Protein requirements during the first year of life¹–⁴

Christophe Dupont

ABSTRACT

The composition of human milk provides the model for estimated total protein and essential amino acid requirements during infancy. However, both the total protein content and the concentrations of individual proteins in human milk change throughout the first year of lactation. Recent reassessments of estimated requirements have resulted in lower total protein recommendations and have emphasized the provision of α-amino nitrogen because most nonprotein nitrogen is not used for maintenance or tissue deposition. In clinical studies, formulas containing various whey-to-casein ratios and having total protein concentrations in the range of 13–15 g/L were shown to promote adequate growth and to result in biochemical measures of protein nutritional status similar to those in breastfed infants. In the second half of infancy, human milk can provide most of the protein needed, provided a modest protein supply is obtained from weaning foods. In special situations in which greater protein intakes are desired, special preparations of protein might be needed. Am J Clin Nutr 2003;77(suppl):1544S–9S.

KEY WORDS Protein, nutrition, infancy, protein requirements

INTRODUCTION

Growth and development between birth and weaning are crucial for long-term well-being. Dietary protein requirements are at their highest during this time period to support both maintenance and the high rates of tissue formation. Protein deposition is highly efficient. About 87% of protein intake over and above that used for maintenance is utilized for tissue synthesis (1). The tissue protein synthesis rate (on a g · kg⁻¹ · d⁻¹ basis), however, is only modestly higher during the first months of life than in older infancy (2). The somewhat slower rates of tissue synthesis in later infancy indicate a diminishing anabolic response to nutrient intake as lactation progresses, which is paralleled by lesser metabolic responses to insulin and amino acids. The total-body protein synthesis rate is ≈4 times greater than actual protein intake at birth and at weaning, which is evidence for extensive reutilization of amino acids in infants. The higher rate of weight gain in formula-fed infants than in breastfed infants may be related to the higher protein content of formula than of human milk. Interestingly, the amino acid composition of human milk protein is distinct from that of the synthesized tissue, which suggests that metabolism of amino acids and nontissue utilization of amino acids must be considered in estimates of protein quantity and quality requirements (3).

THE MODEL OF BREAST MILK

Appropriate protein intake is essential to meet the nutritional needs of infants and should be envisioned in relation to the gold standard of breast milk. According to the World Health Organization, exclusive breastfeeding by a healthy mother is the feeding standard from birth to 6 mo in healthy, term infants. Breastfeeding is encouraged up to 2 y of age, provided that a normal diversification of feeding with good-quality complementary foods is begun at the 7th month. Several assumptions are now well substantiated: breast milk alone can meet nutrient needs during the first 6 mo, with the possible exception of needs for vitamin D in certain populations and for iron in infants of relatively low birth weight. Estimates of protein requirements in the first half of infancy have been based on the protein composition of human milk and assume that the protein requirements of most infants are met by breast milk. However, monitoring of the growth of breastfed infants with the use of national or international growth charts suggests that breastfed infants fall off growth trajectories, especially in the second 6 mo of life. The growth charts themselves were developed from measures of growth of mostly formula-fed infants and thus may not represent the expected normal growth of breastfed infants. New growth charts based on infants breastfed throughout the first year of life are presently being developed by the World Health Organization.

There is no singular standard for breast-milk composition. Total protein varies markedly as a function of the duration of lactation, providing from >2 g/kg to the infant in the first weeks of life to ≈1.15 g/kg at 4 mo. During the same time, the whey-to-casein ratio changes from 80:20 in colostrum to 60:40 in term milk and is even lower in late-lactation milk (4). The different digestibility and kinetics of absorption of amino acids from casein and whey proteins (5) and the different amino acid composition of these fractions means that the protein nutriture of breastfed infants is continuously changing.

