REVIEW: PROTEIN INTAKE DURING BREASTFEEDING, COMPLEMENTARY AND PRE-SCHOOL PERIODS

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Abstract  
Background: In recent years, research in the field of nutrition has broadened from how best to meet nutritional needs and prevent deficiencies to encompassing the impact nutrition has on health in the longer term. Consequently, a convincing body of evidence has been established which demonstrates that the nutrition experienced early in life (a period that is vital in physiological development) is likely to have a long-term impact on a person’s health.  
Purpose: This review investigated the impact of different levels of protein intake concerning three specific periods of early life: the breastfeeding period, the complementary feeding period and the pre-school period.  
Methodology: In searching for applicable articles, the search has been done using PubMed and MedLine searching engines and were limited to search peer-reviewed journals only, both were used to set specific criteria. Also, the basic Boolean search operator “AND” was used to narrow down search results as additional parameters. The citation index searching technique was also used to inspect the citation frequency of author’s work in other publications. Nutrition, Breastfeeding, complementary feeding and pre-school period, all these keywords were used in the search.  
Findings: A growing number of studies are exploring the potential effects of a high protein intake early in life on growth and obesity risk. These studies cover a wide range of ages, from breastfeeding through to complementary feeding and then on to the pre-school period. Some these studies suggest that a higher level of protein intake during infancy and early childhood is
associated with more rapid growth and a higher body mass index (BMI) in later childhood.

**Conclusion:** The age period that is the most sensitive to high protein intake remains unclear, and there is limited information on the effects of different types of protein. These findings are of interest; the role of protein intake in the development of overweight and obesity requires further study.

**Keywords:** Protein Intake, Breastfeeding, complementary feeding and pre-school period

**Introduction**

In recent years, research in the field of nutrition has broadened from how best to meet nutritional needs and prevent deficiencies to encompassing the impact nutrition has on health in the longer term. Consequently, a convincing body of evidence has been established which demonstrates that the nutrition experienced early in life (a period which is vital in physiological development) is likely to have a long-term impact on a person’s health in adulthood (Lanigan & Singhal 2009).

This idea that the nutrition experienced in the early years of a subject’s life has a life-long effect on their health is often referred to as ‘nutritional programming.’ This theory states that a stimulus that is introduced during the critical period of early development will effect long-term changes in the structure or function of an organism (Metges 2001; Riva et al. 2004). The ‘programming’ part of the theory is based on the hypothesis that it is the factors that influence organisms in their critical developmental period that subsequently determine their risk of contracting diseases later in life. It is thought that exposure to these risks can be limited or even avoided entirely by specific, targeted nutrition during pregnancy and the early postnatal period and that a longer term protection of the population’s health can, therefore, be achieved in this way (Mitrova & Bronsky 2013).

This review investigated the impact of different levels of protein intake concerning three specific periods of early life: the breastfeeding period, the complementary feeding period and the pre-school period.

In light of the research by Gunther et al. (2007), which argued that higher levels of protein intake in the earliest months of infants’ lives open them up to a risk of later obesity, it is important to examine the different stages of early life separately, which the following sub-sections go on to do. Gunther and his co-authors identified two main stages as being of key importance: the milk-feeding period (representing the first 4 to 6 months of life) and the complementary feeding period (representing the period when solids are progressively introduced, and which extends up to the end of the second year of life). The present discussion also includes the pre-school
period, as it has been demonstrated that this period is a vital phase of early life, in which a person’s long-term dietary habits are established (Lanigan & Singhal 2009). Each of these three periods will be discussed in turn.

**Protein intake during the breastfeeding period**

Breastfeeding is widely regarded as the optimal type of nutrition intake for infants because it provides the optimal level of nutrients for the development and growth of infants (Agostoni et al. 2005; Koletzko et al. 2012). Breastfeeding has also been shown to have positive impacts on many aspects of health, such as an increased protection against infectious diseases, boosted neurodevelopment and lower exposure to the risk of obesity, diabetes, and hypertension later in life (Koletzko et al. 2012). With these benefits in mind, the current global recommendation is that infants should be exclusively breastfed for the first six months of their lives, with a gradual introduction of solid foods from the age of six months onwards, which should ideally take place alongside continued breastfeeding. These recommendations, which were set out in a World Health Assembly resolution in 2001, were then formally adopted as governmental policy in the UK in 2003 and the US in 2005 (Baird et al. 2008).

