Protein in the Feeding of Term Infants


Abstract

High protein requirements of premature infants during the first weeks of postnatal life are a well-established fact. Those infants gain fat-free mass and protein rapidly during the first weeks of postnatal growth and require a much higher protein/energy ratio than term infants. Recommended protein intakes are 3.5–4.0 g/kg per day. For term infants, on the other hand, FAO and WHO have recently lowered recommended protein intakes to better reflect our current knowledge about the protein concentration in breast milk during the first 12 months of lactation. Longitudinal randomized clinical trials now confirm that term infants who are fed infant and follow-up formulas with protein concentrations >2.25 g/100 kcal (high protein formulas) during the first year of life grow faster than indicated by the WHO growth standards. Rapid weight gain during infancy is a predictor of childhood and adult obesity. Infants fed high protein quality formulas with protein concentrations of 1.6–2.2 g/100 kcal from 3 to 4 months onwards experience weight gain that is very close to that of breastfed infants. Biomarkers (insulin or IGF-1) of infants receiving low protein formulas differ from those of infants receiving high protein formulas. Six-year-old children who received low protein formulas in the first year of life had a lower risk of childhood obesity (BMI >95th percentile of WHO standards) compared with children who received high protein formulas as infants. BMI at 5 years of age is similar in children who were breastfed or received low protein formulas as infants. It is most important that the new low protein formulas are safe and adequate for all healthy term infants. Based on new protein technologies, the levels of essential and branched-chain amino acids in low protein formulas are now close to those in breast milk. Safety has been confirmed by following anthropometric parameters to 5–6 years of age and comparing these parameters with the WHO growth standards. Body composition measurements indicate similar protein...
accretion between 3 and 6 months of age in infants fed high or low protein formulas. Longitudinal data on body composition indicate that children who received a low protein formula until age 12 months gain less fat between 6 and 60 months than children who received a high protein formula. Breastfeeding and the use of low/high protein quality formulas in term infants who cannot be breastfed can help support appropriate metabolic programming during this critical period and reduce the risk of later obesity.

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Growth and Protein Requirements during the First 1,000 Days

In 2006, the WHO published international growth standards for children younger than 5 years, which are now accepted worldwide [1]. The INTERGROWTH-21st Project has recently provided international growth standards for fetuses, newborn infants and the postnatal growth period of preterm infants [2]. These standards can be used to calculate nutritional requirements for growth of premature and term infants and children up to 5 years of age. Weight gain per unit of body weight is high during the fetal period and decreases rapidly during the first postnatal months. Between the 24th and 32nd weeks gestational age, daily weight gain per unit of body weight is 9 times higher than during the 5th month of postnatal age (fig. 1) [3, 4]. Data on body composition of the fetus [3] and children [4] are helpful for estimating the protein requirements for growth. They are 7 times higher between the 24th and 32nd weeks gestational age than during the 5th month of postnatal age (fig. 1).

Protein requirements of low-birth-weight (LBW) infants at 24–32 weeks of gestation are as high as 3.5–4.0 g/100 kcal [5] and can be met only if breast milk is enriched with human milk fortifiers. In contrast, protein requirements of term infants during the first 6 months are met by exclusive breastfeeding. Protein concentration in breast milk decreases from an average of 2.09 g/100 kcal during the first month of lactation to 1.28 g/100 kcal at 3–4 months of lactation and about 1.24 g/100 kcal by 9–12 months (fig. 2) [6]. Protein requirements can be calculated for each age range using the revised recommendations from the Joint WHO/FAO/UNU Expert Consultation [7]. It has been shown that infants who are fed traditional (high protein) formulas and cow’s milk have protein intakes with milk that are 2–5 times higher than those of breastfed infants (fig. 2) [6–11]. As early as 1989, Axelsson et al. [12] demonstrated that feeding term infants with high protein starter formula resulted in high serum levels of branched-chain (‘insulinogenic’) amino acids and insulin. Longitudinal studies indicate that infants fed traditional high protein formulas grow faster than breastfed infants [9, 13]. Rapid weight gain in infancy is associated with higher obesity risk in childhood, adolescence and adulthood [14].
**Fig. 1.** Daily protein gain during the fetal and postnatal period. Calculation based on body composition data of the fetus and children.

