Infant formulas with increased concentrations of α-lactalbumin[1–4]

Eric L Lien

ABSTRACT

Human and bovine milk differ substantially in the ratio of whey to casein protein ($\approx 60:40$ in human milk and $\approx 20:80$ in bovine milk) and in the proportions of specific proteins. Although current infant formulas closely mimic the ratio of total whey to casein in human milk, the concentration of α-lactalbumin (the dominant protein in human milk) is relatively low in formula, whereas β-lactoglobulin, a protein not found in human milk, is the most dominant whey protein in formula. Because of the differences in the protein profiles of human milk and infant formula, amino acid profiles also differ. To meet all essential amino acid requirements of infants, formula concentrations of protein must be higher than those in human milk. Recently, whey sources with elevated concentrations of α-lactalbumin have become available, which permitted the development of formulas with increased concentrations of this protein and decreased concentrations of β-lactoglobulin. α-Lactalbumin is rich in tryptophan, which is typically the limiting amino acid in formula, and as a result, formulas have been developed with lower protein but higher tryptophan concentrations. This type of formula may offer a number of advantages to the neonate, which include producing plasma tryptophan concentrations equal to those found in breastfed infants and obviating the need for the body to dispose of excess nitrogen loads. *Am J Clin Nutr* 2003;77(suppl):1555S–8S.

KEY WORDS α-Lactalbumin, infant formula, human milk

INTRODUCTION

Human milk is the preferred nutrition for the rapidly growing term infant because it provides all required nutrients in properly balanced proportions. If a mother is unable or chooses not to breastfeed her infant, then infant formula is the most appropriate, nutritionally adequate substitute during the first year of life. Infant formula is designed to closely resemble the composition of human milk and produce physiologic responses as close as possible to those in breastfed infants. During the 80 y that infant formula has been available, considerable research has been devoted to improving the protein quality of infant formula and making it more like human milk.

Protein composition of formula

Originally, infant formulas were based on unmodified cow milk protein and therefore had a protein profile comprising 80% casein and 20% whey proteins (1). Compared with the relatively static nature of cow milk, human milk is a dynamic fluid, changing from a high whey-to-casein ratio ($> 80\%$ whey) at the initiation of lactation, to 60:40 whey:casein in mature milk, and approaching equal proportions of whey and casein in extended lactation (1). In 1961, the first whey-dominant infant formula was developed; the protein profile of that formula more closely resembled that of human milk than did previously available formulas. Moreover, the method of producing whey-dominant formula resulted in mineral concentrations that were lower than those in cow milk and previous formulas. The relatively low concentrations of both protein and minerals therefore lowered the renal solute load [ie, the sum of solutes that must be excreted by the kidney, primarily consisting of dietary nonmetabolizable components such as ingested electrolytes in excess of needs, and metabolic end products, predominately excess nitrogen (2)] delivered to the infant.

Whereas whey-dominant formulas are an improvement over previous formulas, the proportion of specific whey proteins in formulas still differs dramatically from that in human milk. The protein profiles as a percentage of total protein of bovine milk, human milk, and current whey-dominant formulas are shown in **Figure 1**. Examination of the concentrations of α-lactalbumin shows that human milk contains a much larger proportion of α-lactalbumin than does bovine milk or whey-dominant formula. In these latter 2 foods, β-lactoglobulin predominates. β-lactoglobulin is not expressed by the human mammary gland and therefore is not found in human milk.