Turning now to nutrient intake during the milk-feeding phase of early life, it is important to note that the key factors that differentiate human milk from infant formula milk are the quantity and quality of protein (Riva et al. 2004). These two forms of milk for infant consumption are now discussed in turn, starting with breast milk.

A steep decrease is observed in the protein content of breast milk over the first month of lactation. The decline then occurs much more gradually in the following months. This total protein content decrease can largely be traced to a decrease in secretory immunoglobulin A (sIgA) and lactoferrin. These substances are two bioactive proteins, and along with lysozyme, they constitute approximately 30% of the total amount of protein found in mature breast milk. The amount of nitrogen found in breast milk therefore also changes sharply, and this is particularly evident in the first few days of life, as it falls from approximately 2–2.5 g/100 ml in colostrum to about 1.0–1.2 g/100 ml in mature breast milk (Agostoni et al. 2005).

With regard to formula milk, most available infant formulas contain a slightly higher energy density than breast milk on average (kcal/100 ml), and as a result of this, formula-fed infants’ aged 3–12 months have reported energy intakes per kilogram of body weight that were 10–18% higher than the intakes of breastfed babies. However, when the difference in protein intakes is expressed as per kg bodyweight, they were much greater, at a 55–80% higher level for formula-fed infants than for breastfed infants (Koletzko et al. 2009).
Protein intake during the breastfeeding period and early growth

Little divergence in growth (expressed as gains in weight and length) is observed to occur when a comparison is made between breastfed and formula-fed infants during the first 6–8 weeks of life. However, it has also been observed that formula-fed infants gain weight and length at a much more rapid rate than breastfed infants from an age of about two months to the end of the first year of their lives (Ziegler 2006). This could create serious problems because, as Mihrshahi et al. (2011) observed, rapid weight gain during infancy is one of the strongest risk factors for obesity in later life. A literature review by Lanigan & Singhal (2009) has also found that at least twenty-one studies (which examined different populations and different ages) have reported associations between rapid weight gain throughout infancy and childhood and later health outcomes and that they specifically connected rapid growth to a greater risk of obesity later in life.

In answering the question of what causes these contrasting growth rates, it has been suggested by more than one study (Hoppe et al. 2004b; Ziegler 2006) that the differences that have been noted may be caused by a higher protein intake among the formula-fed infants, which can be directly linked to the higher protein content of formula milk compared with breast milk, as mentioned in the previous sub-section of the present work.

There has been discussion in several prior studies of the mechanisms that contribute to more rapid growth among formula-fed infants, and these studies have observed that a high intake of protein stimulates the secretion of an insulin-like growth factor 1 (IGF-1), which can promote accelerated growth in young children and create an increased likelihood of overweight and obesity when they are older (Koletzko et al. 2005; Michaelsen et al. 2012 & Rolland-Cachera et al. 1995). Madsen et al. (2011) describe the central role played by IGF-1 in the growth and early development of overweight and obesity. IGF-1 has anabolic effects, as it enhances glucose and amino acid uptake and inhibits protein breakdown (Boyne et al. 2003). It also mediates many of the effects of growth hormone on both embryonic and infant growth (Ben-Shlomo et al. 2005). In a double-blind, randomised controlled trial, Socha et al. (2011) examined the influence of protein intake on the accumulation of IGF-I in infants and discovered a positive dose-response relationship between infants’ protein intake and their total concentrations of IGF-I. The authors also mentioned that an association had also been made between higher protein intake and higher weight in the first two years of life by Koletzko et al. (2009). The latter study will be discussed in more detail below.

In the conclusions of a systematic review of 19 studies which had looked at affluent subject groups, Dewey (1998) stated that the cumulative difference in body weight among infants who had been breastfed for 9
months amounted to around 400 g lower weight by the age of 12 months, and up to 600–650 g lower weight at the age of 1 year in infants who had been breastfed for 12 months when they were compared to formula fed infants.

