasma amino acid concentrations in ELBW infants. This information therefore needs to be obtained by extrapolation from the results of the numerous studies that have been carried out on larger preterm infants studied at various gestational ages and body weights.
NITROGEN ABSORPTION

Various factors may affect nitrogen absorption in preterm infants (6). The stomach does not contribute significantly to overall protein digestion (7). The breakdown of most large-molecular-weight proteins into smaller peptides and amino acids is the result of luminal hydrolysis and subsequently of peptidase activity, located at the level of the microvillus membrane or within the enterocyte (7). In human milk, antibodies, enzymes, and growth factors appear to survive the gastrointestinal environment and can be detected in the stool, decreasing the apparent nitrogen absorption rate (7). Studies in animal preterm neonates suggest that bovine and human whey proteins are hydrolyzed more slowly than casein (8). In addition, the decreasing level of immunoreactive human α-lactalbumin found in the serum of preterm infants with increasing gestational age suggests a relative impairment of protein hydrolysis at the earlier stages of development (9). Because of this, the use of formulas containing partially or more extensively hydrolyzed proteins has been suggested, but their effect on nitrogen absorption is still questionable as the transport of small peptides or individual amino acids may not be as efficient as it is when they have been initially hydrolyzed by microvillus membrane peptidases (10).

Over a period of more than 20 years, we have performed 356 metabolic balance studies (11–16) in preterm infants fed human milk, either unsupplemented or supplemented with various human milk fortifiers (HMF; \( n = 88 \)), European whey-predominant formulas designed before 1980 (WPF1; \( n = 72 \)) and after 1980 (WPF2; \( n = 49 \)), American whey-predominant formulas (WPF3; \( n = 58 \)), hydrolyzed whey formulas (HWF; \( n = 31 \)), and casein-predominant formulas also designed before 1980 (CPF; \( n = 58 \)) (Table 1). In all, almost 30 different regimens were evaluated.

Fecal nitrogen excretion represents the sum of endogenous fecal nitrogen derived from the gastrointestinal tract (desquamation, secretion) and the nonabsorbed fraction of the nitrogen intake. The apparent nitrogen absorption rate may be estimated as the ratio between absorbed nitrogen and nitrogen intake. The apparent nitrogen absorption rate differs significantly according to the feeding regimen. It was higher with WPF1 (89.9%), WPF2 (90.7%), or CPF (89.5%) than with HMF (82.7%), WHF (84.3%), or WPF3 (86.0%) (Table 1). The fractional nitrogen absorption rate (true digestibility) can be estimated by regression analysis between nitrogen absorbed and nitrogen ingested, as the nitrogen coefficient (Fig. 1). Calculated values for this coefficient are (see Fig. 1) 83.4 ± 3.0% for HMF; 93.3 ± 1.0% for WPF1, WPF2, and CPF combined; 76.9 ± 6.1% for WPF3; and 80.6 ± 7.9% for WHF. From these data, the endogenous nitrogen excretion was estimated in the group with the highest nitrogen absorption rate. The mean value lies between 30 and 40 mg/kg body weight per day.

When we evaluated the American whey-predominant formulas (WPF3), which are essentially in-can liquid formulas sterilized with heat treatment that induced a Maillard reaction, our data confirm that the technical process may impair nitrogen absorption (17). Similarly, the technologies necessary to perform partial protein hydrolysis appear to reduce nitrogen absorption significantly in preterm infants.

Reanalyzing the results of numerous metabolic balance studies reported by various groups, Micheli and Schutz (6) suggested that nitrogen absorption could be di-
<table>
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<th>Milk group</th>
<th>HMF</th>
<th>WPF 1</th>
<th>WPF 2</th>
<th>WPF 3</th>
<th>WHF</th>
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<td>Birthweight (g)</td>
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<td>1460 ± 382&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>545 ± 102&lt;sup&gt;df&lt;/sup&gt;</td>
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<td>Fecal excretion (mg/kg/d)</td>
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<td>49 ± 19&lt;sup&gt;abdef&lt;/sup&gt;</td>
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<td>Net prot. utilat.* (%)</td>
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<td>71.5 ± 6.5&lt;sup&gt;abdef&lt;/sup&gt;</td>
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<td>49.3 ± 11.9&lt;sup&gt;abcde&lt;/sup&gt;</td>
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* Values are expressed as mean ± 1 SD.