Estimates by the FAO/WHO for protein requirements (crude nitrogen × 6.25) are likely overestimates based on fixed protein-to-energy ratios in infant diets, ie, the amount of protein per calorie. Expressing protein on a per calorie basis was thought to ensure that protein needs would be met if energy needs were met. At adequate energy intakes, therefore, protein could be utilized for maintenance and tissue deposition, not as an energy source. More

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⁴ Address reprint requests to C Dupont, Service de Neonatologie, Hôpital Saint Vincent de Paul, 82 Avenue Denfert Rochereau, Paris Cedex 14, France 75674. E-mail: christophe.dupont@svp.ap-hop-paris.fr.
ment at 3–4 mo of age has been estimated to be 2% for nutriture among breastfed infants, ie, < 5%, the actual protein requirement may be distinct from the mean protein requirement.

Reassessed (1), resulting in a downward revision in estimated protein requirements during infancy of 10–26%. Part of the difference can be attributed to better measurements of protein intake in breastfed infants and higher estimates of utilization of nonprotein nitrogen (NPN) from breast milk. However, a significant conceptual difference was also introduced: that the mean protein intake may be distinct from the mean protein requirement.

Using the factorial method to reevaluate protein needs, Dewey et al (1) estimated maintenance nitrogen requirements to be 82–93 mg \( N \cdot k g^{-1} \cdot d^{-1} \), which is lower than the 1985 estimate of 120 mg \( N \cdot k g^{-1} \cdot d^{-1} \). Another revision was the elimination of a 50% overage in the daily nitrogen accretion rate to account for day-to-day variability in growth. The new estimates of protein requirements for 1–2-mo-old infants are thus reduced from 2.25 to 1.99 g \( \cdot k g^{-1} \cdot d^{-1} \), those for 5–6-mo-old infants from 1.30 to 0.92 g \( \cdot k g^{-1} \cdot d^{-1} \), and those for 9–12-mo-old infants from 1.15 to 0.78 g \( \cdot k g^{-1} \cdot d^{-1} \).

Assuming that there is a low prevalence of inadequate protein nutriture among breastfed infants, ie, < 5%, the actual protein requirement at 3–4 mo of age has been estimated to be 1.1 g \( \cdot k g^{-1} \cdot d^{-1} \), which is considerably lower than the 1985 WHO/FAO/UNU estimate of 1.47 g \( \cdot k g^{-1} \cdot d^{-1} \) (6). High protein intakes by formula-fed infants, as the result of overestimates of protein requirements, and possibly self-regulation of energy intake, reflected by the lower body temperature and metabolic rate of breastfed infants (7), may explain in part why breastfed infants tend to gain less weight and are usually leaner than are formula-fed infants in the second half of infancy (8).

**WHAT PROTEIN SHOULD BE TAKEN INTO ACCOUNT?**

When assessing the amount of protein available to and required by an infant, the breast-milk content of NPN and some bioactive proteins that are not completely digested should be taken into account. According to some studies, 13–50% of NPN is used for the production of nonessential amino acids, and recycling of ammonia produced from urea secreted into the colon adds to the nitrogen available for the synthesis of amino acids (9). Similarly, the “true” protein available to formula-fed infants is less than estimated by conventional analysis (for cow milk protein, protein is calculated as total nitrogen \( \times 6.38 \)), which includes free amino acids, peptides, and other NPN.

NPN in mature human milk contributes more significantly to total nitrogen (24% of total nitrogen) than does NPN in infant formulas. Casein-dominant formulas contain only 5–7% of total nitrogen as NPN; electro dialyzed whey has 14–18% of total nitrogen as NPN, and ultrafiltrated whey has 6–8% (10). Not all the NPN is available for protein synthesis. About one-half of the NPN of human milk is urea. In adults, about one-quarter of urea nitrogen may be utilized by colonic bacteria in the synthesis of amino acids available to the host (11). Studies in infants suggest that 3% of milk urea is incorporated into serum proteins (12), and 13% of total urea nitrogen is retained in the body. Formula urea may have a different bioavailability than does human milk urea as a result of different colonic bacterial populations in breastfed and formula-fed infants. At the incorporation rate of adults, urea nitrogen might provide 2% of the protein requirements of infants. Small peptides and free amino acids in human milk are in micromolar concentrations (13). Consequently, NPN from urea, low-molecular-weight \( \alpha \)-amino nitrogen sources, and minor compounds of milk such as creatine and creatinine, choline, polyamines, nucleotides, and amino sugars do not contribute quantitatively to satisfying nitrogen needs for maintenance or growth.

