Relevance of pre- and postnatal nutrition to development and interplay between the microbiota and metabolic and immune systems

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ABSTRACT
Early-life programming is becoming an established concept that states that the environment during early development affects health and disease in adulthood, probably via epigenetic mechanisms such as DNA methylation, histone modifications, RNA silencing, or a combination. Accumulating evidence suggests that nutrition during pregnancy and early postnatal life is one of the most important environmental cues that programs microbiological, metabolic, and immunologic development. The neonatal period is crucial for the early microbial colonization of the almost sterile gastrointestinal tract of the newborn infant. These first colonizers play an important role in host health because they are involved in nutritional, immunologic, and physiologic functions. Exposure to environmental microbial components is also suggested to have a key role in the maturation process of the immune system, and in turn the immune system shapes the composition of the microbiota. Therefore, the use of nutritional strategies to program the microbiota composition to favor a more beneficial bacterial population and to support the development of the metabolic and immune systems may provide a good opportunity to prevent later health problems such as obesity, diabetes, and allergy. Am J Clin Nutr 2013;98(suppl):586S–93S.

INTRODUCTION
Accumulating evidence from epidemiologic and animal studies suggests that environmental factors play a critical role during early life development, with potential long-term effects on health (1). Early-life programming is becoming an accepted scientific concept and leads some to suggest that the genetic impact is perhaps overestimated (2). It has been recognized that ontogenetic development involves developmental plasticity, which implies that specific conditions give rise to later life outcomes.

The concept of programming during early life was prompted by studies of Hales and Barker (3), who proposed the “thrifty phenotype hypothesis” or the “fetal origins” hypothesis, which postulated that under conditions of suboptimal in utero nutrition, the fetus must adapt to its environment to ensure survival of the organism. This phenomenon has evolved to nutritional, developmental, or metabolic programming that involves alterations in growth and development during this sensitive time period, with long-term consequences. These adaptations can predispose the individual to lifelong health problems such as metabolic syndrome or related diseases, including glucose intolerance, insulin resistance, cardiovascular disease, and obesity (4).

Epidemiologic studies provide support for the strong link between prenatal and postnatal nutritional status and the risk of developing diseases in adulthood. Many studies support the notion that poor fetal growth resulting in low birth weight is associated with higher prevalence of type 2 diabetes and glucose intolerance than is normal birth weight (reviewed in references 3 and 5). It is now thought that most of these risks are also associated with the period of rapid catch-up growth after compromised birth status (6). In addition, it has been shown that increased nutritional availability in utero (through, eg, gestational diabetes or maternal obesity) increases the risk of obesity and metabolic syndrome for the offspring (7). Evidence also suggests that early postnatal growth and nutrition, independent of birth weight, can play a critical role in the risk of diseases later in life.

Rapid postnatal growth during infancy and childhood has been found to be associated with an increased risk of cardiovascular disease, hypertension, obesity and adiposity, and glucose intolerance, although there is much debate about the stage of child development during which accelerated postnatal growth is most deleterious (reviewed in references 4 and 8). Slower growth during the postnatal period has been shown to benefit long-term metabolic health. Breastfed and formula-fed infants appear to follow a different growth pattern during the first year of life. Breastfeeding is associated with slower growth, specifically during the second half of the first year, and has been suggested to have a modest protective effect against obesity risk in later life (9, 10).

POTENTIAL MECHANISMS OF EARLY-LIFE PROGRAMMING
The mechanisms that control programming are very complex and involve both genetic and environmental factors. One of the
underlying mechanisms is through permanent structural changes in tissue resulting from suboptimal conditions of an important factor during a critical period of development. Epigenetic programming during development that results in functional changes in gene expression is now widely thought to form integral underlying mechanisms in early-life programming. Epigenetics can be defined as any change in phenotype or gene expression caused by modifications independent of a change in genotype. These modifications include methylation of DNA and modification of histones, including acetylation. Epigenetic changes can be triggered by environmental and genetic influences. Exposure to environmental compounds or behaviors, placental insufficiency, maternal (inadequate) nutrition, and metabolic disturbances can promote improper epigenetic programming, leading to susceptibility to various diseases in the first generation and sometimes subsequent generations, i.e., transgenerational effects (11). Transient nutritional stimuli (or lack thereof) that occur at sensitive ontogenetic stages may have long-lasting influences on expression of various genes via epigenetic mechanisms. Emerging evidence indicates that epigenetic regulation of transcription factors, including the peroxisome proliferator-activated receptor α and Pdx1, is a common mechanism of early-life programming (4). Transcription factors represent attractive targets of nutritional programming, because alterations in transcription factor expression can affect a wide range of other downstream target genes and have lasting effects on immune and metabolic set points.