<sup>a</sup> p < 0.05 versus HMF
<sup>b</sup> p < 0.05 versus WPF 1
<sup>c</sup> p < 0.05 versus WPF 2
<sup>d</sup> p < 0.05 versus WPF 3
<sup>e</sup> p < 0.05 versus WHF
<sup>f</sup> p < 0.05 versus CPF

* nitrogen retention/nitrogen intake
® nitrogen retention/nitrogen absorbed
Nitrogen balance

Absorption

Retention
rectly related to gestational age and that immaturity of ELBW infants could significantly reduce metabolizable nitrogen. Multivariate analysis of our data does not confirm this hypothesis but suggests that nitrogen absorption is independent of weight or gestational age at the time of the balance study (Fig. 2). Thus data we obtained in VLBW infants could probably be extrapolated to ELBW infants.

NITROGEN UTILIZATION

During the second part of gestation, lean body mass and protein accretion increase faster than weight gain owing to a progressive reduction in total water content (18). The fetus has a much higher fractional protein turnover rate than the term infant, because of the increased ratio of organs with high rates of protein synthesis over other tissues. Body nitrogen content increases exponentially during this period, whereas protein gain represents around 20% to 25% of protein synthesis (18).

Many factors are known to affect protein utilization in ELBW infants: protein and nitrogen intakes, energy-to-protein ratio, biological value of ingested proteins, nutritional status, hormones, and clinical factors (6). Various studies have shown that daily protein gain increases linearly with protein supply up to around 4 g/kg·d, at which point the effect of protein gain appears to decrease. However, an additional effect of metabolizable energy has been demonstrated. This effect appears to be more pronounced at suboptimal energy intakes (<100 kcal/kg·d). The efficiency of protein gain can be estimated by the ratio between retained and absorbed nitrogen, as well as by the slope of the regression line calculated between nitrogen retained and nitrogen absorbed (see Fig. 1D–F).

In our results the efficiency of protein gain differs according to the feeding regimen (Table 1); the highest values were obtained in preterm infants fed WPF2 (77.7%) and WPF3 (77.5%). The efficiency of protein gain was significantly reduced in infants fed WHF (74.0%) and HMF (72.1%). The lower value obtained with HMF may be related to the nonprotein nitrogen (NPN) fraction of human milk, representing 20% to 25% of the total nitrogen content of human milk but 13.5% to 17% of the total nitrogen content of HMF. As demonstrated for urea nitrogen, the contribution of this metabolizable NPN fraction to protein gain is lower than that of α-lactalbumin or casein in human milk (19). By contrast, the significantly lower value obtained with WHF suggests that the process of hydrolysis itself reduces the bioavailability of whey protein. The low efficiency values obtained with the use of WPF1 (62.6%) and CPF (49.3%) may be related, on the one hand, to the higher nitrogen supply provided by those formulas reaching the protein gain plateau and, on the other hand, by a relative reduction in metabolizable energy caused by the use of a poorly absorbed cow’s milk fat blend in most of the formulas made before 1980.
The efficiency of whey-predominant and casein-predominant formulas has been demonstrated more recently by Cooke et al. (20), who compared three preterm formulas with 60:40, 35:65, and 20:80 whey/casein ratios. The absorption rate was slightly lower with the whey-predominant formula (83% versus 86% and 85%), whereas efficiency was around 80% and similar in the three groups.
Thus, with respect to net protein utilization (N retained/N ingested), cow's milk protein formulas, whether whey-predominant or casein-predominant, appear to be more efficient than human milk, with or without protein supplementation. However, our studies confirm that in formulas the protein bioavailability can be altered by various technical processes such as heat treatment or hydrolysis (21).