A recent and comprehensive systematic review by Hornell et al. (2013) provided an overview of the most recent scientific evidence on the short- and long-term health impacts of higher intakes of protein during infancy and later childhood. In this sub-section, the findings of this systematic review regarding the effects of higher intakes of protein during early infancy are given, and subsection 3, below, presents the findings of this systematic review that relate to the effects of higher intakes of protein during late infancy and early childhood.

The systematic review by Hornell et al. (2013) identified a total of two studies (which were both clinical trials) which had found an association between higher protein intake during early infancy and increased growth, while, in contrast, the findings of one study (i.e. one of the clinical trials) showed no such effect. Each of these studies is now discussed in more detail. In the former group, which found the association, a large, double-blind, randomised controlled trial conducted by Koletzko et al. (2009) generated the strongest evidence. In this study, infants who had been fed solely with formula in the first 2 months of their lives were randomised to be given formulas with higher or lower levels of protein, in order to establish whether or not a high protein intake early in infancy leads to a more rapid level of growth during the first 2 years of life. The results were that the higher protein content in infant formula was found to be associated with higher weight, but had no effect on length (when compared to infants who had been fed with a low-protein formula) in the first two years of life. The authors, therefore, concluded that the limiting of protein intake in infancy may be a valid method of decreasing the risk of overweight and obesity later in life. In a similar vein, Sandstrom et al. (2008) also performed a randomised control study and found that infants who had been fed with standard formula milk gained significantly more weight than breastfed infants. It should also be noted at this point that one of the studies identified by Hornell et al. (2013) (i.e. one of the clinical trials) found no association between protein intake and growth. A randomised control study by Raiha et al. (2002) found that protein intake had no influence on gains of weight or length, or on body mass index (BMI), in any of four different feeding groups (with variations of breastfed vs. formula-fed involving different protein contents and ratios of whey/casein) over the first 4 months of life. Similarly, two recent cohort studies have found that formula feeding is a likely risk factor in relation to excessive weight gain in early life. The first of these, by Gunnarsdottir et al. (2010), looked at the relationship between the duration of time in early life
spent exclusively breastfeeding and rapid weight gain during infancy, using data from two Nordic countries: Denmark (n=85) and Iceland (n=100). The study found that infants who had exclusively been breastfed for ≤2 months gained 348g more weight [95% Confidence Interval: 69, 626] between 2 to 6 months of age than infants who had exclusively been breastfed for 3–4 months.

The second study that is relevant here was carried out by Mihrshahi et al. (2011), which used birth data and baseline assessment information of infants (n=612) from a randomised controlled trial in Austria to identify the modifiable risk factors that could be associated with rapid weight gain during early infancy. The study found that, after adjusting for several variables (i.e. the mother’s age, smoking in pregnancy, BMI, and education and infant birth weight, age, gender and introduction of solid foods), the only two modifiable factors which could be clearly associated with rapid weight gain having attained statistical significance were formula-feeding [odds ratio: 1.72; 95% Confidence Interval: 1.01, 2.94] and feeding on schedule [odds ratio: 2.29; 95% Confidence Interval: 1.14, 4.61]. Mihrshahi et al. (2011) concluded that the key mechanisms that explain these findings may include the actual content of formula milk (especially its higher protein content) or different feeding styles (such as the option of feeding according to a set schedule chosen by some parents, which is thought to increase the risk of overfeeding).

Protein intake during the breastfeeding period and later obesity

A growing body of evidence has found that a shorter duration of breastfeeding is associated with a higher childhood BMI and that the inverse is also true, i.e., that a longer duration is associated with a lower childhood BMI (Mihrshahi et al. 2011). A comprehensive meta-analysis conducted by Harder et al. (2005) examined the association between the duration of breastfeeding and the risk of overweight. The authors concluded that the duration of breastfeeding could be inversely and linearly associated with the risk of becoming overweight in future, in that each additional month of breastfeeding was seen to be linked to a 4% lower level of obesity prevalence at later ages. Three other systematic reviews have also linked the two and found that when breastfeeding is longer in duration, a reduced risk of obesity results (Arenz et al. 2004a; Owen et al. 2005a & Owen et al. 2005b). Recently, Scott et al. (2012) examined the relationship between the duration of breastfeeding and children’s weight status using data from a national sample of children and adolescents in Australia. Their findings were that children who were breastfed for ≥6 months had a significantly lower risk of becoming overweight (adjusted odds ratio: 0.64, [95% Confidence Interval: 0.45, 0.91]) or obese (adjusted odds ratio: 0.51, [95% Confidence Interval:
0.29, 0.90]) in later childhood, when compared to children who were never breastfed at all, after adjustments were made for certain maternal factors (i.e. age, education and ethnicity) and for the children’s age, gender, total energy intake, level of physical activity, screen time and sleep duration. In line with this, as Robinson & Fall (2012) observed in their literature review, other epidemiological studies have also found a lower risk of overweight and obesity among children and adults who were breastfed as infants. For this reason, infancy has become a focal point in the public health debate, as it represents a critical period in the prevention of obesity.