PROGRAMMING DURING INFANCY

Microbiota

Different observational and interventional studies suggest that the composition of the microbiota plays an important role during pregnancy and early in life and has an influence on the metabolic and immune profiles and the risk of disease development in offspring later in life (12).

The human gut harbors ~10^{14} bacterial cells compared with 10^{13} human cells for the entire body and constitutes an exceptionally diverse and dynamic microbial ecosystem [reviewed by Martin et al (13)]. The composition of the gut microbiota is complex and is crucial for human health (14). The gut microbiota contribute to human health in many ways, such as enhancing digestive efficiency, improving the bioavailability of nutrients and absorption processes, promoting proper immune development, and limiting pathogen colonization (15).

The microbial colonization of the human gut by microorganisms starts at birth and continues afterward. Bacteria from the maternal vaginal and gastrointestinal tract as well as the surrounding environment are the first colonizers of the infant’s gut. However, it has recently been suggested that the colonization process or exposure to microbial compounds may start before birth and that infants may also receive microorganisms from the mother during gestation (16–18). The colonization is influenced by several factors such as genetic background, gestational age, mode of delivery (cesarean section compared with vaginal delivery), type of feeding (breastfeeding compared with formula-feeding and weaning to solids), and antibiotic therapy (19–23). Importantly, it has been suggested that stress and diet during late pregnancy also play a role in the initial colonization of the newborn (24, 25) and thereby play an important role in early-life programming.

Immune system

Evidence is accumulating that programming is an important factor in the development of the immune system and that events or specific exposure during pregnancy can modify gene expression through epigenetic mechanisms and thereby determine the functionality of the immune system. Differences in the immune response are already detectable at birth, suggesting that in utero environmental exposures have the capacity to modify the set point of the immune system at birth (26). The differentiation of T cells is under epigenetic control and plays an important role in preventing fetal rejection during pregnancy, resulting in skewing compared with T helper (Th)^{2} immune responses and suppression of Th1-mediated immunity. Recent studies indicate that Treg (regulatory T cell) function is also impaired during pregnancy. The bias against proinflammatory cytokines leaves the newborn susceptible to microbial infection (27), and therefore optimal regulation of gene expression is required to obtain a balanced postnatal immune response. Immaturity in Th1 and Treg activity may increase the risk of an inappropriate persistence of a Th2 allergy-prone immune phenotype. Environmental exposures are key in the regulation of appropriate gene expression, and alterations (phenotypic) will affect disease susceptibility. The rise in different immune-mediated diseases, including allergy, shows the potential impact of changes in our environment on immune development (28).

Although in utero events will most likely have the greatest impact on gene expression, postnatal exposures are also important in determining immune development. Environmental changes that have been implicated in the rise of immune-mediated diseases include decline in infectious exposures, dietary changes, and increased exposure to pollutants and toxins. An important role for microbes in the maturation of the immune system is also suggested by the so-called hygiene hypothesis (29). Different epidemiologic studies support this hygiene hypothesis and have clearly shown that modifications in the pattern of microbial exposure represent a critical factor underlying the rise in the prevalence of atopic disorders and immune disorders later in life, such as allergy, obesity, and diabetes. Moreover, this increase in immune-mediated diseases appears to be associated with a decrease in infections during childhood (30), suggesting a different build-up of experience.