**Fig. 2.** Protein concentrations (g/100 kcal) in breast milk, new experimental formulas (3–12 months) and cow’s milk. The recent lower and higher limits for protein concentration in the EU are also shown.
A review of data from epidemiological studies [15] and randomized controlled trials [9, 12, 16] indicates that children who are fed infant formulas with protein concentrations >2.10 g/100 kcal (high protein formulas) during the first year of life grow faster than indicated by the WHO standards. How can formula-fed infants be prevented from rapid growth? A randomized controlled trial [17] indicated that infants fed a whey-based formula with a protein content of 1.8 g/100 kcal have growth and metabolic outcomes that are similar to those of breastfed infants through 4 months of age. A meta-analysis (n = 1,150 infants) recently confirmed that at 4 months of age the weight of infants fed the 1.8 g/100 kcal formula corresponds to the median of the WHO standard [15]. The formula tested in those studies had an amino acid pattern that was close to the pattern in breast milk [15].

Growth of infants through 12 months of age was evaluated in 3 randomized trials of high and low protein formulas [9, 13, 16]. The trials had similar experimental designs and each study included a nonrandomized reference group of exclusively breastfed infants. The 3 trials followed the children until 5–6 years of age. Additionally, the CHOP study (EU Childhood Obesity Program) [18] was a multicenter European trial which compared outcomes of infants fed formulas containing 1.77 and 2.2 g of protein/100 kcal. Corresponding protein concentrations in follow-up formulas were 2.9 and 4.4 g/100 kcal. BMI was already significantly lower between 6 and 12 months of age in the group that received the low protein formulas. A follow-up examination at 6 years indicated a significantly lower BMI in the low protein group (p = 0.009) and a lower prevalence of childhood obesity (relative risk –2.87; 95% CI –1.22 to –6.75; p = 0.016) [13]. Blood urea nitrogen, insulin and IGF-1 in serum were lower at 6 months of age in the low protein group [19]. IGF-1 at age 6 months was a strong predictor of weight-for-length ratios at 6, 12 and 24 months. Our group performed 2 randomized controlled trials to evaluate a modified whey formula containing a lower protein concentration (1.6 g/100 kcal; fig. 2) [15]. Experimental design and results of the trials through 1–2 years have recently been published [9, 15, 16]. Follow-up data from the US multicenter trial on weight, height and BMI through 5 years of age are now available (table 1) [20]. At 5 years, weight and BMI in the high protein (2.15 g/100 kcal) formula group were significantly higher than in the breastfed reference group. Differences between the low protein (1.61 g/100 kcal) formula and breastfed groups were smaller and not statistically significant. Height was not significantly different between the 3 groups at 5 years. It should be mentioned that the difference in protein concentrations between the high and low protein formulas was as small (0.55 g/100 kcal), and there was no difference between groups in the amount of formula consumed between 3 and 12 months. At age 5 years, chil-
children who had received the low protein formula had mean weight-for-age scores of +0.57 (SD 0.84) and BMI z-scores of +0.42 (SD 1.06) that were above the WHO median. This indicates adequate growth of children who received a formula with a protein concentration of 1.6 g/100 kcal between 3 and 12 months of age.

In a longitudinal analysis of data from a Chilean study [9], which included infants of overweight and obese mothers, weight over the course of time from 3 months to 3 years of age was significantly lower in the low protein (1.65 g/100 kcal) formula group compared with the high protein (2.7 g/100 kcal) formula group (global test: p = 0.0142). Over the course of time from 3 months to 3 years of age, weight did not significantly differ between the breastfed and low protein groups (global test: p = 0.77), but high protein group was significant higher than breastfed (global test: p = 0.005). Over the course of time from 3 months to 3 years of age BMI z-scores of the high protein formula group were significantly higher than those of the breastfed reference group (global test: p = 0.001), but not the low protein formula group than the breastfed reference group (global test: p = 0.21). Visit wise ANCOVA on BMI z-scores displays significant differences (p < 0.05) at 6, 9, 12 and 24 months between the low protein formu-