**WHICH AMINO ACIDS SHOULD BE TAKEN INTO ACCOUNT?**

Although there is a relation between total protein intake and the growth rate, this relation does not apply to each amino acid in milk proteins. Some amino acids, notably glycine and arginine, are supplied in milk in quantities that are much less than the needs of the neonate. As a result, milk-fed neonates carry out a tightly regulated transfer of nitrogen from amino acids in excess to the deficient ones (3). The designation of which amino acids are essential or indispensable has not changed substantially over time (Table 1), although some disagreement exists over the possible requirements for some amino acids, including for roles other than in protein synthesis. The most notable difference is that histidine is now considered essential,

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**TABLE 1**

<table>
<thead>
<tr>
<th>Indispensable (essential)</th>
<th>Conditionally indispensable</th>
<th>Dispensable (nonessential)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>Valine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Isoleucine</td>
<td>Cystine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leucine</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lysine</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Methionine</td>
<td>Methionine</td>
<td>Proline</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phenylalanine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Threonine</td>
<td>Taurine</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Tryptophan</td>
<td>Histidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 From Young et al (14).

2 The National Academy of Sciences (15) Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids suggests that glutamate, alanine, or aspartate may be required as a source of \( \alpha \)-amino nitrogen.
The minimum protein quantity of 1.8 g/100 kcal is specified by the EC for cow-milk-based formulas, although higher amounts of protein are required for hydrolysate formulas (2.25 g/100 kcal, or 15 g/L) and soy-protein-based formulas (2.25 g/100 kcal). In addition, the EC specifies protein quality by setting minimum concentrations of each essential and semiemssential amino acid on a mg/g total protein basis, requiring that not less than the concentrations of human milk protein amino acids be met (Table 2; 17).

On the basis of advice from the American Academy of Pediatrics, the US Infant Formula Act and the FDA’s regulations also stipulate a minimum protein concentration of 1.8 g/100 kcal, which at a standard energy concentration of 667 kcal/L is 12 g/L. The quality of protein must be ≥70% of that of casein in a PER; if the PER is less than that of casein, the total amount of protein must be increased in a reciprocal manner. For example, if the protein quality was 95% of that of casein, then 1/0.95 times the minimum of 1.8 g/100 kcal, or 1.89 g/100 kcal would be the minimum allowed. The specification, according to original guidance from the American Academy of Pediatrics, is that infant formula “contain an amount and quality of protein similar to that of human milk.” Thus, CODEX, the EC, and the United States specify minimal protein quality specifications in addition to minimal quantity specifications, and both quantity and quality may be seen as practical ways to ensure that infant formula protein closely approximates human milk protein.

More recently, the FDA commissioned a review of the minimum specifications for infant formula (18), conducted by the Life Sciences Research Office (LSRO). The LSRO noted that a safe intake typically was the amount that meets or exceeds the requirements of practically all the individuals in a group, whereas the minimum protein content in infant formula was based on the minimum protein content of human milk. A new type of specification was recommended for the first time by the LSRO: namely, that the minimum protein concentration be expressed on the basis of the amount of α-amino nitrogen rather than on the basis of the estimated amount of protein calculated from total nitrogen. Thus, the recommendation was a minimum specification of 1.7 g/100 kcal rather than 1.8 g/100 kcal. Because the 1.7 g value excludes NPN components that are unavailable for protein synthesis, the same amount of true protein is recommended. The LSRO modified the PER test for protein quality because it encompasses digestibility and bioavailability of amino acids.

Another new recommendation by the LSRO was the establishment of minimal requirements for essential amino acids, following guidelines set up by the EC (17). The LSRO used data from 4 recent publications on the protein composition of human milk to develop a table of amino acid requirements and also recommended that twice the minimum value of any indispensable amino acid be a maximum allowable level. The LSRO did not include the conditionally essential amino acids arginine and histidine, and their estimates of total isoleucine, valine, and leucine in human milk are slightly greater than the values set by the EC. The LSRO concluded that no correction need be made for commonly used protein sources for infant formulas, because there was no significant difference in the digestibility of these proteins, but that digestibility and clinical demonstration of protein adequacy would be needed for new protein sources.