THE EFFECT OF GUT MICROBIOTA ON GROWTH AND HOST METABOLISM

The gut microbiota provides additional metabolic capacities to their host and regulate expression of genes involved in lipid and carbohydrate metabolism, which thereby influences nutrient supply, energy balance, and body weight (31, 32). Indeed, the gut microbiota has been shown to contribute to the host metabolism through numerous mechanisms, including increased energy harvest from the diet, modulation of lipid metabolism, altered

4Abbreviations used: AA, arachidonic acid; FO, fish oil; HMOS, human-milk oligosaccharide; LC-PUFA, long-chain PUFA; Th, T helper.
endocrine function, and increased inflammatory response (33). The first evidence for a role of the gut microbiota in the regulation of host energy homeostasis was derived from experiments with germ-free mice. In comparison with conventional mice, germ-free mice had 40% less total body fat, despite higher caloric intake. The colonization of the germ-free mice with a normal gut microbiota resulted within 2 wk in an increase in total body fat, an increase in hepatic triglycerides, and a dramatic increase in insulin resistance without affecting feed pellet consumption (34). The underlying mechanism of the observed resistance of germ-free mice to diet-induced obesity includes the effect of the gut microbiota on energy harvest from the diet, energy storage of triglycerides, and energy expenditure through fatty acid oxidation (35). Fecal transplant to germ-free mice with an “obese microbiota” resulted in a significantly greater increase in total body fat than did colonization with a “lean microbiota,” which shows the importance of the intestinal microbiota as an environmental factor that can promote obesity and other metabolic diseases (36). Recently, Ajslev et al (37) showed that a number of early exposure factors, such as delivery mode, maternal prepregnancy BMI, and antibiotic use early in life, have an impact on the risk of overweight in later childhood, most likely through a disturbed gut microbiota composition.

It has been reported that differences in the gut microbiota during the first year of life may precede the onset of obesity (38). In this study, the numbers of *Bifidobacterium* spp. were higher and the numbers of *Staphylococcus aureus* were lower in children who remained at normal weight than in children who became overweight at 4 y of age. Thus, a microbiota profile in favor of a higher number of bifidobacteria and a lower number of *S. aureus* in infancy may provide protection against overweight and obesity development. The microbiota of the mother is an important source of intestinal bacteria for the infant, and any alterations may be transferred to infants and lead to an increased risk of obesity. Different observational studies in humans suggest a link between the composition of the gut microbiota during pregnancy and body weight and metabolic biomarkers. In particular, *Staphylococcus* and *Escherichia coli* numbers were higher in women with excessive weight gain during pregnancy (38, 39). More recent studies showed that weight gain during pregnancy had an effect on the microbiota acquisition, composition, and activity during early infancy (40). These data suggest that gut colonization early in life is most likely to be crucial for preventing metabolic disease later in life.

**LINK BETWEEN METABOLIC AND IMMUNE STATUS**

Obesity is associated with chronic low-grade inflammation, underlying the pathogenesis of different obesity-associated diseases, including diabetes and asthma. Epidemiologic and animal models have shown a strong association between early-life environment and obesity-associated diseases (41, 42). It has been postulated that the changed environmental factors in the obese state may cause epigenetic alterations that lead to increased susceptibility to allergy (43). However, limited data are available on the relation between BMI (pre)pregnancy and the risk of atopic disease in the offspring. Placental inflammation is a characteristic of maternal obesity that confers an increased risk of obesity in offspring (42).

Burcelin et al (44) postulated a new paradigm that specific diets, including a fat-enriched diet, induce a modification in the intestinal microbiota. The translocation of bacteria and bacterial antigens into the host, via the innate immune system, toward metabolically active tissues can trigger a chronic inflammatory status and consequently impaired metabolic functions such as insulin resistance and excessive adipose development via direct communication between inflammatory cells and metabolic cells as described in a recent review on underlying cellular and molecular mechanisms (45). The resulting organ capacity and cellular functionality that is built in early life in response to environmental cues such as nutrition will determine the flexibility of the system to cope with challenges in later life.