Role of α-lactalbumin

α-Lactalbumin plays an essential role in milk formation in all species (3). During lactation, the mammary gland expresses both the enzyme β-1,4-galactosyltransferase (GT-1) and α-lactalbumin. These 2 proteins form an enzyme complex, lactose synthase, that is present in the Golgi vesicles during lactogenesis. This enzyme complex couples activated galactose to the receptor sugar glucose with high specificity, and thus lactose becomes the primary carbohydrate formed during lactation. The α-lactalbumin–GT-1 complex has an affinity for its substrate, glucose, that is 1000-fold that of GT-1 in the absence of α-lactalbumin. The formation of lactose is essential for milk production because it provides the driving osmotic force behind the fluid formation of milk: as the lactose synthase enzyme system synthesizes lactose, water is drawn from the mother's circulation and the aqueous component of milk is formed. The important role of α-lactalbumin in lactose formation
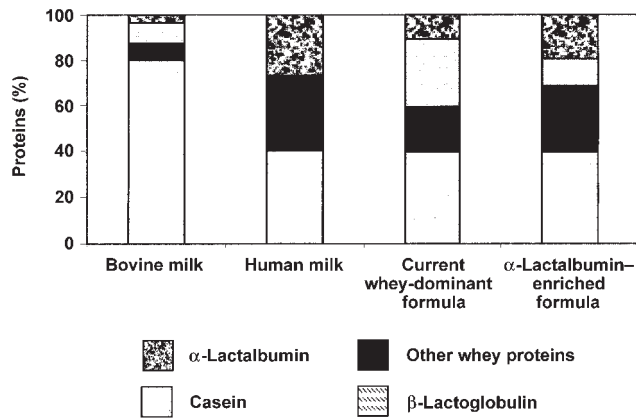
FIGURE 1. Proportions of proteins in bovine milk, human milk, and infant formula.

FIGURE 2. Biochemical and nutritional roles of α-lactalbumin.

has been shown in experiments involving transgenic animals. Lactation is disrupted in α-lactalbumin knockout mice, but it can be restored by human α-lactalbumin gene replacement (4). The association between α-lactalbumin and GT-1 is relatively weak, and α-lactalbumin readily dissociates from the enzyme complex and is secreted into milk in high concentrations. Thus, α-lactalbumin becomes a substantial source of amino acids and helps provide the appropriate balance of essential amino acids in human milk (*see* below). The dual activities of α-lactalbumin with its biochemical and nutritional roles are shown in **Figure 2**.

Amino acid concentrations in human milk and infant formula

Differences in whey and casein protein composition among cow milk, human milk, and infant formulas result in the delivery of different amounts of amino acids to the infant during feeding. Dupont (5) described the essentiality of specific amino acids for the infant. For protein synthesis to proceed at optimal rates, all essential amino acids must be present in the diet in appropriate proportions. If even one essential amino acid is present at a suboptimal concentration, the growth rate and the development of the neonate will be impaired. Published amino acid requirements for the neonate are based on the assumption that human milk will meet all of the amino acid requirements of the infant. If the infant is not breastfed, the alternative feeding system must provide at least equivalent amounts of amino acids. The amino acids tryptophan and cysteine are worthy of specific discussion. Cow milk tryptophan and cysteine concentrations are approximately one-half of those found in human milk, when expressed as a proportion of total protein (6). These amino acids have important roles beyond protein synthesis. Tryptophan is a precursor of serotonin, a neurotransmitter that may regulate the sleep-wake rhythm (7), the response to stress, and other physiologic processes (8). Cysteine is a component of the tripeptide glutathione, an important element of the antioxidant system of the neonate (6). Cysteine is also the precursor to taurine (9), an amino acid that is not incorporated into proteins but is a component of bile salts and that may also play a role in brain development (10). The endogenous production of taurine may be limited, at least in preterm infants, by the relatively slow maturation of the enzymes involved in taurine synthesis (11). To compensate for essential amino acid differences between human milk and formula, formulas generally have total
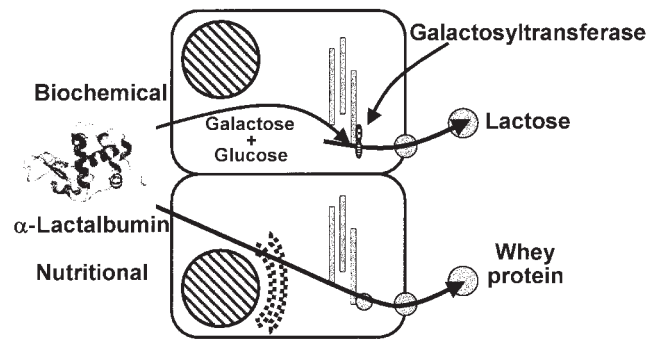
concentrations of ≥15 g protein/L, whereas human milk has concentrations of 9–11 g protein/L (1). This excess formula protein meets all the essential amino acid needs of the infant to support growth, but some studies using such formulas reported that plasma tryptophan concentrations are still lower in formula-fed infants than in breastfed infants (12, 13). However, other studies did not report such an effect (14, 15), which suggests that differences in formulation or processing may play a role.

