Does Nutrition during Infancy and Early Childhood Contribute to Later Obesity via Metabolic Imprinting of Epigenetic Gene Regulatory Mechanisms?

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Introduction

An epidemic of obesity is occurring in the US and many other developed countries, and appears to be responsible for an associated increase in the prevalence of type-2 diabetes, dyslipidemia, and hypertension. Alarmingly, this trend for increasing adiposity and its comorbidities is not limited to adults, but is also threatening children at younger and younger ages. Over the last three decades, the prevalence of overweight among children age 2–19 years has nearly doubled in the US [1]. What is the cause of the dramatic increase in adiposity among our children and adults? The increasing availability of highly palatable foods combined with more ‘modern’ lifestyles involving less physical work and play are creating a more obesigenic environment. But why do some individuals respond to these environmental changes with increased adiposity, while others seem naturally resistant to increases in body weight?

It has long been presumed that individual genetic variation alone explains individual differences in obesity susceptibility. According to the ‘thrifty genotype’ hypothesis [2], individuals whose genomes evolved to maximize energy intake in times of plenty, and minimize energy expenditure, developed an evolutionary advantage allowing them to survive prolonged periods of food deprivation. In our modern society, these same ‘thrifty’ genes now promote excessive weight gain and type-2 diabetes. A complementary explanation for individual variation in obesity susceptibility, the ‘thrifty phenotype’ hypothesis [3], proposes that individual differences in satiety mechanisms,
endocrine interactions that regulate metabolism, and neurological and behavioral mechanisms affecting physical activity are determined not only by genes, but also by environmental influences during development. This chapter will review briefly evidence from human studies and animal models that during infancy and early childhood nutrition serves as an important signal for ‘fine-tuning’ various metabolic systems, and thereby influences obesity susceptibility throughout life. The primary focus of this article will be an evaluation of the hypothesis that nutrition during infancy and early childhood modifies obesity susceptibility by perturbing epigenetic mechanisms.

**Metabolic Imprinting**

Several years ago, Waterland and Garza [4] proposed the term ‘metabolic imprinting’ to encompass a subset of adaptive metabolic responses to early nutritional influences. Metabolic imprinting is characterized by: (1) susceptibility limited to a specific ontogenic period early in development (i.e. a ‘critical window’); (2) a persistent effect lasting through adulthood; (3) a specific and measurable outcome, and (4) a dose-response or threshold relation between exposure and outcome [4]. When this definition was proposed, no phenomenon characterized in either human populations or experimental animal models met the stringent criteria of metabolic imprinting. The postulation of metabolic imprinting was thus intended as a challenge to researchers in this field. Once specific examples of metabolic imprinting are characterized we will, by definition, have gained substantial insight into the underlying developmental processes. Considering that obesity can result from dysregulation of myriad physiological systems, it is not appropriate to postulate metabolic imprinting of obesity. Obesity not a specific outcome. This point underscores the most important distinction between metabolic imprinting and ‘programming’ [5]. Whereas it would be reasonable to propose nutritional programming of obesity, metabolic imprinting can be postulated only in the context of a specific candidate mechanism.

**Early Postnatal Nutrition and Adult Obesity Susceptibility**

Several excellent reviews [3, 6–8] have considered whether adult obesity susceptibility is influenced by nutrition during critical periods of development. Of particular relevance here, retrospective studies have reported that adiposity is lower in individuals who were breastfed as infants, relative to those who were formula-fed. These data have led to the provocative hypothesis that breastfeeding protects against later obesity [9, 10]. Because socioeconomic status is associated with both obesity and breastfeeding rates, these studies
adjust for the influence of socioeconomic status. It is impossible, however, to rule out potential residual confounding. Recent findings that obesity physiologically impairs lactation [11] suggest yet another cause for an inverse relation between breastfeeding and obesity. For these reasons, and because of the discordant findings among apparently similar studies, the effect of breastfeeding on later obesity remains highly controversial [12]. Other human observational approaches suggesting that early postnatal nutrition contributes to later obesity may likewise be explained by alternative pathways. For example, several studies link rapid weight gain in infancy to overweight in adulthood [13]. Although consistent with the hypothesis that infantile overnutrition causes adult obesity, such relations can more parsimoniously be explained by an individual tendency for excessive food intake manifesting in infancy and persisting to adulthood.