**ROLE OF NUTRITION IN EARLY-LIFE PROGRAMMING**

A growing body of evidence from epidemiologic studies in humans and animal studies shows that genome regulation is largely modified by the nutritional environment. The quantity and quality of nutrition during the very early stages of life (from conception until the neonatal stage) are suggested to have an impact on the development of later chronic diseases. Breastfeeding is suggested to overcome some of the adverse effects related to fetal malprogramming and to confer protection from metabolic diseases and related diseases (including insulin resistance, cardiovascular disease, and obesity) (2).

Human milk is the first dietary exposure in infancy. It is considered the best nutritional option for growth and healthy development of the newborn, because it contains a wide range of health-promoting compounds including human-milk oligosaccharides (HMOs), nucleotides, fatty acids, immunoglobulins, cytokines, immune cells, lysozyme, lactoferrin, growth factors, and other immune-modulating factors (46–50). In recent years, human milk has been shown to be a consistent continuous source of bacteria, including staphylococci, streptococci, bifidobacteria, and lact acid bacteria, to the infant gut as well as described in a recent review on underlying cellular and molecular mechanisms (45). The resulting organ capacity and cellular functionality that is built in early life in response to environmental cues such as nutrition will determine the flexibility of the system to cope with challenges in later life.

Dietary fats provide energy for growth, supply the essential fatty acids linoleic acid and α-linolenic acid, and ensure adequate absorption of the fat-soluble vitamins required for healthy growth and development. The composition of human milk is influenced by maternal diet, which therefore shows the importance of maternal nutritional status. One of the key ingredients that may modulate long-term effects is the supply of long-chain PUFAs (LC-PUFAs). It is thought that the shift toward increased dietary omega-6 (n-6) fatty acid intake and decreased n-3 fatty acid intake as the results of changes in the Western food chain and dietary habits may be a risk factor for obesity in later life. The n-6-derived LC-PUFA arachidonic acid (AA) as well as its metabolites especially have the capacity to directly stimulate adipogenesis, thereby leading to increased adipose tissue gain (56, 57).

As a result of the reported beneficial effects of human milk, several attempts have been made to develop infant formulas that, for instance, stimulate similar gut colonization to that of breastfed infants. The main strategies to modulate the gut microbiota of infants have been the administration of prebiotics, probiotics, symbiotics, and LC-PUFAs during prenatal and postnatal life.
**Prebiotics**

Prebiotics are nondigestible oligosaccharides that reach the colon intact and are known for their ability to selectively stimulate the growth and activity of bacteria that exert positive health effects (58). Nondigestible HMOSs are one of the major constituents in human milk and are regarded as important immunomodulatory components. HMOSs are structurally very complex and have a huge diversity (59–62). The beneficial effects on the immune system are commonly ascribed to the stimulation of the growth and metabolism of protective commensal intestinal bacteria (63, 64). Different studies have reported that oligosaccharide mixtures were able to modulate the microbiota of bottle-fed infants, making the composition of the microbiota more similar to the bifidobacteria-dominated microbiota in breastfed infants (65, 66). Available evidence suggests that HMOSs may have systemic effects in infants because they have been found in urine (67, 68). In line with these observations, the immunomodulatory effect is not restricted to the gastrointestinal tract. Beneficial effects have been observed on parameters of allergy, infection, and inflammation in both animal studies and clinical trials with oligosaccharides (reviewed in reference 69). To date, there are limited data to support the therapeutic potential of prebiotics against allergic diseases and infections. There is one study that reported beneficial effects of prebiotics in the treatment of atopic dermatitis (70), and only a few studies describing benefits in the treatment of infants with infections or inflammatory conditions (71).

More trials are clearly needed, but the promising early results support the importance of prebiotics in achieving a beneficial gut microbiota and host-microbe interactions at critical periods for the development and potentially prevention of human disease.

