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Protein quality in early infancy and long-term health outcomes

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ABSTRACT

Nutrition in early life plays vital role in development of metabolic diseases in adulthood, especially obesity and its complications. Conventional infant formulas having protein supply of 55–80% as compared to breastfed infants could enhance early weight gain and later obesity risk. An improved amino acid profile along with a recommended protein quantity of 1.8 g protein per 100 kcal enables whey predominant starter formula to meet requirement of normal term infants during the first four months of life. Therefore infant formulae with reduced protein contents, high protein quality and the whey-predominant are recommended in infants who are not completely breastfed.

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1. Introduction

Globally, childhood obesity is one of the major public health concerns in the 21st century with its increase in prevalence at an alarming rate. It has been estimated that, globally the number of overweight children under the age of five is greater than 42 million. Among them, nearly 31 million of these children reside in developing countries.¹ Prevailing evidence suggest that childhood obesity is associated with the development of metabolic diseases like diabetes, obesity, hypertension and cardiovascular diseases at a younger age. As the

metabolic diseases related with overweight and obesity is usually preventable, prevention of childhood obesity is very essential in this context.²

The prevalence of overweight and obesity among children has increased rapidly in many low- and middle-income countries.¹ Moreover, in the developing countries, children of high socioeconomic status are affected more than the low socioeconomic status. However, in developed countries, children in lower socioeconomic status are affected more than the children in higher socioeconomic status.³

Genetic factors affect the vulnerability of a given child to an obesogenic environment; however, it is not the only factor that

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affects childhood and adult obesity. It is the net outcome of factors like genetic influence, early life programming, individual lifestyle choices and the environment that results in childhood and adult obesity.⁴

The purpose of this article is to review the role of early life metabolic programming and the impact of protein intake in early life on long term health outcomes.

2. Early metabolic programming and long term health outcome

The Barker's or Fetal Origins of Adult Disease" (FOAD) hypothesis suggest that prenatal exposures affect the fetus' development and consequently, it increases the risk of specific diseases in adult life. Initially, this association was observed for adult coronary heart disease, later it was demonstrated with a range of chronic conditions including Type II diabetes mellitus (T2DM), cancer, and osteoporosis to various psychiatric illnesses. The FAOD hypothesis is based on the evidence that certain intrauterine exposures can influence a single genotype to produce different phenotypes at specific developmental periods.⁵

Epigenetics, which is defined as somatically heritable states of gene expression resulting from changes in chromatin structure without alterations in the DNA sequence, also plays an important role in the metabolic make up of an individual. Prevailing evidence suggest that metabolic events during limited and sensitive windows of prenatal and postnatal development have marked modulating effects on health in later life, which is a concept often referred to as programming or metabolic programming.⁶ In addition, environmental exposures during the acute time window can affect the profile of epigenetics and results in obesity. It has been observed that nutrition in early life, an environmental factor, play a vital role in the development of metabolic diseases in adulthood, especially the obesity and its complications (see Fig. 1).⁷

Transgenerational transmission of epigenetic markers was first demonstrated by Morgan and colleagues using the Avy-dependent coat color mouse. Although methylation of the intra-cisternal A particle retrotransposon (IAP) region was associated with a wild-type coat color phenotype, the mice were genetically agouti. In addition, it has been observed that DNA methylation markers could be transmitted across multiple generations.⁸ Other studies also showed that methyl supplemented diets during pregnancy were adequate to increase methylation of the Avy-allele and produce offspring of pseudoagouti appearance regardless of the parents phenotype and this phenotype was then passed on to the F2 generation.⁹ These studies suggest that manipulations of the maternal condition potentially cause epigenetic modifications in the offspring and produce a variety of phenotypic outcomes even if the offspring were genetically identical.^{8,9}

Maternal protein restricted diet during pregnancy, which induces an altered phenotype in the offspring, involves alterations in DNA methylation and histone modifications in particular genes. For example, the honeybees that grow to be either queens or workers based on if they are feed on royal jelly or beebread demonstrated the epigenetic effects of diet on the honeybee phenotype. In the honeybee model, the different

Fig. 1 – Interplay of genetic, environmental and epigenetic factors in the metabolic programming. SNPs, single nucleotide polymorphisms; DNA, Deoxyribonucleic acid; mRNA, messenger ribonucleic acid.