| Table 1. Differences in weight, BMI, and BMI-for-age z-scores by infant feeding group (intention-to-treat analysis) |
|---|---|---|---|
| Age | Weight, g | BMI | BMI-for-age z-score |
| | difference (95% CI) | p | difference (95% CI) | p | difference (95% CI) | p |
| CTRL vs. HM |
| 0.5 y | 238.9 (118.3 to 359.6) | 0.0001 | 0.16 (0.11 to 0.42) | 0.253 | 0.10 (−0.07 to 0.28) | 0.251 |
| 1 y | 617.3 (380.6 to 853.0) | <0.0001 | 0.51 (0.14 to 0.87) | 0.007 | 0.34 (0.10 to 0.57) | 0.006 |
| 3 y | 389.6 (−68.2 to 847.5) | 0.095 | −0.02 (−0.41 to 0.36) | 0.898 | −0.03 (−0.32 to 0.25) | 0.809 |
| 5 y | 853.9 (12.9 to 1,695.0) | 0.047 | 0.56 (0.09 to 1.02) | 0.021 | 0.37 (0.01 to 0.72) | 0.043 |
| EXP vs. HM |
| 0.5 y | 167.9 (47.7 to 288.0) | 0.006 | 0.07 (−0.20 to 0.34) | 0.598 | 0.06 (−0.12 to 0.24) | 0.510 |
| 1 y | 385.6 (148.7 to 622.5) | 0.002 | 0.25 (−0.12 to 0.62) | 0.193 | 0.17 (−0.07 to 0.41) | 0.161 |
| 3 y | 172.4 (−299.6 to 644.4) | 0.472 | −0.07 (−0.47 to 0.32) | 0.715 | −0.08 (−0.38 to 0.21) | 0.590 |
| 5 y | 678.6 (−173.1 to 1,530.4) | 0.118 | 0.47 (−0.01 to 0.95) | 0.053 | 0.30 (−0.06 to 0.67) | 0.102 |
| EXP vs. CTRL |
| 0.5 y | −71.1 (−193.4 to 51.25) | 0.254 | −0.08 (−0.36 to 0.19) | 0.542 | −0.04 (−0.22 to 0.13) | 0.628 |
| 1 y | −231.7 (−473.2 to 9.86) | 0.060 | −0.26 (−0.64 to 0.12) | 0.174 | −0.16 (−0.41 to 0.08) | 0.184 |
| 3 y | −217.2 (−717.2 to 282.84) | 0.393 | −0.05 (−0.47 to 0.37) | 0.818 | −0.05 (−0.36 to 0.26) | 0.771 |
| 5 y | −175.3 (−1,100.0 to 749.24) | 0.709 | −0.08 (−0.60 to 0.43) | 0.753 | −0.07 (−0.46 to 0.33) | 0.744 |

CTRL = Children fed formula with a protein concentration of 2.15 g/100 kcal from 3 to 12 months of age (n = 47 at age 5 years); EXP = children fed formula with 1.61 g/100 kcal from 3 to 12 months of age (n = 47 at age 5 years); HM = exclusively breastfed until 4–6 months of age (reference group; n = 79 at age 5 years); y = years. Statistics: ANCOVA; correcting for maternal prepregnancy BMI, sex and value at 3 months (study start).
la and high protein formula. Recently, we received preliminary results of the follow-up at 5 years. Unfortunately, only 34 of 89 and 37 of 87 infants who were enrolled in the low and high protein formula groups, respectively, could be followed up at 5 years. There was no difference in BMI z-scores (0.12; 95% CI –0.23 to +0.47). In addition, BMIs of both formula groups did not differ from the BMI of the breastfed reference group (n = 34). In our studies [9, 16], IGF-1 at the age of 6 months was found to be strongly associated with weight gain at 3–6 months of age (p < 0.0008). However, IGF-1 cannot be utilized in clinical practice as a predictive biomarker of accelerated growth during the first 6 months of life because it explains only 5–6% of the variability in weight gain [9, 16] or weight-for-length ratios [15]. IGF-1 at 6 months [9], which was the time when exclusive formula feeding ended, was significantly lower in the low protein formula group (p = 0.010) and breastfed reference group (p = 0.005) compared with the group that received the high protein formula. No differences in IGF-1 were observed between the low protein formula and breastfed infants (p = 0.7).