The fractional protein synthesis rate decreases with gestational age in fetal sheep at a greater rate than the fractional growth rate, but both curves indicate a much higher protein turnover and presumably a greater utilization in ELBW infants (18). Animal studies and metabolic balances performed in preterm infants show that the rate of protein accretion per unit body weight decreases throughout gestation (18). Reanalyzing the results of numerous metabolic balance studies in preterm infants, Micheli and Schutz (6) showed that there is a gestational-age-related change in the percentage of protein energy needed to achieve optimal protein gain. However, the efficiency of protein gain—the ratio between protein gain and metabolizable protein—seems to be independent of gestational age. In contrast, in preterm infants fed HMF or formulas with the highest protein efficiency (WPF2, WPF3), we calculated that protein gain was dependent not only on the absorbed protein supply but also on the body weight at the time of the balance study. The efficiency of protein gain (ratio of protein retained to protein absorbed) was inversely related to body weight (see Fig. 2), suggesting a greater efficiency of protein deposition in ELBW infants.

**PLASMA AMINO ACID CONCENTRATIONS**

ELBW infants have incomplete development of several amino acid metabolic pathways and require high-quality protein with an adequate nitrogen supply to prevent deficiency or overload of various essential or semi-essential amino acids. It is still a matter of debate whether the plasma amino acid disturbances commonly observed in preterm infants on oral or parenteral nutrition are harmful for development. Optimal values for plasma amino acid concentrations in preterm infants are also a matter of discussion. At least three different gold standards have been proposed for premature infants:

1. amino acid concentrations from the umbilical cord obtained at fetal cord puncture or after birth;
2. amino acid concentrations obtained in rapidly growing preterm infants receiving their own mother's milk or human milk supplemented with human milk proteins;
3. amino acid concentrations in healthy breastfed term infants (22–27).

For ELBW infants, levels that obtain during the last trimester of gestation or in growing infants with optimal intake of human milk protein appear to be safe. However, since large differences are observed for some amino acids (threonine, valine, tyrosine, phenylalanine, lysine, and histidine) between fetal and postnatal values, a combined reference standard has been proposed (Fig. 3), taking into account the mean ± 1 SD of the values obtained in cord blood and in preterm infants fed human milk supplemented with human milk protein (28,29).
According to this reference, preterm infants fed human milk fortified with whole cow’s milk protein or casein hydrolysate have plasma amino acid concentrations within the normal range. On the other hand, the use of whey hydrolysate as a fortifier induces a significant increase in plasma threonine and a relative reduction in phenylalanine (29,30).

In view of the fact that bovine casein has a different amino acid composition from human milk, that bovine whey is quite different from human whey, and that human milk contains a relatively large proportion of nonprotein nitrogen partially available for protein synthesis, it is virtually impossible in cow’s milk-based formulas to obtain a nitrogen and amino acid pattern identical to that found in human milk. Thus there have been numerous studies evaluating indices of protein metabolism and plasma amino acid concentrations in preterm infants fed formula with various whey/casein ratios (20,31–33). Recent data (20,32,33) suggest that the type of protein has no effect on metabolic acidosis, uremia, or hyperammonemia, in contrast to data reported in preterm infants receiving older preterm formulas (34). However, the supply of individual amino acids differs significantly according to the whey/casein ratio, thereby influencing the plasma amino acid concentrations. Thus, compared with reference values, threonine is increased and histidine is relatively decreased in infants fed WPF, whereas methionine and aromatic amino acids are increased in infants fed CPF. However, these disturbances of plasma amino acid concentrations do not reach the level previously reported with older formulas (35,36).
Whey-hydrolyzed formulas have recently been evaluated in preterm infants (5,37). Indeed, various technological processes necessary to reduce protein antigenicity may modify amino acid content and amino acid bioavailability (21). The use of a higher percentage of whey in protein-hydrolyzed formulas aggravates the distortion of plasma amino acids previously observed with the use of whey-predominant formulas, with an increase in threonine and a decrease in aromatic amino acid concentrations. Moreover, a sharp decrease in plasma histidine and tryptophan concentrations was also observed, which could be related to a relative reduction in amino acid bioavailability. Therefore histidine and tryptophan supplementation seems to be required for these formulas (37).

Whey protein separation is obtained by acidic or enzymatic casein precipitation from cow’s milk proteins. In contrast to enzymatic precipitation, in the acidic precipitation process the κ-casein and its glycomacropeptide rich in threonine are eliminated from the soluble phase with the casein (38). Therefore it is now possible to design whey-predominant or whey-hydrolyzed formulas with a lower threonine content.