Numerous studies have evaluated the alteration of whey:casein in formulas or the addition of free amino acids to formula to more closely match the human milk concentrations of total protein and amino acids as well as the plasma amino acid concentrations in breastfed infants. Several studies evaluated formulas with protein concentrations below the standard 15 g/L and found acceptable amino acid profiles (14, 15). Other studies have identified tryptophan as an amino acid worthy of specific examination. For example, Hanning et al (12) evaluated plasma amino acid concentrations in breastfed infants and infants fed a control formula containing 15 g protein/L with whey:casein of 60:40 or a tryptophan-fortified experimental formula with 13 g protein/L and whey:casein of 40:60. The tryptophan concentration in the experimental formula was higher than that in either human milk or the control formula. Energy intake and growth did not vary between groups. At all time points studied (4, 8, and 12 wk), plasma tryptophan concentrations were similar in the breastfed and experimental groups and significantly higher in both these groups than in the control group. In addition, at all time points, the ratio of tryptophan to large neutral amino acids (LNAAs: leucine, isoleucine, valine, tyrosine, tryptophan, and phenylalanine) was significantly higher in the breastfed group than in either formula group, whereas ratios in the experimental group were significantly higher than those in the control group. Fazzolari-Nesci et al (13) also compared breastfed infants to infants fed a control or tryptophan-supplemented formula. As in the work of Hanning et al (12) discussed above, the control group had significantly lower plasma tryptophan concentrations than did the breastfed group, and the concentrations in the experimental group did not differ significantly from those in the breastfed group.

With regard to tryptophan, both the absolute concentration and the concentration of tryptophan relative to other LNAAs are of importance. The ratio of tryptophan to other LNAAs influences the uptake of these amino acids into the brain, because a single amino acid transporter is responsible for the movement of all LNAAs (including tryptophan) from the peripheral circulation into

the brain. Therefore, the relative abundance of tryptophan compared with that of other LNAAs is critical in determining the amount of tryptophan that is transported into the brain. In adults who consume a mixed diet high in carbohydrates, the insulin-induced transfer of LNAAs into muscle tissue produces a relatively high plasma tryptophan:LNAAs that enhances the transfer of tryptophan into the brain. In infants, however, a milieu of contrainsulin hormones, such as glucagon and epinephrine (16), limits the insulin-induced flow of LNAAs into muscle tissue. Combined with the lower muscle mass of infants (12% compared with 20% for adults) and their higher protein intake per unit of body mass per day (breastfed infants: 2 g/kg; formula-fed infants: 2–4 g/kg; adults: 0.8–1.2 g/kg), infants must rely more heavily on the dietary balance of tryptophan:LNAAs to maintain adequate brain tryptophan uptake (17).

The above studies show that current formulas may compositionally meet all requirements, but infants fed those formulas still had plasma amino acid profiles that differed from those in breast-fed infants. Plasma concentrations of tryptophan were significantly lower in formula-fed than in breastfed infants in some studies (discussed above). These differences in plasma tryptophan can be eliminated by the introduction of tryptophan-rich feeding systems.