By far the most compelling human data demonstrating that early postnatal diet can influence metabolic parameters relevant to obesity are from Lucas [14] who performed a longitudinal study of preterm infants randomly assigned to one of two formulas or breast milk during the first few weeks of life. At 15 years of age, the ratio of serum leptin to fat mass was 25% higher in subjects who were fed a special preterm formula in early infancy, compared to those who received either the standard infant formula or banked breast milk [15]. Leptin is secreted by adipose tissue and acts as a satiety signal in the hypothalamus. Thus, although there were no significant differences among the groups in body mass index or fat mass at age 15, the effect on serum leptin normalized to fat mass indicates that early postnatal diet caused a permanent change in adipose tissue leptin secretion and/or hypothalamic leptin sensitivity. It is likely that these physiological differences will cause group differences in adiposity as the subjects get older.

Several animal models provide strong support for the hypothesis that nutrition in the early postnatal period influences developmental pathways that affect adult obesity susceptibility. In the rodent suckling-period litter size model, the offspring from several litters of rats or mice born on the same day are randomized and redistributed to foster dams in small, normal, or large litters. These animals experience, respectively, overnutrition, normal nutrition or undernutrition during the suckling period. At weaning, small-litter pups are heavier and fatter than pups suckled in normal-sized litters, and these differences persist to adulthood [16, 17]. Animals suckled in small litters also display a persistent impairment in glucose-stimulated insulin secretion in vivo and in vitro [16, 18, 19]. Patel and co-workers [20] have for several years been developing the ‘pup in a cup’ model for early postnatal induction of obesity in the rat. Within a few days of birth, rat pups are cannulated and fed enterally until postnatal day 18. The rate of formula delivery is adjusted so that formula-fed pups gain weight at the same rate as mother-fed animals, and after day 18 all animals are provided free access to the same standard rat chow. Rats fed the high-carbohydrate formula during
the suckling period become heavier, fatter, and hyperinsulinemic as adults, relative to rats who are either enterally fed a high-fat formula (similar to rat milk) or suckled by their mothers [20]. In another model, it was recently shown that male mice whose mothers are fed a low-protein diet during the suckling period only are thereafter protected from the induction of obesity by a highly palatable diet [21].

Compared to nutritional exposures during gestation, nutrition in the early postnatal period appears to have a greater impact on metabolic imprinting of physiological parameters relevant to body weight regulation [4]. In fact, in many of the models purporting to show 'fetal programming' of obesity, such as the maternal caloric restriction model [22], it is unclear whether adult obesity results from the prenatal restriction per se or from dietary compensation (catch-up growth) in the early postnatal period.

**Epigenetics, Development, and Nutrition**

The vast majority of cells in the human body contain the same complement of DNA: the entire human genome. Yet hepatocytes express a very different subset of genes from, say, neurons or adipocytes. These tissue-specific patterns of gene expression are maintained by ‘epigenetic’ mechanisms. Epigenetics is the study of mitotically and/or meiotically heritable alterations in gene expression that are not associated with changes in DNA sequence. Epigenetic processes include DNA methylation, various modifications of the histone proteins that ‘package’ DNA in the nucleus (including acetylation, ubiquitination, and methylation) and feed-forward autoregulation by specific transcription factors [23]. All of these processes interact synergistically to maintain specific regions of the genome in an open, transcriptionally active state and others in a highly condensed and transcriptionally silent state. By definition, these cell-type-specific epigenetic alterations, once established during development, are maintained through successive rounds of cellular proliferation throughout life and can in some cases be transmitted through the germ line. Of the various epigenetic alterations, DNA methylation is among the best characterized. For this reason, and because of the direct influence of diet on the DNA methylation pathway [24], we have focused on nutritional influences on DNA methylation during mammalian development.