Table 2. Essential and conditionally indispensable amino acids in human milk

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount (mg/100 kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>69</td>
</tr>
<tr>
<td>Cystine</td>
<td>24</td>
</tr>
<tr>
<td>Histidine</td>
<td>45</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>72</td>
</tr>
<tr>
<td>Leucine</td>
<td>156</td>
</tr>
<tr>
<td>Lysine</td>
<td>122</td>
</tr>
<tr>
<td>Methionine</td>
<td>29</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>62</td>
</tr>
<tr>
<td>Threonine</td>
<td>80</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>30</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>59</td>
</tr>
<tr>
<td>Valine</td>
<td>80</td>
</tr>
</tbody>
</table>

1 From Annex 5, reference 17.

whereas previously it was deemed conditionally essential (14, 15). Arginine and histidine are included by the European Community (EC) in their list of human milk amino acids that must be matched in infant formulas. The recent National Academy of Sciences dietary reference intakes for amino acids cite new studies that suggest α-amino nitrogen in the form of glutamate, alanine, or aspartate may be nutritionally essential, perpetuating the controversy over just which amino acids are essential (15).

A formula with a low protein concentration is acceptable if the intake of each constituent amino acid is not less than that required, with use of the reference of the breastfed infant (9). If the quantity of protein in the diet decreases, quality and digestibility become more important. Whole-fat cow milk protein contains less cysteine and tryptophan per gram than does breast-milk protein, so that these may become limiting amino acids as total protein intake is reduced. Whey-dominant formulas provide more cysteine and more tryptophan than do casein-dominant formulas. Milk proteins and peptides also have nutritional properties somewhat different from the simple provision of their constituent amino acids, in part because the rates of digestion and absorption of free amino acids differ from those of amino acids bound to protein. Whey-dominant formulas, which are more readily digested than are casein-dominant formulas, with a true protein concentration of 13 g/L (ie, excluding NPN) and processed with ultrahigh temperature to improve digestibility, support plasma tryptophan concentrations seen in breastfed infants (16).

Protein systems for infant formula may be devised that provide increased amounts of tryptophan and would thereby enable further reductions in total protein concentration.

PROTEIN REGULATIONS

The requirements of 3 major regulatory bodies—CODEX, the EC, and the Food and Drug Administration (FDA)—determine the minimal specifications for protein and amino acids in infant formulas. CODEX specifies the minimum protein quantity at 1.8 g/100 kcal (12 g/L) and specifies that the total amount of protein be calculated as nitrogen content × 6.38. CODEX also specifies that the protein quality not be <85% casein, as assessed by the protein efficiency ratio (PER). Drafts of revisions of the CODEX standard now contain language about use of a chemical score (ie, the minimum ratio among all ratios of the amounts of an amino acid in a test product to the amount of that amino acid in human milk) for protein quality assessment rather than the PER.
human milk, a standard starting formula should have a minimum protein content of 1.8 g/100 kcal or 12 g/L in a 670-kcal/L formula. Available protein will thereby represent nutritionally a minimum of 25% more than provided on average through the first 6 mo of breastfeeding. Formulas with more protein will not provide any advantages but will stress the metabolic and excretory functions of the infant. Compared with higher-protein formulas, reduced-protein formulas with a whey-to-casein ratio of 60:40 or 50:50 will produce plasma amino acid patterns more similar but not identical to those of breastfed infants (19).