α-LACTALBUMIN–ENRICHED INFANT FORMULAS

Two important goals in the improvement of infant formulas are to more closely match the total protein content and the protein profile of human milk. Recent advances in dairy technology permitted the isolation of whey fractions with a higher concentration of α-lactalbumin than was previously available. This provides an attractive avenue for the development of improved formula protein systems, because α-lactalbumin contains a relatively high concentration of tryptophan and other essential amino acids compared with whole cow milk. However, the addition of α-lactalbumin alone is not the ideal modification for infant formula. For example, the proportion of arginine in bovine α-lactalbumin is one-fourth that in human milk (6). If an infant formula were created with whey:casein of 60:40, with the whey fraction being entirely α-lactalbumin, a substantial arginine deficit would occur. Therefore, the goal of reducing the total protein concentration in infant formulas to bring it closer to that observed in human milk cannot be met solely via the extensive enrichment of formulas with α-lactalbumin. Revised formulas should contain a balanced enrichment of α-lactalbumin, preferably coupled with a reduction in β-lactoglobulin (not found in human milk) and selective retention of other proteins. This will yield a formula that will be lower in total protein but that will retain the necessary balance of essential amino acids.

The advantage of infant formulas with increased concentrations of α-lactalbumin was shown by Heine et al (18) in a study in which a standard infant formula (18 g/L, low α-lactalbumin) was compared with 2 lower-protein experimental formulas (13 g/L), one containing an intermediate concentration of α-lactalbumin and one containing a high concentration of α-lactalbumin. As a percentage of total protein, tryptophan increased progressively from the low to the high α-lactalbumin formulas. In a crossover design, infants received all 3 formulas for 2 wk. Only infants receiving the formula with a high concentration of α-lactalbumin had serum tryptophan concentrations as high as those of breastfed infants. The above study showed that the development of low-protein
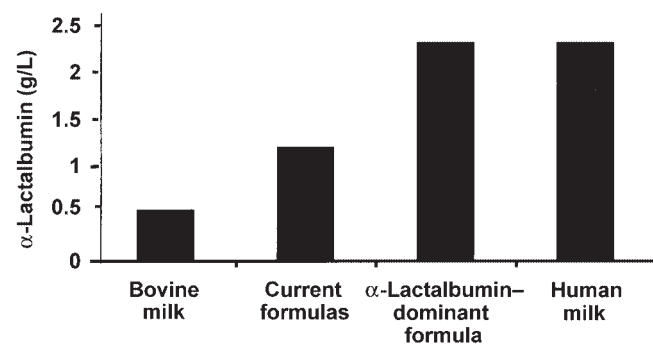

FIGURE 3. Concentrations of α-lactalbumin in bovine milk, infant formula, and human milk (human milk data from reference 20).

formulas enriched in α-lactalbumin is a step closer to the creation of a formula with the composition of human milk. However, the study was not designed to address questions on the effect of such formulas on the normal growth and protein status of term infants (the study was of short duration in a limited number of infants). To evaluate whether a lower-protein formula with concentrations of α-lactalbumin similar to those of human milk would support normal growth and improve protein utilization, a 12-wk, multi-center, randomized controlled trial was conducted (19). In this trial, an α-lactalbumin–enriched experimental formula (total of 14 g protein/L) was compared with a whey-dominant control formula (total of 15 g protein/L). The proportions of proteins in the control and α-lactalbumin–enriched formulas are shown in Figure 1. The total whey:casein remained unchanged (60:40). The dominant protein in the experimental formula was α-lactalbumin, and that in the control formula was β-lactoglobulin. Note that, in the experimental formula, there was a concomitant decrease in β-lactoglobulin as the concentration of α-lactalbumin was increased. The α-lactalbumin concentration in the experimental formula was 2.2 g/L, which is similar to the concentrations in human milk (2.4 g/L; 20) and almost twice as high as the concentrations in current formulas (**Figure 3**). Growth (length, weight, and head circumference) was assessed every 4 wk and found to be typical for term infants and similar in infants fed either formula. Serum albumin, a global measure of protein adequacy, showed a similar mean increase from baseline in the 2 groups during the course of the study (control group: 0.6 g/dL; experimental group: 0.5 g/dL). Blood urea nitrogen concentration, an indicator of recent protein intake, was significantly lower in the experimental group than in the control group at the end of the study, which is consistent with a lower intake of dietary nitrogen. The above data show that the α-lactalbumin–enriched formula supports normal growth and maintains protein status at lower amounts of protein consumption. An increase in protein utilization is therefore documented.