Using a crossover study design, we recently evaluated plasma amino acid concentrations in 14 preterm infants receiving either a conventional enzymatic or an acidic whey-predominant formula (39). A sharp reduction in plasma threonine concentration was observed in infants fed the acidic WPF (27.9 ± 8.5 μmol/dl) compared with those receiving the conventional WPF (37.5 ± 8.4 μmol/dl). All the other plasma amino acid concentrations were similar with the exception of valine, which was also reduced in the infants fed with acidic WPF. Similarly, in another study in preterm infants receiving an acidic whey-hydrolyzed formula (40), the plasma threonine concentration was significantly lower (35.7 ± 9.2 μmol/dl; n = 13) than the value observed in those fed the enzymatic whey-hydrolyzed formula (48.7 ± 11.3 μmol/dl; n = 11). Considering that threonine metabolism is highly dependent on gestational age (41) and that the increase of brain threonine concentration related to plasma concentrations is higher than that of the other essential amino acids (42), it is highly advisable to use acidic whey rather than enzymatic whey in formulas for ELBW infants.

Glutamine is the most abundant amino acid in the human body and the predominant amino acid supplied to the fetus through the placenta. It is thought to be an important fuel for rapidly dividing cells such as enterocytes and lymphocytes. In ELBW infants who undergo numerous stresses during the first weeks of life and are fed by parenteral and oral nutrition, the provision of glutamine is limited at a time of increased demand. It could indeed be a conditionally essential amino acid. It has recently been suggested that glutamine supplementation in formula may decrease hospital-acquired sepsis and improve tolerance of enteral feedings in ELBW infants (43). Therefore glutamine supplementation in formulas specially designed for ELBW infants should be considered and carefully evaluated.

CONCLUSION

There is much evidence that early enteral feeding, by enhancing the maturation of the gastrointestinal tract, decreases the number of days of feeding intolerance without
adverse effect in ELBW infants. However, owing to the immaturity of numerous metabolic pathways and their precarious state, there is a need for more appropriate guidelines on early enteral support. Several studies support the use of human milk, but attention needs to be focused on specific nutrient limitations. Calcium, phosphorus, proteins, and energy supplements are available, but they markedly increase the osmolality of the feed. When human milk is not available, specifically designed formulas can be used. A review of numerous metabolic balance studies has shown that nitrogen absorption and utilization appear satisfactory. However, absorption and utilization may be impaired by the technical processes involved in protein isolation, protein hydrolysis, or sterilization. The immaturity of amino acid metabolic pathways in ELBW infants may easily induce an amino acid overload or deficiency, which may be deleterious for development. Therefore protein sources, amino acid composition, and the bioavailability of amino acids require careful evaluation and adaptation to optimize plasma amino acid concentrations.

REFERENCES


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DISCUSSION

Prof. Lucas: Do you believe that there are adequate efficacy and safety data on hydrolyzed preterm formulas in general, or the one you are talking about in particular, to make their use recommendable in clinical care?

Dr. Rigo: Hydrolyzed formulas have been used in term infants for many years, and there have been many improvements in their composition. About 10 years ago, we made the first study on the nutritional efficiency of protein-hydrolyzed formula, and we found significant differences between some of the formulas on the market at that time and cow's milk-based formulas. Since then, however, there has been a great improvement in the quality of the hydrolyzed protein. There are still some differences from conventional formulas with respect to absorption and perhaps utilization of nitrogen. And there may also be components that are present in normal formulas but not in hydrolyzed formulas. We now have sufficient data on energy utilization and on calcium and phosphorus retention, but data on zinc and trace element accretion with hydrolyzed formulas are still insufficient. Although the quality of hydrolyzed formulas has improved, we need further nutritional studies.

Prof. Haschke: I would extend that question. Are sufficient safety data available for the presently used nonhydrolysate premature infant formulas? The long-term outcome data presently available are for premature formulas that are no longer on the market and are completely outdated. We know nothing about the long-term outcome of premature formulas that are on the market at present. In clinical practice both the hydrolysates and the nonhydrolysates are well tolerated, but we have not studied them with an adequate sample size as in pharmaceutical trials.

Dr. Rigo: There are still some differences between hydrolysate and nonhydrolysate premature infant formulas that request evaluation. The difficulties are illustrated in relation to the threonine concentration. We know that with previous whey hydrolysate formulas the threonine concentration was quite high. We had two possibilities: one was for the industry to change the composition of the formula by using acidic whey, which decreased the threonine concentration; the other issue was to carry out large developmental studies to determine the effect of a high threonine concentration. The work involved for developmental studies would be more extensive than decreasing the threonine concentration by using acidic whey.