types of honey that induced the epigenetic changes in DNA methylation pattern resulted in different honeybee phenotype. Therefore, diet can alter DNA methylation and thereby, alter gene expression that possibly affects both physiologic and pathologic pathways.⁷

Accelerated early postnatal growth which occurs as a result of relative over nutrition in infancy has been suggested to increase the risk of later obesity. A study by Singhal et al. assessed the effect of early growth promotion on later body composition in two randomized trials of infants born small for gestational age who have been assigned at birth randomly to receive either a nutrient-enriched formula or a standard formula. The study demonstrated that the fat mass was lower in children assigned to the standard formula as compared with the children who received the nutrient enriched formula with a mean (95% CI) difference after adjustment for sex for study 1 was -38% (-67% , -10%), $P = 0.009$ and for study 2 was -18% (-36% , -0.3%), $P = 0.04$. The study has provided the evidence that there is a causal link between faster early weight gain and a later risk of obesity. The prevailing evidence suggests that early nutritional environment interacts with an individual's genetic disposition to program metabolism and development.¹⁰

Adipogenesis which is regulated by peroxisome proliferator-activated receptor (PPAR) that is activated by cytosine-adenosine-adenosine-thymidine (CAAT) enhancer binding proteins (C/EBPs) and regulatory element-binding protein 1 (SREBP-1) occurs predominantly during late fetal and early postnatal life. However, it can also occur at later stages of life under certain conditions like obesity. During early life, nutritional environment greatly influence adipocyte proliferation and differentiation, especially the concentrations of circulating hormones and nutrients such as insulin-like

growth factors, glucose, insulin, and glucocorticoids. Although various factors can initiate the differentiation process, insulin is the essential factor for the differentiation and formation of mature adipocytes from preadipocytes.⁵ Insulin like growth factor-1 (IGF-1) which is structurally and functionally similar to insulin also plays important role in the regulation of postnatal human growth and intrauterine growth. Previous studies with normal human neonates demonstrated a correlation between cord serum IGF-I levels and birth weight. In addition, IGF-1 mediates proliferation and differentiation of preadipocytes to mature adipocytes.¹¹

3. The impact of protein intake on growth and development

Protein, one of the essential macronutrients, is the main building block during tissue synthesis and it up-regulates the immune system, stimulates key metabolic pathways and also contribute to the development of the vital organs. Despite protein quantity, protein quality is one of the vital characteristics of the nutritional value of infant feeding. Human milk that contains predominantly the whey proteins than bovine's milk is the gold standard in early infant nutrition.^{12,13} Numerous factors affect the quality of proteins; however, the amino acid profile is considered as a major contributor to overall protein quality.¹³

For instance, α -lactalbumin (LA) in infant nutrition is important as it constitutes a large part of the whey (36%) and total protein in human milk. However, in bovine milk, LA is only a minor part of the milk protein content (17%) and glycomacropeptide (GMP) constitutes 12% of the whey κ -casein is released in whey during cheese making by the action of chymosin. GMP being a biologically active component is significantly rich in the branched chain amino acids (BCAAs) threonine but depleted in tryptophan.¹³ Modification of the protein composition of infant formula by increasing its proportion of LA with GMP that accounts for 15% or 10% of the protein resulted in growth patterns in infants that were similar to those of breastfed infants. These results suggest that it is potentially owing to the decrease in plasma concentrations of insulinogenic amino acids.¹⁴

As per the early protein hypothesis, a high-protein intake in early infant period may increase the levels of insulin-releasing amino acids (BCAAs) and this in turn may stimulate insulin and insulin-like growth factor 1 (IGF-1) secretion. These factors which are all upstream of the mammalian target of rapamycin (mTOR) growth signaling network possibly stimulate growth and adipogenic activity. Therefore, the increased protein intake early in life that stimulates weight gain can be translated into an increased later obesity risk.¹³

It has been observed that the 5 BCAAs including isoleucine, leucine, valine, tyrosine, and phenylalanine are significantly associated with increased risk for diabetes. A combination of three amino acids predicted >5-fold higher risk for diabetes. Thus, the circulating amino acids may directly promote insulin resistance, possibly via disruption of insulin signaling in skeletal muscle.¹⁵