POSSIBLE BENEFITS OF FORMULAS RICH IN α-LACTALBUMIN OR TRYPTOPHAN

Feeding infants α-lactalbumin–rich formulas resulted in higher plasma tryptophan concentrations than did feeding infants standard formulas. An emerging research area relates to the effect of tryptophan feeding on neurobehavioral outcomes. Tryptophan is the precursor of the neurotransmitter

serotonin and the neurosecretory hormone melatonin. Serotonin and melatonin regulate many neurobehavioral effects such as appetite, satiation, mood, pain perception, and the sleep-wake rhythm (6, 7). When tryptophan is fed in low amounts relative to other LNAAs, the transport of tryptophan across the blood-brain barrier is decreased by competition for the transport system.

Feeding α-lactalbumin as a source of tryptophan has shown neurobehavioral benefits in adults. Markus et al (21) compared the effects of α-lactalbumin with those of casein on stress response in adults. Among stress-vulnerable subjects, those receiving α-lactalbumin had significantly lower depression scores than did those receiving casein. In a related study, these same authors also observed that α-lactalbumin improved cognitive performance in stress-vulnerable subjects (22).

Newborns and infants may be at risk of insufficient bioavailability of tryptophan for optimal serotonin synthesis in the brain. In term infants, Yogman et al (7) evaluated the acute effects of enterally administered tryptophan or valine (an amino acid that is not a precursor of neurotransmitters but that does compete for the LNAA transport system). Infants who received tryptophan in a glucose solution entered both active and quiet sleep significantly more rapidly than did infants who received a standard infant formula or infants who received valine in glucose. In contrast, the time required by infants receiving valine to enter these sleep states was significantly longer than that required by infants receiving the standard formula. Steinberg et al (23) assessed infant behavior during an 8-wk feeding period of low-protein infant formulas (< 13 g protein/L) supplemented with 3 concentrations of tryptophan. A breastfed group and a standard casein-dominant formula (15 g protein/L) group were included. The groups receiving tryptophan-fortified formula had a latency to sleep that was significantly shorter than that in the unsupplemented formula groups and similar to that in the breastfed group. These results show the potential for amino acid status to influence processes beyond requirements for normal protein synthesis, but much more research is needed to understand the specific effects in infants.

In conclusion, among the more pronounced differences between infant formula and breast milk are differences in protein concentration and composition. Human milk, which is low in protein compared with current formulas but rich in essential amino acids, is ideal for the term neonate. Formulas with amino acid profiles similar to those of human milk will permit the lowering of total protein content to a concentration nearer to that in human milk at the same time that they retain the nutritional benefits of current formulas.