Although they are not yet fully understood, the potential mechanisms by which breastfeeding apparently lowers the risk of overweight and obesity can broadly be classified into a) those who influence behaviour and b) those which are related to the unique nutritional composition of breast milk (Arenz et al. 2004b; Bartok & Ventura 2009; Lanigan & Singhal 2009; Singhal & Lanigan 2007). Each of these factors is now discussed in turn.

Explanations that centre around the influence which breastfeeding has on infant behaviour often include the theory that breastfed infants display different suckling patterns and higher suckling frequencies in comparison to infants who have not been breastfed. They, therefore, appear to have a greater degree of control over the size and intervals of their intakes than those fed with formula. These differences may be a factor in modulating body weight in later life (Koletzko et al. 2005). On the other hand, nutrition-based explanations of the inverse association between breastfeeding and obesity tend to focus on the bioactive nutrients in breast milk that are not present in some formulas (e.g. long-chain polyunsaturated fatty acids). It has also been observed that differences in protein intake in early life (which can mean up to a 70% greater volume of protein for formula-fed infants than the amount consumed by breastfed infants) may also influence their respective levels of overweight or obesity in later life, due to the higher protein content of formula milk, as discussed above (Singhal & Lanigan 2007).

**Protein intake during the complementary feeding period**

WHO published a worldwide recommendation in 2001 that for the first six months of life, infants should exclusively be breastfed, and from the age of 6 months onwards, they should be introduced to solid foods in addition to continued breastfeeding. In reality, of course, solid foods are introduced at a wide range of points in time, depending on parental preference and culture. In the UK (as well as in other countries), for example, solid foods are sometimes introduced earlier (e.g. in some cases before infants are four months old), whereas introduction is also often delayed beyond six months of age (Moorcroft et al. 2011a).
The term ‘complementary feeding’ denotes an infant’s intake of all foods (solid and/or liquid) which are additional to breast milk or infant formula (Cameron et al. 2012 & Przyrembel 2012). A recent literature review by Przyrembel (2012) highlighted that the early introduction of complementary food is inversely related to the following factors: the mother’s education, age and socioeconomic status, as well as the mother’s smoking, duration of breastfeeding and access to information on health care. These, whether alone or in combination, may have an impact on an infant’s health outcomes later in their life.

Although a complete consensus has not yet been reached, some evidence has pointed towards an inverse relationship between an infant’s age when complementary feeding commences and their later risk of adiposity (Grote et al. 2012; Moorcroft et al. 2011b & Przyrembel 2012). Two recent cohort studies have found associations to exist between infants’ ages at the introduction of solid foods and their subsequent development (or otherwise) of overweight and obesity. These studies are now briefly discussed in turn. Firstly, Seach et al. (2010) monitored 307 Australian children from birth to 10 years of age and observed that for each month by which solid foods were delayed, the odds ratio of the child becoming obese at 10 years of age was lowered by 0.66 [95% confidence interval: 0.49–0.88], regardless of the nature of the feeding or the duration of the breastfeeding.

Secondly, Schack-Nielsen et al. (2010) examined the impact of infants’ ages at the introduction of complementary feeding on their BMI during childhood and to trace their data all the way through to adulthood, based on a subsample of the Copenhagen Perinatal Cohort, which was established in 1959–1961 (n = 5068). They found that the infants’ risk of overweight by the age of 42 decreased or was inclined to decrease in line with their increasing age (in months) at the point of introduction of complementary feeding [odds ratio: 0.94; 95% Confidence Interval: 0.86, 1.02], while statistically controlling for the following variables: breastfeeding, sex, maternal age at birth, pre-pregnancy BMI, gestational weight gain, smoking during pregnancy, and social class.