Fomon et al (20) reported data on male infants fed low-protein, whey-dominant formulas containing 11 g/L from days 8–13; from days 14–55, the first 240 mL of formula each day was an 11-g/L formula, with subsequent feedings alternatively using formulas with 8 or 11 g/L. From days 56–83, the formulas were similarly alternated but the first feeding was with the 8-g/L formula. From days 84–112, the 8-g/L formula was fed. Control infants were fed a formula containing 15 g/L. A reference group from a historical database of infants was used as a comparison. The historical control subjects were fed one of a wide range of formulas that had protein concentrations from 1.98 to 2.67 g/100 kcal (13.2–17.8 g/L). Weight gain was not affected by the low-protein diet, and length gain of the infants fed the low-protein formula did not differ significantly from that of the control group, although it was less than that of the historical reference group. Furthermore, blood albumin concentrations did not differ significantly among groups. However, blood urea nitrogen values were low in the low-protein group and were described as being lower than those of breastfed infants. The authors suggested that the low urea concentration might indicate inadequate total protein intake, but the concentration of urea in human milk is quite high and some blood urea nitrogen in breastfed infants may be related to milk urea intake. Because of the complex feeding schedule used in this study, it is difficult to know at which point protein intake was inadequate, if indeed it was. Urea nitrogen concentrations were lower in the low-protein group at each time point. But the absence of an effect on growth or on albumin concentrations suggests that there was no clinical consequence of the low-protein diet. The fact that the length gain difference was significant only in comparison with the historical control group and not the concurrent control group fed a fixed formula containing 15 g/L suggests that the current sample of infants differed in some environmental way from the historical reference group. One possibility is that the reference group comprised subjects fed a variety of formulas, including some with protein amounts substantially >15 g/L, which resulted in greater length gains.

In a more recent study of low protein intake (21), male infants were fed a casein-dominant formula with a concentration of 11.5 g/L (calculated from protein nitrogen plus nonprotein a-amino nitrogen × 6.38, or total nitrogen × 6.11) for 112 d. Growth and serum amino acid concentrations were compared with those of a historical reference group of male infants aged 28 or 84 d fed either casein-dominant or whey-dominant formulas with protein contents ranging from 1.8 to 2.7 g/100 kcal (12–18 g/L). The outcome was also compared with a second reference group of breastfed male infants aged 56 and 112 d. The experimental group lost 6 of 22 enrolled, and the mean weight gain of those lost to the study was less than that of those who completed the study. This attrition may have introduced a bias in that the infants who completed the study tended to be the faster-growing infants from the initial group. Indeed, they consumed more energy over the course of the study than did the reference formula-fed infants. They had a larger BMI than did the reference formula-fed infants from the start, a difference that was thereafter maintained, and they gained more body weight than did the reference formula-fed infants. Length, serum albumin, and urea nitrogen did not differ significantly between the low-protein group and the reference breastfed group, suggesting that protein status was not adversely affected at 11.5 g total alpha-protein/L. It is unknown whether there was any difference between the reference infants fed the casein-dominant formula and the reference infants fed the whey-dominant formula in any of the outcome measures. The authors indicated that the infants receiving the low-protein formula received sufficient protein but could not conclude that the formula was safe because of the possibility of increased weight gain. As indicated above, the loss of infants during this study raises questions that can only be answered by further research. In the United Kingdom, casein-dominant formulas are promoted for hungrier infants, perhaps related to the slower rate of nitrogen absorption from casein than from whey proteins (4).

Lönnerdal and Chen (22) measured growth and serum amino acid concentrations of infants fed formulas with 55% whey and 45% casein and total protein contents of 15.5, 14.2, or 12.9 g/L (14.7, 13.3, or 12.2 g true protein/L, respectively) and also whey-dominant (60:40) and casein dominant (20:80) control formulas containing 14 g total protein/L. There was no significant difference in growth outcomes among the groups and only occasional differences in amino acid concentrations. Furthermore, in most instances where there were differences in amino acid concentrations between the breastfed reference group and the infants fed test formulas, the differences were less for the lowest protein group than for the others. The exception to this was that at 12 wk of feeding, all formulas resulted in lower plasma tryptophan concentrations, and the difference was most notable in the 12.9-g/L (55:45) group. Blood urea nitrogen and serum protein also did not vary among the formula-fed groups. These results indicate that lower-protein formulas might be developed successfully to more closely match the responses of breastfed infants, provided that the quality of protein is ensured by clinical results.