**Body Composition**

The CHOP study [18] provides estimates of body composition based on isotope dilution methods in a subgroup of children fed high or low protein formula [21]. Weight gain velocity strongly correlated with fat mass (FM) z-score (r = 0.564, p < 0.001) but showed no association with fat-free mass (FFM) z-score. FM correlated with BMI at 6, 12 and 24 months. FFM and FM z-scores did not differ significantly between infants receiving the high and low protein formulas.

In the Chilean study [9] in children of overweight or obese mothers, there was no difference in percent FM and FFM at 12 and 60 months of age using dual energy X-ray absorptiometry (DEXA). In the US multicenter study [16], we compared changes in body composition between 3 and 6 months of age by employing air displacement plethysmography (Pea Pod®). Comparisons between 6 and 36, and 6 and 60 months of age were made by DEXA. Changes in body composition at selected age intervals were compared with the respective changes in reference children [4]. Between 3 and 6 months of age, the period when the high and low protein formulas were fed exclusively, protein accretion was of major interest. Protein is part of FFM [4]. Mean (SD) FFM gain in high protein (n = 15) and low protein (n = 17) was 1,089 g (315) and 1,032 g (233), respectively (p = 0.1). Mean daily protein accretion per kilogram of body weight was calculated by utilizing mean weights and FFM of the infants and published protein concentrations in FFM [4] at 3 and 6 months of age. Daily protein gain between 3 and 6 months was 0.32 and 0.31 g/kg per day in the high and low protein groups, respectively,
which is close to the value of 0.29 g/kg per day in reference children (fig. 1) [4].
Similar protein accretion rates in high and low protein formula groups between 3 and 6 months of age indicates that infant formula with 1.6 g/100 kcal of modified whey protein [15] is sufficient to support healthy growth. Also of interest are longitudinal DEXA data between 6 and 60 months of age, which indicate changes in body fatness after breastfeeding or high or low protein formula feeding in infancy. In reference children, percent body fat decreases 7.9 and 10.9% between 6 and 36, and between 6 and 60 months, respectively [4]. Figure 3 indicates changes in percent body fat in children who had been breastfed, or fed high or low protein formula during infancy. The high protein formula group differed significantly from the breastfed and low protein formula groups at both age intervals. No significant difference was observed between the breastfed and low protein formula groups. The longitudinal data on percent body fat could indicate a programming effect in the high protein formula group.

**Conclusions**

Protein requirements of LBW infants, in particular very LBW infants, are difficult to meet. If LBW infants are receiving breast milk, better fortification strategies are urgently needed. Conversely, most formula-fed infants still receive too
much protein, which can result in rapid growth during infancy and an increased obesity risk in later life. Clinical trials confirmed that feeding low protein formulas slows infant growth and results in BMI at 5–6 years of age that is comparable to BMI of breastfed children. Biomarkers (IGF-1 or insulin) in infants fed low protein formulas are lower than those in infants fed high protein formulas. Body composition measurements indicate adequate protein accretion rates in infants fed a formula with protein of 1.6 g/100 kcal between 3 and 6 months of age. Changes in percent body fat through 5 years of age differ in high and low protein formula groups. Collectively, these data indicate differences in metabolic programming in children who were fed high or low protein formula as infants.

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**References**

20 Ziegler EE, Fields DA, Chernausek SD, et al: Effect of infant formula with protein content of 1.6 g/100 kcal fed between 3–12 months on growth at 3 and 5 years of age. 9th World Congress of Developmental Origins of Health and Disease, Cape Town, November 10, 2015.