Dr. Atkinson: In support of hydrolysate formulas for premature infants, you suggested first, improved digestibility over whole protein, though your data do not support that; second, prevention of allergy, and I wonder if you have any data showing that there is less allergy in premature infants fed on hydrolysates; and third, improved gastric motility, and again do you have any data to support that? You also said that the acidic processing of whey reduces the threonine content. Do you really feel that the level of threonine in standard whey-predominant formulas is potentially toxic?

Dr. Rigo: Protein digestibility was a little lower than for whole protein because the absorption rate was significantly different. Regarding atopic disease, there are data from Lucas suggesting that premature infants may develop atopy, but we have no follow-up data in preterm infants fed protein hydrolysate to evaluate the efficacy of whey hydrolysate protein in preventing atopic disease. Regarding gastric emptying, there is some evidence that it is more rapid with protein hydrolysate than with whole protein.

In relation to your question about threonine, there are no current data suggesting that threonine could be toxic during development. However, with whey protein hydrolysate formulas we are reaching levels not previously attained with whey-predominant formulas, and we know that there is a close relation between the threonine concentration in plasma and in brain. Thus there is a need for caution and follow-up studies.
Prof. Lucas: Our data on allergy are being misused in the context of hydrolyzed formula. We showed that it was only subjects with a positive family history of allergy who benefited from exclusion of cow’s milk protein. The majority of infants with a negative family history probably benefit from being given whole protein. My other point is that we’ve had much experience with using whole-protein formulas but very little experience with using hydrolyzed formulas, in terms of clinical trial testing. From everything I’ve heard, it would seem that the use of these formulas should be regarded as theoretical and experimental (but see discussion of Ziegler’s Chapter 16—ED).

Dr. Atkinson: I have another point I would like your opinion on. Traditionally, one purpose of hydrolysate formulas was to rest the gut in case of gastrointestinal problems. Do you think that the preterm infant’s gut needs rest? Or maybe by giving them a hydrolysate are we doing harm by suppressing the induction of proteases?

Dr. Rigo: Such formulas have been used for quite a long time now without apparent ill effects on the gut, but I don’t have the data to give you a complete answer on the effect on protease activity.

Dr. Guesry: I can give some partial answers to Dr. Atkinson’s questions. With regard to the toxicity of threonine, we have no human studies, but there are animal studies that have been published [1] and show that baby rats with up to four times the normal level of threonine in the brain are perfectly normal in their behavior after 3 months. So there is no observable change in the behavior of rats submitted to high threonine levels. Your question about resting the intestine of the premature baby by giving hydrolyzed protein was also investigated in an animal experiment. We did studies in minipigs and showed that you don’t rest the intestinal enzymes when you feed hydrolyzed protein—trypsin, chymotrypsin, all the pancreatic enzymes and intestinal enzymes were normal.

Prof. Moro: Does the acidic methodology influence other amino acids apart from threonine?

Dr. Rigo: The only significant difference was a small reduction in valine concentration. All the other amino acids were similar in the two groups.

Prof. Pohlandt: I would like to address the question of absorption and the derived values of efficiency. I think your data are based on nitrogen retention, but you didn’t mention whether you had taken into account the different urea concentrations in human milk and formula. You tried to explain the surprisingly low absorption rate of human milk protein by the presence of immunoglobulins, but quantitatively the immunoglobulins are less important than urea. Urea accounts for about 25% of the nitrogen in breast milk but only 15% in commercially available formulas on the European market. I think this 10% difference in urea nitrogen could easily explain the apparently lower protein absorption from human milk.

Dr. Rigo: I disagree. Urea is very well absorbed. The difference in absorption must be due to a protein component of human milk that are not digested like immunoglobulins, transferrin, and so on. Urea is not well utilized, but 95% is absorbed, and some goes directly into the urine. Urea does not interfere with absorption rate but it does influence significantly the utilization rate and therefore the nitrogen balance.

Prof. Pohlandt: But you haven’t taken it into account in the efficiency calculation.