PROTEIN-TO-ENERGY RATIOS AND PROTEIN IN THE MIXED DIET DURING THE SECOND HALF OF INFANCY

Pragmatic and physiologic considerations justify expressing protein requirements in relation to energy intake, ie, g/100 kcal, for situations as diverse as weaning diets, catch-up growth, and hypercatabolic states (9). Human milk provides generous quantities of some nutrients, eg, vitamin A, folate, vitamin B-12, vitamin C, iodine, and selenium. Consequently, even as the total volume of human milk consumed is slowly displaced by complementary foods, there is little dependence on the complementary foods in the infants’ diet to provide these nutrients.

The concentration of protein in breast milk at 1–3 mo, at typical volumes of intake, provides ~1.25 g · kg⁻¹ · d⁻¹, more than the estimated requirements for protein for maintenance and growth of 1.13 g · kg⁻¹ · d⁻¹ (1). At 6 mo of lactation, protein intake from breast milk is ~1.10 g · kg⁻¹ · d⁻¹, whereas estimated needs for growth and maintenance are 0.86 or 0.74 g · kg⁻¹ · d⁻¹ for 3–6-mo-old and 6–12-mo-old infants, respectively (1). Protein needs are met by breast-milk protein if the infant is exclusively breastfed, but at the time of weaning, the most suitable protein-to-energy ratio in a milk or formula will depend on the protein-to-energy
ratio of the weaning foods available and will therefore vary by country. The protein-to-energy ratio of the solid weaning food in many developed countries is high, reaching 2.5 g/100 kcal after correction for protein quality (9); thus, a very-high-protein milk is not needed to achieve satisfactory intakes. However, modest displacement of breast milk by low-protein weaning foods can result in inadequate total protein intake (Figure 1). In many developing countries, the only weaning food is maize or rice, which has a low protein-to-energy ratio. When the protein concentration of the weaning food drops below that of milk, ie, when it is < 1 g/100 kcal (such as for cassava), it is impossible to meet total protein needs. The alternate approach to meeting protein needs in situations where complementary foods contain no or only low amounts of protein is to use follow-on formula (formula designed for infants aged 5–12 mo according to EC regulations) containing more protein (23).

Dewey (8) calculated that by 12–23 mo, complementary foods typically contribute 57% of the protein needed. Others have experimentally lowered the protein content of follow-on formula to determine the nutritional resiliency of such diets to protein-poor complementary foods. Karlsland Åkeson et al (19, 24) fed infants who had been exclusively breastfed for 3 mo follow-on formulas containing 13, 15, or 18 g protein/L. Infants who were either breastfed or partially breastfed were additional groups. All infants were permitted the same complementary foods. There were no significant differences among the formula groups in growth or serologic measures of protein status (albumin, prealbumin, transferrin, and creatinine). There were few differences in plasma amino acid concentrations, although the 18-g/L group had many essential amino acid values that were higher than those of the breastfed reference group. In addition, the sum of essential amino acids was higher in both the 15- and 18-g/L groups than in the 13-g/L group at 6 mo of feeding. The authors concluded that 13 g/L is adequate in all regards for follow-on formula, provided high-protein complementary foods, like those in Sweden, are used. The same investigators also studied Italian infants in a similar paradigm and reported similar results (25). Follow-on formulas are designed specifically to meet the child’s demands when food diversification is limited.

CONCLUSIONS

Continued research on the dietary protein and amino acid requirements of breastfed infants and comprehensive reassessments of earlier recommendations have resulted in lower quantitative recommendations for protein during infancy. Clinical studies of formulas containing amounts of protein have shown good growth and serologic responses of protein status, but as the quantity of protein is reduced, special attention must be paid to ensure that the quality of the protein is adequate to meet essential amino acid requirements. In the second half of infancy, when protein requirements are even lower, continued breastfeeding in combination with feeding most weaning foods, or use of formulas containing ≥ 13 g protein/L, satisfies the protein needs of infants. 2

The author had no conflicts of interest.

REFERENCES