**Probiotics**

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (72). The use of probiotics in the pediatric setting has tripled in the past 5 y, with bifidobacteria and lactobacilli as the most common bacterial groups used (73). There is a considerable and evolving body of evidence for possible short-term benefits of some probiotic strains in the treatment of infants and young children with infectious diarrhea as well as in prevention of antibiotic-associated diarrhea (74). It has been shown that maternal intake of probiotics plus dietary counseling resulted in lower blood glucose concentrations and improved glucose tolerance (75). Moreover, perinatal probiotic intervention appeared to moderate the initial phase of excessive weight gain and seemed to reduce the birth weight–adjusted mean BMI (76).

Other postulated effects of probiotics, such as prevention of common childhood infections, reduction in food allergy, atopic dermatitis, and primary prevention of atopy, even if promising, remain to be convincingly established (77). Multiple mechanisms of action have been suggested, but for most probiotics little is known about their precise mode of action, and effects will mostly depend on the specific strain used. Clinical studies that investigated the effect of probiotic supplementation on the risk of allergy and infections have been performed (78, 79). Although gut microbiota manipulation in experimental models has shown promising results in controlling obesity, findings from clinical trials in human subjects are conflicting and potentially confounded by dietary habits, antibiotic use, nutritional supplementation, and physical activity. Findings from a randomized, double-blind, controlled trial of prenatal and postnatal administration of *Lactobacillus rhamnosus* suggest that probiotics might modify the growth pattern of a child by restraining the excessive weight gain that occurs in the first 1–2 y of life but not that that occurs between ages 2 and 4 y (80). Maternal probiotic supplementation of 265 pregnant women in the first trimester did not show significant differences in either prenatal or postnatal growth rates (80, 81). So far, probiotics have shown more promise, albeit limited, in the primary prevention of disease rather than in the treatment of established disease (82).

With respect to programming, evidence from animal studies has shown that supplementation with probiotics early in life can influence immune development and attenuate allergic responses later in life. However, clinical trials in humans have been highly variable, and it remains to be determined whether probiotics play a role in the programming of allergic disease, including asthma.

**Symbiotics**

The mixture of pro- and prebiotics, the so-called symbiotics, has been suggested to have a synergistic effect by ensuring the viability of the delivered probiotic bacteria and stimulating the growth or metabolism of health-promoting bacteria, and thus improving the host welfare (58, 83). So far, studies that evaluated the role of symbiotics in modulating the immune system are limited, although several authors have shown a beneficial effect of symbiotics on the prevention and/or treatment of allergy and infections. Recently, several symbiotics mixtures have been shown to have a beneficial role in preventing infectious diseases (84, 85).

Pre- and perinatal supplementation of galacto-oligosaccharides in combination with probiotics has been shown to reduce the incidence of eczema at 2 y of age, although the study failed to show long-term effects at age 5 y (86). Moreover, it has been suggested that infants with IgE-associated allergy might benefit from a symbiotic mixture containing a combination of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides and *Bifidobacterium breve* M-16V (87). In this study, supplementation with the symbiotic-specific mixture early in life suggested a potential programming effect and showed a preventive effect on asthma-like symptoms and possibly on subsequent development of asthma (88).

**LC-PUFAs**

Essential fatty acids must be acquired from the diet and are the precursors for LC-PUFAs that have been implicated as being important for the development of the immune system as well as for growth and metabolic profiles. In different studies in humans and animals, dietary n–3 LC-PUFAs have been shown to improve insulin sensitivity, counteract dyslipidemia, and reduce body weight gain and adiposity (89). Data on nutritional programming by postnatal n–3 LC-PUFAs, however, are limited to beneficial effects on brain development (90) and allergy prevention (91), whereas data on body weight are limited and suggest differential effects. In human preterm infants, dietary DHA reduced growth (92, 93), whereas addition of both DHA...
and AA enhanced growth (93). Rat offspring fed fish oil (FO) mainly consisting of n−3 LC-PUFAs during both pregnancy and lactation had reduced birth weight and decreased birth weight gain during lactation (94).