During the first 2 years of life, there is a remarkable increase in protein intake with the mean protein intake $\sim$ 3 times as

high as the physiologic requirement. However, in some children, it is 4–5 times their physiologic requirement. A large variation in the protein content of foods provided for infants and toddlers exists; the most important and typically largest source of protein is milk, most suitably is human breastmilk or in absence of human milk, it is milk from other sources like milk from human milk bank, bovine's milk or infant formulas. The source of protein (PE %) from breast milk, infant formula, and whole bovine's milk are 5%, 7–9% and 20 PE%, respectively. As the PE% is very high in bovine's milk with reduced fat (28 PE % in 2% milk and 39 PE% in skimmed milk), the reduced-fat milks are usually not recommended for infants and toddlers.¹⁶

Generally, conventional formula-fed infants have higher protein intakes and higher concentrations of many plasma amino acids from 4 to 6 month of age than breast fed infants. IGF-I concentrations which increases with the high protein intake and due to presence of cGMP, as mostly infant formulas are produced from bovine's milk, accelerates growth from 4 to 6 months of life. However, multifactorial influence in observational studies does not prove cause-effect relationship.^{17–19}

Given the prevailing evidence, Codex Alimentarius and the European Union directive defined an acceptable protein range from 1.8 to 3.0 g/100 kcal.^{20,21} The European Food Safety Authority (EFSA) and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) panel suggest that a minimum protein intake of 1.8 g/100 kcal from (infant formula) based on intact milk protein is sufficient to ensure adequate growth and development.^{22,23}

4. Clinical studies on infant formula

A meta-analysis which involved epidemiological studies have demonstrated that breastfeeding is associated with $\approx$ 15–20% less obesity in adulthood.²⁴ Conventional infant formulas which usually have a protein supply of 55–80% as compared to breastfed infants could enhance both early weight gain and later obesity risk. As a result, infant formulae with reduced protein contents however with high protein quality, the whey-predominant formulas, are recommended in infants who are not fully breastfed.²⁵

A multicenter pooled analysis which involved 11 randomized controlled trials of healthy term infants assessed the effects of whey-predominant formulas with a protein content of 1.8 g/100 kcal on infant growth by comparing against WHO growth standards and breastfed infants. The study demonstrate that the 95% CIs in the overall estimates of weight-for-age (WAZ) were within $\pm$ 0.5 SD of WHO growth standards for the infants fed lower protein formula (LPF) [0.07 (–0.16, 0.29)] and LPF with active ingredients. However, WAZ was higher in the LPF ($P < 0.001$) and LPFA ($P = 0.003$) groups than in the breastfed infants. This is owing to the fact that breastfed infants had a relatively low WAZ [–0.23 (–0.51, 0.05)] as compared with WHO growth standards. The study concluded that term infant formula with a lower protein content which is closer to that of breastfed milk, with or without active ingredients, supports a healthy growth pattern comparable to the WHO growth standards and close to breastfed infants.²⁶

In a multicenter European study which involved 1138 healthy, conventional formula-fed infants who were randomly assigned to receive bovine's milk-based infant and follow-on formula with lower (1.77 and 2.2 g protein/100 kcal, respectively) or higher (2.9 and 4.4 g protein/100 kcal, respectively) protein contents for the first year. For comparison, 619 exclusively breastfed children were also followed. Weight, length, weight-for length, and BMI were determined at inclusion and at 3, 6, 12, and 24 months of age. The study showed that at 24 months, the weight-for-length z score of infants in the lower protein (LP) formula group was 0.20 (0.06, 0.34) lower than that of the higher protein (HP) group and did not differ from that of the breastfed reference groups. The study also found that a higher protein content of infant formula is associated with higher weight gain in the first 2 y of life but has no effect on length. The study concludes that lower protein intake in infancy might reduce the later risk of overweight and obesity, with adequate and appropriate growth centiles similar to breast-fed babies.²⁷

Numerous studies have demonstrated that conventional formula-fed infants have increased plasma or serum urea concentrations as compared to the breast-fed infants. A study by Raiha et al. investigated if feeding formulas with the recommended minimum protein content (1.8 g/100 kcal) and an improved protein quality enable patients to achieve normal growth and plasma urea concentrations. The study involved healthy term infants who were randomly assigned to breast-fed or three formula-fed groups including one group received standard formula with a protein/energy ratio of 2.2 g/100 kcal and two other groups received formulas with a protein/energy ratio of 1.8 g/100 kcal that differ mainly by their source of protein. The study found no significant differences between

the four feeding groups for weight- and length-gains or for body mass indices (BMI). Although no differences in energy intakes between the formula fed groups were found, protein intakes were less in infants fed with the 1.8 g/100 kcal formulas. The plasma urea level with the lower protein content (1.8 g/100 kcal) group was significantly lesser as compared with higher protein content group (2.2 g/100 kcal); however, it is comparable with the breast-fed group ($P < 0.001$) (see Fig. 2). The results of the study implicate that an improved amino acid profile along with a recommended protein quantity of 1.8 g protein per 100 kcal enables the whey predominant starter formula to meet the requirement of normal term infants during the first four months of life.²⁸

