

## CALL FOR PAPERS | *Fetal and Neonatal Programming: Epigenetic Modification of Phenotype*

### More than just a gut instinct—the potential interplay between a baby’s nutrition, its gut microbiome, and the epigenome

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**Mischke M, Plösch T.** More than just a gut instinct—the potential interplay between a baby’s nutrition, its gut microbiome, and the epigenome. *Am J Physiol Regul Integr Comp Physiol* 304: R1065–R1069, 2013. First published April 17, 2013; doi:10.1152/ajpregu.00551.2012.—Substantial evidence links early postnatal nutrition to the development of obesity later in life. However, the molecular mechanisms of this connection must be further elucidated. Epigenetic mechanisms have been indicated to be involved in this process, referred to as metabolic programming. Therefore, we propose here that early postnatal nutrition (breast and formula feeding) epigenetically programs the developing organs via modulation of the gut microbiome and influences the body weight phenotype including the predisposition to obesity. Specifically, the early-age food patterns are known to determine the gross composition of the early gut microbiota. In turn, the microbiota produces large quantities of epigenetically active metabolites, such as folate and short chain fatty acids (butyrate and acetate). The spectrum of these produced metabolites depends on the composition of the gut microbiota. Hence, it is likely that changes in gut microbiota that result in altered metabolite composition might influence the epigenome of directly adjacent intestinal cells, as well as other major target cell populations, such as hepatocytes and adipocytes. Nuclear receptors and other transcription factors (the PPARs, LXR, RXR, and others) could be physiologically relevant targets of this metabolite-induced epigenetic regulation. Ultimately, transcriptional networks regulating energy balance could be manipulated. For these reasons, we postulate that early nutrition may influence the baby epigenome via microbial metabolites, which contributes to the observed relationship between early nutrition and adult obesity.

bacterial metabolites; epigenetic programming; gut flora; microbiome; baby nutrition

CHILDHOOD OBESITY is a growing problem in industrialized countries. For example, in The Netherlands, the prevalence of being overweight or obese has doubled in specific age groups (4–15 years) between 1997 and 2002/2004 (49), with about 17% of all boys and 30% of all girls becoming overweight by the age of 8. This has long-term consequences for public health, since overweight and obese children have a high risk to be overweight or obese as adults (25, 42) and to develop accompanying morbidities of the metabolic syndrome (e.g., Type 2 diabetes) (19).

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Early nutrition has been specified as risk factor for developing and manifesting obesity throughout life. For example, breast-fed children are on average leaner than children fed formula diets in the first year of life (2, 12, 44, 51). After weaning, the body mass index of children generally decreases. However, in formula-fed children this effect is considerably less prominent than in breast-fed children. This positive correlation of breast-feeding with a leaner body weight phenotype continues until school age and is still present, even when confounding factors like socioeconomic status of the parents are taken into account (44). This effect is a prime example of a metabolic programming process that may be influenced and generated by epigenetic factors.

It has been convincingly demonstrated that maternal and neonatal (mal)nutrition not only influences the metabolism of the developing offspring directly but also has a major impact on its epigenome (summarized in Ref. 23). Therefore, it is likely to influence the offspring’s metabolism far beyond its reach into postnatal life. In addition, plasticity of the epigenome is frequently observed postnatally in still developing and renewing tissues (53).

Certain diets and food patterns are known to modulate the composition of the gut microbiota and thus consequently its metabolites. Differences in availability of short-chain fatty acids (e.g., butyrate) and bacterial metabolites essential for one-carbon metabolism (folate) occur, depending on the nutritional habits and microbiota composition. As these substrates exert epigenetic activity (explained below), a change in their balance may lead to a shift in the epigenetic coding of the genome. Primarily, this would affect the epigenome of intestinal cells and possibly the first line of immune cells but subsequently also that of other tissues that come into contact with these metabolites, such as hepatocytes and adipocytes.

Given these studies, it is self-evident that early postnatal nutrition could shape the developing epigenome of target tissues via modulation of the gut microbiome (visualized in Fig. 1), thereby determining the predisposition to obesity. In this hypothesis paper, we focus on two major bacterial metabolites that are known to modulate the epigenome of the cell: 1) folate, which is crucially involved in one-carbon metabolism and can influence DNA methylation to disable gene transcription; and 2) butyrate, a short chain fatty acid and potent inhibitor of histone deacetylases. We take a closer look on how breast feeding versus formula feeding might influence the bacterial population of the gut and subsequently the indicated critical metabolites.