REFERENCES

1. Rudloff S, Kunz C. Protein and nonprotein nitrogen components in human milk, bovine milk, and infant formula: quantitative and qualitative aspects in infant nutrition. J Pediatr Gastroenterol Nutr 1997; 24:328–44.
2. Fomon SJ. Nutrition of normal infants. St Louis: Mosby, 1993:94.
3. Brew K, Grobler JA. α-Lactalbumin. In: Fox PF, ed. Proteins. Vol 1, Advanced dairy chemistry. London: Elsevier Applied Science, 1992: 191–229.
4. Stacey A, Schnieke A, Kerr M, et al. Lactation is disrupted by α-lactalbumin deficiency and can be restored by human α-lactalbumin gene replacement in mice. Proc Natl Acad Sci U S A 1995;92: 2835–9.
5. Dupont C. Protein requirements during the first year of life. Am J Clin Nutr 2003;77(suppl):1544–9.
6. Heine WE, Klein PD, Reeds PJ. The importance of α-lactalbumin in infant nutrition. J Nutr 1991;121:277–83.
7. Yogman MW, Zeisel SH, Roberts C. Assessing effects of serotonin precursors on newborn behavior. J Psychiatr Res 1982;17: 123–33.
8. Anisman H, Zacharko RM. Multiple neurochemical and behavioral consequences of stressors: implications for depression. In: File SE, ed. Psychopharmacology of anxiolytics and antidepressants. New York: Pergamon, 1991:57–82.
9. Lo CW. Human milk: nutritional properties. In: Walker WA, Watkins JB, eds. Nutrition in pediatrics. Hamilton, Canada: Decker, 1996: 436–48.
10. Sturman JA. Taurine in development. J Nutr 1988;118:1169–76.
11. Gaull GE, Rassin DK, Räihä NCR, et al. Milk protein quantity and quality in low-birth-weight infants: effects on sulfur amino acids in plasma and urine. J Pediatr 1977;90:348–55.
12. Hanning RM, Paes B, Atkinson SA. Protein metabolism and growth of term infants in response to a reduced-protein, 40:60 whey:casein formula with added tryptophan. Am J Clin Nutr 1992;56:1004–11.
13. Fazzolari-Nesci A, Domianello D, Sotera V, Räihä NC. Tryptophan fortification of adapted formula increases plasma tryptophan concentrations to levels not different from those found in breast-fed infants. J Pediatr Gastroenterol Nutr 1992;14:456–9.
14. Lönnerdal B, Chen C-L. Effects of formula protein level and ratio on infant growth, plasma amino acids and serum trace elements. Acta Paediatr Scand 1990;79:257–65.
15. Lönnerdal B, Hernell O. Effects of feeding ultrahigh-temperature (UHT)–treated infant formula with different protein concentrations or powdered formula, as compared with breast-feeding, on plasma amino acids, hematology, and trace element status. Am J Clin Nutr 1998;68:350–6.
16. Girard J, Pegorier JP. An overview of early post-partum nutrition and metabolism. Biochem Soc Trans 1998;26:67–152.
17. Heine WE. The significance of tryptophan in infant nutrition. In: Huether G, Kochen W, Simat TJ, Steinhard H, eds. Tryptophan, serotonin and melatonin: basic aspects and applications. New York: Kluwer Academic/Plenum Publishers, 1999:705–10.
18. Heine W, Radke M, Wutzke KD, Peters E, Kundt G. Alpha-lactalbumin-enriched low-protein infant formulas: a comparison to breast milk feeding. Acta Paediatr 1996;85:1024–8.
19. Lien E, Davis A, and multi-center study group. A multicenter study of the growth, acceptability and protein status of a lower protein term infant formula with increased bovine alpha-lactalbumin. J Pediatr Gastroenterol Nutr 2002;34:A479 (abstr).
20. Jackson J, Kuhlman C, Lönnerdal B, et al. Variations in concentrations of alpha lactalbumin (A Lac) in human milk: a nine-country survey. FASEB J 2002;16:A663 (abstr).
21. Markus CR, Olivier B, Panhuysen GE, et al. The bovine protein α-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. Am J Clin Nutr 2000;71:1536–44.
22. Markus CR, Olivier B, de Haan EH. Whey protein rich in α-lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. Am J Clin Nutr 2002;75: 1051–6.
23. Steinberg LA, O'Connell NC, Hatch TF, et al. Tryptophan intake influences infants' sleep latency. J Nutr 1992;122:1781–91.