Perhaps unsurprisingly, the risk of later overweight or obesity which arises from the early introduction of complementary food may vary between breastfed and formula-fed infants (Przyrembel 2012). In support of this view, a recent observational cohort study by Huh et al. (2011) followed the progress of the subjects three years later and reported that the impact of the introduction of solid foods differed among the 568 breastfed, and 279 formula-fed children included in the study. In the group of breastfed infants, the timing of solid food introduction was not observed to be associated with the infants’ chances of obesity (odds ratio: 1.1 [95% confidence interval: 0.3– 4.4]). In contrast, among the group of formula-fed infants, the
introduction of solid foods prior to 4 months of age was seen to be associated with a sixfold increase in their chances of obesity at the age of 3 (odds ratio: 6.3 [95% confidence interval: 2.3–6.9]).

It is important to clarify here that the introduction of solid foods has been associated with significant changes in dietary intake, and with levels of protein intake in particular (Przyrembel 2012). A review of the literature in this area reveals that many cohort studies in developed countries have reported that it is common for infants to experience a rapid rise in their protein intake during the introduction of solid foods (Gunther et al. 2007). Taking one of these as an example, a cohort study conducted in Germany by Gunther et al. (2007) found that protein intake increased dramatically during the period of complementary feeding. Specifically, for infants of 6 months, 12 months, and toddlers of 18–24 months of age, the mean of daily protein intakes were 11.9 g/day, 29.7 g/day and 33.8 g/day respectively. The same study found that the mean percentages of energy from protein were 7.8%, 14.7% and 14.8% respectively. In addition to this, several epidemiological studies in different forms, which have included clinical trials as well as a cohort and cross-sectional studies, have found a high protein intake during this period to be strongly associated both with accelerated early growth and an increased risk of overweight and obesity during later childhood. These are discussed in the following paragraphs.

A comprehensive systematic review by Hornell et al. (2013), which is referenced above in section 2, provided an overview of the most recent scientific evidence regarding the short- and long-term health impacts of higher intakes of protein during infancy and later childhood. The subsections which follow in this thesis, on protein intake during complementary feeding and early growth and on protein intake during complementary feeding and later obesity respectively, use the studies identified by Hornell et al. (2013) as points of reference in their discussion. Nine studies were identified, which were published between January 2000 and December 2011.

**Protein intake during the complementary feeding period and early growth**

The systematic review by Hornell et al. (2013) identified one study (a cohort study) which had found an association between higher protein intake during complementary feeding and increased growth, while the findings of two other identified studies (one clinical trial and one cohort study) showed no such effect. The study identified by Hornell et al. (2013) as finding a positive association was an observational cohort study carried out by Morgan et al. (2004), which aimed to determine whether or not meat consumption influences the growth of infants of up to two years of age, and it found that protein intake through the consumption of meat by infants of between 4 to 12
months of age, possibly via its impact on the total level of protein intake, can be associated with a rapid weight gain in those infants over the first 2 years of their lives.

Two of the studies identified by Hornell et al. in 2013 (one clinical trial and one cohort study) found no association between protein intake and growth. These studies are now discussed in turn. The first study which was identified as having found no association was that of Larnkjaer et al. (2009), who performed a randomised controlled trial, and did not observe any evidence of an association between the milk type used in feeding (i.e. whole milk or infant formula) on infants’ growth between the age of 9 and 12 months. They concluded that this lack of association might be due to the relatively short intervention period that they had studied. Secondly, a cohort study by van Vught et al. (2009) also reported that higher protein intake had no effect on growth, although they did find a positive relationship between amino acids (e.g. arginine) and linear growth.

**Protein intake during the complementary feeding period and later obesity**

The recent systematic review by Hornell et al. (2013) introduced in the previous sub-section also identified a total of six studies (specifically, six cohort studies) which had found a positive association between protein intake during complementary feeding and later obesity. The following paragraphs discuss each of these studies in turn.