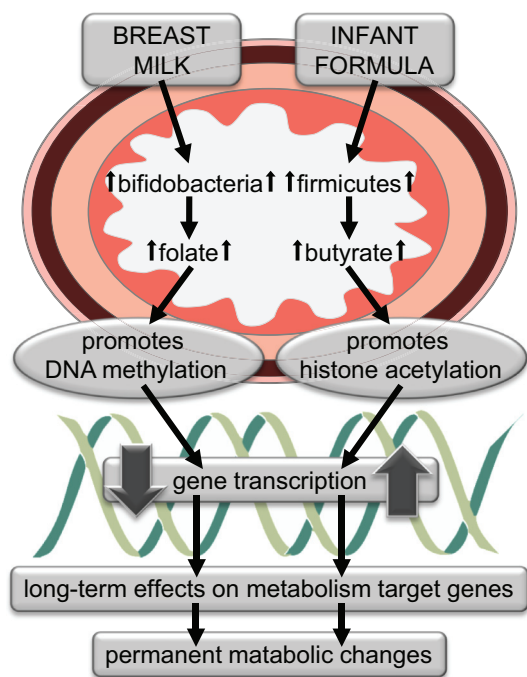


Fig. 1. A proposed molecular mechanism linking early nutrition, bacterial metabolites, epigenetics, and obesity.

*The First Clue: Early Nutrition Determines the Composition of the Gut Microbiota and Its Metabolites*

The fetal gastrointestinal tract is virtually sterile. Colonization begins at birth from the maternal microbiota of the genital tract and colon and the environment in general (16, 36). This is further supported by the observation that babies delivered by Caesarian section possess a characteristic microbiota in comparison to those that are naturally delivered. In addition, breast milk is a source for new bacterial species (34). Later on, the composition of the infant’s colonic microbiota is heavily influenced by diet. By the age of two, the bacterial population of the gut is considered adult-like and remains relatively constant (36).

The microbiota of breast-fed infants is generally dominated by bifidobacteria and ruminococci (15, 20). Bifidobacteria produce a large spectrum of metabolites contributing to their putatively beneficial effects (38). One important secondary metabolite of bifidobacteria is folate, which contributes to the one-carbon metabolism of the host and therefore enables maintenance of DNA methylation marks as explained below (41).

The protein content of commonly used formula diets is matched to provide an amount of essential amino acids equivalent to breast milk (27). However, the overall protein content is higher to compensate for the qualitatively inferior protein sources. It can therefore be assumed that relatively more protein reaches the colon, leading to enrichment in proteolytic bacteria at the expense of carbohydrate-fermenting bacteria. Therefore, the microbiotic community of formula-fed infants is generally more complex, with a pronounced larger proportion of firmicutes (lactobacilli, clostridia, and streptococci) being present (14, 15, 20, 26, 36). Interestingly, non-breast-milk diets promote the production of butyrate. Breastfeeding therefore stimulates the development of a bacterial microbiota that produces large amounts of folate, while formula diets facilitate a

more proteolytic microbiota that produces butyrate. Furthermore, a study in rats has shown that addition of human milk to their diet not only stimulates growth of bifidobacteria but also enhances intestinal folate bioavailability (28).

*The Second Clue: The Composition of Gut Bacteria Can Cause Obesity in Mice*

It has been observed in mouse models that germ-free mice consume more food but accumulate less body fat than conventional, non-germ-free mice (5). Colonization of germ-free mice by conventional murine microbiota induces hepatic lipogenesis in the host. Moreover, germ-free mice are protected from obesity induced by a high-fat diet. Finally, it has been demonstrated that the colonic microbiota of obese mice (*ob/ob*) varies from that of lean control mice. When *ob/ob*-derived microbiota is transferred to germ-free mice, the recipients show a significantly higher fat gain (48).

Similarly, recent human studies have shown that fecal transplants from lean to obese volunteers lead to improved insulin sensitivity in the recipients, which was attributed to acute changes in the intestinal microbiota (52). Moreover, Kalliomäki and colleagues (24) observed that overweight and obese children, aged 7 years, had been preceded by a lower content of bifidobacteria and a higher content of *Staphylococcus aureus*, also indicating a programming role of the intestinal microbiota.

As we have previously mentioned, the microbiota produces large quantities of specific metabolites. It has been proposed that these metabolites directly influence gene expression and metabolism (e.g., ghrelin stability) in the host and influence metabolic rates (4, 13). It is important to note that these substances include several epigenetic modifiers (folate, butyrate), which in large quantities reach the intestinal epithelium, epithelial stem cells, and, via the portal system, the liver.

*The Third Clue: Nutrition Shapes the Epigenome*

Fetal nutrition, including malnutrition due to placental dysfunctions, is linked to the development of chronic diseases in adulthood [DOHaD hypothesis, Developmental Origins of Health and Disease (6–8)]. In epidemiological studies, as well as animal experiments, the nutritional supply of the developing embryo and fetus has been shown to influence the risk to develop cardiovascular disease and the metabolic syndrome (summarized in Ref. 23). Along with other researchers, we have proposed that epigenetic mechanisms like DNA methylation or histone modifications are involved in this metabolic programming: Several key regulators of metabolism (the PPARs, LXR, RXR, POMC) can be epigenetically modified in animal models of fetal undernutrition or overnutrition (31, 32, 40, 50). Ultimately, changes in the expression of these key regulators will lead to changes in the physiology of the organism or to modified responses to environmental stimuli. For instance, epigenetic modification of the *POMC* promoter may induce changes in its transcription, which may ultimately lead to changes in satiety regulation.