Dr. Rigo: I showed you the global efficiency—that is, the ratio between nitrogen retention and nitrogen absorption. I explained that one of the differences between formula and human milk was the higher urea content of human milk, which is well absorbed but not well utilized, so it decreases the apparent efficiency of human milk.

Dr. Walker: Why were whey predominant formulas 2 and 3 so much more efficient than formula 1 with respect to absorption and retention?

Dr. Rigo: WPF1 was a very old formula that was used before 1980, and it had reduced en-
ergy content. The fat blend was also completely different, and we showed that the fat absorption was sometimes very low. So the metabolizable energy available with formula 1 was relatively poor. In addition, nitrogen content was also relatively high. There was no significant difference in protein absorption, but utilization is a function of the energy available.

**Dr. Walker:** Several years ago, it was suggested that certain amino acids are essential in premature infants that are not essential in term infants. Is this taken into consideration when providing nitrogen for the premature infant?

**Dr. Rigo:** I think the essential amino acids for the preterm infant are the same as for the term infant. The formula used by Räihä 20 years ago is completely different from the formulas we have now. There was a big difference in the protein content and also in the fat blend. There was also a higher phosphorus content. The amino acid content of the old formulas was not well balanced, and they were low in cysteine and taurine. Current formulas are much better balanced and are adequate in terms of their amino acid content.

**Prof. Heird:** As valuable as those earlier studies by Räihä, Gaull, and coworkers were at the time, they have one major problem: the mineral and particularly the sodium, potassium, and phosphorus contents of those formulas were the same, and also the same as in human milk. So our calculations lead us to the conclusion that there is no way that the higher protein intake could in fact have been retained. In fact, 2.25 g/kg·d was about the maximum that could have been retained, and that obviously is important in terms of this whole issue of the utilization of protein intake.

**Prof. Koletzko:** You referred to the disadvantages of heat-treated liquid formulas and implied that the Maillard reaction was a possible explanation. Do you believe we should not use heat-treated liquid formula, or is there a way to avoid the protein damage?

**Dr. Rigo:** We need to consider heat treatment not only in terms of protein absorption, but also in terms of amino acid bioavailability. There is certainly some reduction in amino acid bioavailability with heat treatment, and we need to bear that in mind. But the overall protein efficiency of the liquid formula was similar to the powdered formula, and the only difference we found was in absorption. Plasma amino acid concentrations were also similar to powdered formula; there were slight differences, but the profile was exactly the same.

**Prof. Moro:** If I understood correctly, your group of babies receiving fortified human milk was a mixture of those receiving supplementary human protein and those receiving supplementary bovine protein. I don't think you can make valid comparisons with such a composite group. You should separate babies receiving only human milk protein from the babies receiving human milk protein plus protein deriving from the fortifier.

**Dr. Rigo:** I agree with you, we could have split this group into two, but then I would also have had to present comparisons of seven groups, and since there was not a large difference between the two fortified human milk groups, I preferred to combine them. Also in the group fed cow milk human milk fortifier, the range of nitrogen intake would be smaller than in those fed human milk alone and with addition of human milk protein.

**Prof. Wu:** Several studies support the use of human milk. I think it would be better to use preterm human milk. Preterm milk has a higher content of total nitrogen, protein nitrogen, sodium, calcium, magnesium, zinc, copper, iron, as well as IgA and other protective factors.

**Dr. Rigo:** Most of the time we try to use own mother's milk, but if we don't have it, we also use banked human milk for feeding preterm infants. It may be difficult to obtain sufficient preterm mothers' milk.

**Prof. Endres:** A group in Berlin has shown that formulas containing hydrolyzed protein do not contain IGF-1. There have been few reports on this, but one study claimed that the endogenous production of IGF-1 was sufficient in premature babies. Do you have a definitive answer to this question?
Dr. Rigo: I have no complete answer to that question and the exact role of IGF-1 in the gastrointestinal tract is not known. This is something that probably needs studying.

Dr. Filho: You mentioned the use of glutamine as a supplement for preterm formulas. Do you have any further information on this?

Dr. Rigo: There are some data showing a reduction in sepsis in the preterm infant given supplementary glutamine [2]. However, there are serious technical difficulties because glutamine is very unstable. More work needs to be done.

REFERENCES