Three of the cohort studies examined protein intake in the first year of life and related it to outcomes that were recorded at between 5 and ten years of age. The first of these cohort studies, by Scaglioni et al. (2000), identified a positive association between an infant’s protein intake at 1 year of age, and their risk of overweight at 5 years old; however, they also found parental overweight to be an important risk factor that affects the likelihood of a child being overweight in the first years of their life. Secondly, Gunnarsdottir & Thorsdottir (2003) reported a positive relationship between the intake of protein in infancy and an increased risk of childhood obesity at the age of 6 years; however, this was only the case among boys. They also found that rapid growth during the first twelve months of life could be associated with an increased BMI at the age of 6 years in both genders. Thirdly, Hoppe et al. (2004a) identified that protein intake (both in absolute terms and as a percentage of energy) at nine months of age was a predictor of a child’s weight and height at ten years of age.

The remaining three cohort studies of the six mentioned above looked at protein intake in early childhood and its consequent outcomes at between 4-10 years. The first of these, by Skinner et al. (2004), found that the average intakes of protein and fat consumed at between 2 and eight years of age were
positively associated with BMI at eight years of age. Similarly, Gunther et al. (2007) observed that the consistently high levels of protein intake at 12 and 18-24 months of age (with the exception of the level at 6 months old), were positively related with higher average BMI z-scores at 7 years of age, as well as an increased risk of BMI at above the 75th percentile. The last of the cohort studies, by Ohlund et al. (2010), found a young child’s BMI at 6-18 months to be the most accurate predictor of their BMI at 4 years of age, while also noting that other factors (specifically, the child’s protein consumption at 17-18 months and at 4 years old, their energy intake at 4 years old, and their fathers, but not their mothers, BMI) were also independent predictors.

**Protein intake during the pre-school period**

Having previously discussed the role of protein intake in accelerated early growth and the development of obesity during the breastfeeding period and the complementary feeding period, this sub-section continues the discussion by examining the effects of higher intakes of protein during the pre-school period.

It has been demonstrated that the pre-school period is a vital phase of early life, and is the time in which a person’s long-term dietary habits are established. This means that potential life-long levels of appetite, the likelihood of obesity, and other risk factors for cardiovascular diseases (CVD) are established at an early age (Lanigan & Singhal 2009).

A recent extensive literature review by Lanigan et al. (2010) looked at pre-school ages to examine the key factors influencing the development of obesity. The evidence evaluated by the authors of this review suggested that most of the excess weight gained by obese children accrues before the age of 5 years and that their overweight or obesity by that age tracks into their later lives. Importantly, the authors also observed that the associations between dietary factors and obesity in pre-school children had rarely been studied, a viewpoint that is supported by the small number of studies which were identified to have done so by a very recent review of the literature.

This systematic review by Hornell et al. (2013), as mentioned above, gave an overview of the most recent scientific evidence regarding the short- and long-term health impacts of higher intakes of protein during infancy and later childhood. Although the authors did not specifically comment on the small number of relevant studies, only three studies were identified as having examined these associations (which were published between January 2000 and December 2011). These are now discussed in turn.

The first of the identified studies was a cohort study of 127 Swedish children (Ohlund et al. 2010), which found a positive association between the children’s BMI at 4 years of age and the intake of protein not only at 17–18
months but also at 4 years of age, both when expressed in grams and when expressed as a percentage of energy intake (EI). The authors concluded that dietary protein intake in early childhood should be reduced where possible as part of a wider strategy aiming for obesity prevention in Western countries. In agreement with these findings, two cross-sectional studies (Kourlaba et al. 2008 & Manios et al. 2008) were also identified in the review by Hornell et al. (2013), and both reported that toddlers’ and pre-schoolers’ dietary intakes of protein were higher among those classified as being ‘at risk of being overweight’ or ‘overweight’ than their peers who were at or around normal weight levels.

**Summary of prior studies on the effects of protein intake**

A growing number of studies are exploring the potential effects of a high protein intake early in life on growth and obesity risk. These studies cover a wide range of ages, from breastfeeding through to complementary feeding and then on to the pre-school period. Some these studies suggest that a higher level of protein intake during infancy and early childhood is associated with more rapid growth and a higher BMI in later childhood. However, the age period that is the most sensitive to high protein intake remains unclear, and there is limited information on the effects of different types of protein. These findings are of interest; the role of protein intake in the development of overweight and obesity requires further study.

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