While fetal programming has been extensively studied in the past, only limited data are available to demonstrate how long this critical window extends into neonatal development. Independent studies detected postnatal DNA methylation changes in developing organs, such as the liver and brain (56, 57). Interestingly, recent data from McKay et al. (35) show that postnatal dietary

folate concentrations can modify DNA methylation in intestinal cells at several loci in a mouse model. Therefore, it is conceivable that programming may take place in developing organs even after birth.

#### *The Link: Bacterial Metabolites as Potential Mediators Between Nutrition and the Epigenome*

A plethora of data shows the influence of nutrients and dietary compounds on the epigenome. However, very little is known about the potential impact of bacterial metabolites. Here, we present two major regulatory elements of the epigenome, DNA methylation and histone modifications, and their potential for modification by the microbiome.

**DNA methylation.** The DNA methylation status of a gene, which includes its promoter, intragenic, and surrounding regions, is crucial for its transcriptional activity. This was also shown for loci being especially vulnerable to epigenetic modifications, such as the *agouti viable yellow* ( $A^{vy}$ ) and the *axin fused* locus in mice. Methylation of these loci depends on dietary methyl group availability to the mother and, consequently, leads to the agouti ( $A^{vy}$ ) or tail-kinked (*axin fused*) phenotype in the offspring, respectively (37, 54, 55). Now it is also evident for various genes involved in metabolic regulation, including lipid metabolism (*LXR*, *PPAR $\alpha$* ), glucose metabolism (glucocorticoid receptor), and energy expenditure (*CIDEa/c*, *POMC*) (29, 31, 32, 40, 50). DNA methylation of a gene promoter region is in general associated with a decreased transcriptional activity (22).

For adequate DNA methylation, the availability of methyl groups in the form of the methyl substrate *S*-adenosyl methionine (SAM) is indispensable. For regeneration of SAM, methyl groups are provided by the organism's one-carbon metabolism. More importantly, the complex network of one-carbon metabolism is centered on folate and its derivatives in humans (17). Since the human body cannot synthesize folate, it has to be taken up from external sources, such as the daily diet. Additionally, folate-synthesizing gut bacteria contribute largely to an adequate folate supply of the host (3, 47). Bifidobacteria seem to be especially potent in producing folate, as mentioned previously (30, 41). This indicates that not only dietary folate content per se, but also the diet's selective potential for certain gut microbiota is important for an adequate folate supply.

**Histone modifications.** A second layer of complexity is added by the fact that methylated areas of DNA attract binding of specific protein complexes that subsequently modify histones in their vicinity, which affect the accessibility of the DNA for transcription. Histone modifications include acetylation, methylation, phosphorylation, and ubiquitination at various amino acid residues of histone tails. In general, histone acetylation is associated with stimulation of gene expression (21). Several agents are known to influence histone acetylation, including butyrate (10, 45).

Butyrate is a short chain fatty acid produced by intestinal microbial fermentation of undigested dietary carbohydrates and dietary, as well as endogenous, proteins. Besides being an energy substrate contributing to fat accumulation upon absorption (48), butyrate also induces hyperacetylation of histones by inhibiting histone deacetylase (11, 43) and thereby regulates the activity of host genes. In vitro and in vivo studies demonstrated this mechanism for various genes associated with dis-

ease, such as cancer and sickle cell anemia (9, 39), but importantly, also for genes involved in cholesterol and lipid metabolism and storage (1, 13, 18). As one example, the gene encoding cholesterol 7 $\alpha$ -hydroxylase (*Cyp7a1*), which catabolizes cholesterol to bile acids, has been shown to be regulated by histone modifications upon maternal protein restriction in rats (46). Although this has not yet been shown for postnatal nutrition, histone deacetylase inhibition may therefore be a key factor of epigenetic regulation of lipid metabolism. In the human gut, Gram-positive firmicutes are indicated to contribute largely to butyrate production (33). The firmicute ratio of the gut microbiota is strongly increased by formula feeding (14), which makes it plausible that butyrate-mediated effects on the cholesterol and lipid metabolism may be epigenetically programmed in formula-fed infants.

#### *Perspectives and Significance*

We have proposed here that early nutrition significantly influences the composition of the baby's first gut microbiota, which in turn programs the epigenome of the host via epigenetically active bacterial metabolites. This may contribute to the epidemiological observations linking baby nutrition and increased risk of obesity. While we are well aware of the fact that multiple other factors are crucially involved in this process, among others the immune system, we believe that the epigenetic contribution is a relevant player and deserves further investigation. On the other hand, research currently focuses on metabolic consequences of early nutrition for mainly historical reasons. Extending this research to other areas, especially immunology, will hopefully give more insight into this complex interaction of the environment and the epigenome.

In this context, it should be emphasized that the processes proposed here influence the organism for life, which makes them extremely important from a health policy point of view. If our hypothesis is supported by further research, it should be carefully considered for the development of new generations of formula diets that imitate the beneficial epigenetic actions.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

#### AUTHOR CONTRIBUTIONS

Author contributions: M.M. and T.P. prepared figures; M.M. and T.P. drafted manuscript; M.M. and T.P. edited and revised manuscript; M.M. and T.P. approved final version of manuscript.

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