Essential amino acids, especially BCAAs, IGF-I, and urinary C-peptide:creatinine ratio, were significantly ($P < 0.001$) higher in the high protein group than in the low protein group, whereas IGF-binding protein (IGF-BP2) was lower and IGF-BP3 did not differ significantly. Lowering the protein intake with infant formula resulted in a metabolic and endocrine response comparable to that of breastfed infants who had lower plasma amino acid concentrations and a less active IGF-1 axis and lower insulin production.²⁹

In an attempt to prevent the childhood obesity, early nutrition is considered as the target to achieve this goal of prevention of childhood obesity. A European multicenter, double-blind, randomized clinical trial which involved healthy infants who were randomized ($n = 1090$) to receive the high protein formula or lower protein content formula (within recommended amounts) in the first year of life. Breastfed infants ($n = 588$) were enrolled as an observational control group. The study demonstrated that high protein children had a significantly higher BMI (by 0.51; 95% CI: 0.13, 0.90; $P = 0.009$)

Fig. 2 – (a) Weight and length gains; (b) plasma urea concentration among two formula-fed and breast-fed groups.

at 6 y of age. The risk of becoming obese in the higher protein group was 2.43 (95% CI: 1.12, 5.27; $P = 0.024$) times greater than the lower protein group. Although there was a tendency for an increased weight in higher protein children (0.67 kg; 95% CI: 20.04, 1.39 kg; $P = 0.064$), no significant difference in height between both the groups was found. Anthropometric measurements were comparable between the lower protein group and breastfed groups. The study concludes that infant formula with a lower protein content reduces BMI and obesity risk at the age of 6 years.³⁰

Guenther et al. investigated if a critical period of protein intake for later obesity exist early in childhood and the relation between protein intakes from different sources and body mass index SD score and body fat (BF) at 7 y of age. The study included 203 participants of the Dortmund Nutritional and Longitudinally Designed Study with information on diet at 6 months, 12 months, 18–24 months, 3–4 years, and 5–6 years. The study, using the life-course plots, showed that the ages of 12 months and 5–6 years were identified as critical ages at which higher total and animal, but not vegetable, protein intakes were positively related to later body fatness. The study also demonstrated that animal protein intake at 12 months was associated with BF% at 7 y ($P = 0.008$). With respect to food groups, dairy, but not meat or cereal protein intake, at 12 months was related to BF% at 7 y ($P = 0.07$). Animal protein at 5–6 y yielded similar results ($P = 0.01$), but food group associations were less consistent. A higher animal, especially dairy, protein intake at 12 months may be associated with an unfavorable body composition at 7 y. The age of 5–6 y might represent another critical period of protein intake for later obesity risk.³¹

Given the large support of evidence, in healthy term infants who do not receive breast milk, infant formula with protein content closer to breast milk in early life could reduce obesity risk in childhood without compromising growth and thereby, it could decrease the risk of obesity-related complications.

5. Summary

Human breastmilk is the gold standard for appropriate nutrition of infants. Its qualitative and quantitative composition is the benchmark for nutrition requirements in early life. Protecting infants from overweight and childhood obesity, thus possibly from metabolic diseases in later life are emerging concepts in the science of nutrition in infancy. Intake of bovine's milk or conventional formulas, which are adapted from bovine's milk, possibly can increase the risk of developing obesity and consequent associated negative health outcomes such as metabolic syndrome later in life.

Infant formulas have evolved over the years to bring protein quality and quantity closer to breast milk. One of the key steps in bringing formula milk closer to breast milk has been to reduce insulinogenic amino acids to match up aminogram closer to breast milk and optimizing the whey: casein ratio. Improving protein quality in infant formulas will be advantageous to early metabolic programming and thereby, have a positive impact on long term metabolic health outcome.

Conflict of interest

The authors have none to declare.

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