Recently, the effects of fat quality in the early postnatal diet on body fat development have been studied in a mouse model (95, 96). The results indicate lasting effects of differences in dietary fat quality during early postnatal life on adult body composition and metabolic homeostasis. Feeding mice n−3 LC-PUFAs postnatally reduced body fat mass significantly without affecting lean body mass or body weight when challenged with a moderate Western-style diet from mouse puberty into (young) adulthood. Animals fed a diet low in n−6 LC-PUFA showed an even more pronounced reduction in (adult) fat accumulation. In addition, lowering n−6 LC-PUFA intake during neonatal development resulted in reduced fasting triglyceride concentrations, improved insulin sensitivity (HOMA-IR), and lower fasting resistin and leptin plasma concentrations in adulthood. It was suggested that the dietary changes in n−3 and n−6 LC-PUFAs altered adipocyte proliferation and differentiation in the postnatal period, whereas n−6 LC-PUFAs including AA and its metabolic products stimulated preadipocyte proliferation and adipogenesis (97). Interestingly, the effect of the postnatal dietary changes in n−3 or n−6 LC-PUFAs on body composition only became evident after switching to a moderately obeseogenic diet during adolescence/adulthood, suggesting a programming effect.

LC-PUFAs have been shown to influence the immune system via different mechanisms (56). Although epidemiologic studies support the hypothesis of a relation between increased intake of n−6 PUFA and increased prevalence of allergic disease, clinical beneficial effects from intervention studies are more conflicting. Moreover, to date, only a few intervention studies have been reported to assess the effects of LC-PUFA intervention on infection in infants. A recent study in animals showed a programming effect of maternal diet supplemented with LC-PUFAs on the offspring’s immune response, and the lactation period appeared to be the period that conferred most susceptibility to immune programming (98).

However, knowledge on the association of LC-PUFA with the modulation of the gut microbiota is lacking. In a recent study, Nielsen et al (99) showed that administration of FO to 10-mo-old infants either fed a cow-milk or standard infant formula resulted in a distinct microbiota composition as measured by denaturing gradient gel electrophoresis, a molecular-based method to decipher the diversity and the overall composition of the gut microbiota. The authors showed that the effect of FO supplementation on the microbiota was more pronounced in the group of infants who were fed a cow-milk formula and not in the group of infants who were fed a standard infant formula. Likewise, Andersen et al (100) performed a study in infants and young children in which they investigated the effect of supplementation with FO or sunflower oil on the temporal dynamics of the microbial ecosystem. Their results showed a significant impact on the modulation of the gut microbiota of infants who received the FO supplementation; this effect was not observed in subjects who received the sunflower oil supplementation. Moreover, the changes in the microbiota in the FO group were primarily observed in children who stopped breastfeeding before entering the study. In addition to these scarce examples on the possible effect that LC-PUFAs might have on the microbial ecosystem, more data from animal studies have highlighted the potential role of LC-PUFAs on gut integrity and epithelial barrier function (101–104). In a stress-induced rat model, nutritional intervention with a mixture of prebiotics, probiotics, and LC-PUFAs induced a restoration of gut permeability that had been impaired by neonatal stress; furthermore, a significant catch-up on the growth rate in the animals supplemented with LC-PUFA/prebiotics/probiotics was reported. The positive effect on the gut barrier observed in this study might have not been solely associated to a single ingredient but more likely to the synergistic interaction between the probiotic, prebiotic, and LC-PUFA mixture (102). More studies are needed to elucidate the beneficial effect of LC-PUFAs on the gut microbiota and gut homeostasis and its link to immune and metabolic programming.

CONCLUSIONS

It is now widely accepted that environmental exposures during early life, in particular nutrition, can influence long-term health. It is necessary to define the protective and predisposing effects of early nutrition on the development of later chronic diseases. An understanding of the biological mechanisms involved, including the important role of the intestinal microbiota, the relative contributions of individual components of the diet, and the time constraints (window of opportunity) is important to enable the design of effective prevention or treatment strategies and to combat the burden of common diseases such as obesity and its comorbidities, allergy, and inflammatory diseases.

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REFERENCES


83. van de Heijning BJ, Van der Beek EM. n-3 Long-chain polyunsaturated fatty acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula containing DHA. J Pediatr Gastroenterol Nutr 1999;69(suppl):105S–98S.