In mammals, methylation of cytosine to 5-methyl-cytosine occurs on both DNA strands within palindromic CpG dinucleotides. (The ‘p’ denotes the intervening phosphate group.) CpG methylation contributes to transcriptional regulation by affecting the binding of methylation-sensitive DNA-binding proteins. CpG methylation in a gene’s promoter region is generally associated with transcriptional repression, but CpG methylation in discrete regulatory regions can also augment transcription. During development, the high levels of genomic CpG methylation in the sperm and oocyte are largely erased following
fertilization, and around the time of implantation a wave of de novo methylation occurs in the embryonic genome [25]. Tissue-specific patterns of CpG methylation are established during prenatal and early postnatal development, and are thereafter maintained through cellular replication by a ‘maintenance methylase’ DNA methyltransferase-1 (DNMT1). The DNA methylation pathway is directly dependent on dietary methyl donors and cofactors (fig. 1). Hence, during critical ontogenic periods, a dietary excess or deficiency of key nutrients such as folic acid, methionine, and vitamin B12 may affect the establishment of CpG methylation in specific regulatory regions of genes [26]. These induced epigenetic alterations can be maintained to affect adult gene expression and phenotype. Importantly, just as genetic variation influences individual susceptibility to various diseases, it is becoming increasingly clear that individual differences in epigenetic regulation can affect disease susceptibility [27–29]. Unlike genetic variation, however, which is determined by parental inheritance, we currently know very little about the factors that determine individual variation in epigenotype.

**Perturbation of DNA Methylation by Environmental Influences during Development**

Recent studies in animal models have demonstrated early environmental influences on mammalian developmental epigenetics. Wolff et al. [30] showed that maternal dietary methyl donor supplementation affects the coat color of

![Fig. 1. Mammalian one-carbon metabolism provides the methyl groups for methylation of DNA and other substrates (indicated as ‘X’). DNA methylation is directly dependent on nutrients including folate (shown here as 5-CH3-tetrahydrofolate), cobalamin (vitamin B12), choline, and methionine.](image-url)
viable yellow agouti (\(A^{vy}\)) mice. The \textit{agouti} gene encodes a paracrine-signaling molecule that regulates the formation of a yellow pigment. \textit{Agouti} is normally expressed only in hair follicles during a specific stage of hair growth, causing a yellow band on an otherwise black hair; this results in the brown (agouti) coat color of a normal mouse. In \(A^{vy}\) mice a retrotransposon (intracisternal A particle, IAP) has inserted into the \textit{agouti} gene. The IAP insertion introduces a cryptic promoter, and destabilizes the establishment of DNA methylation at \textit{agouti} [31]. Consequently, within a single litter of isogenic \(A^{vy}/a\) animals, some animals will have a high level of CpG methylation at \(A^{vy}\), while others will display systemic hypomethylation at \(A^{vy}\). Hypomethylation of \(A^{vy}\) allows ectopic agouti expression from the IAP cryptic promoter and, not surprisingly, these animals have yellow coats. Because the agouti protein binds antagonistically to the melanocortin-4 receptor that contributes to hypothalamic regulation of satiety, ectopic agouti expression also causes hyperphagia, obesity, and hyperinsulinemia [32]. In \(A^{vy}/a\) animals with hypermethylation at \(A^{vy}\), ectopic agouti expression is silenced, recapitulating the brown, lean phenotype of an \(A/A\) mouse (fig. 2a).

Waterland and Jirtle [33] demonstrated that dietary methyl donor supplementation of female \(a/a\) mice before conception and during pregnancy shifts the coat color distribution of their \(A^{vy}/a\) offspring by increasing \(A^{vy}\) CpG methylation (fig. 2b, c). The nutritionally induced change in offspring epigenotype affected all tissues and was maintained into adulthood. Hence, a transient nutritional stimulus during a critical ontogenic period caused an epigenetic alteration that persisted to influence the adult phenotype. Because viral-derived transposable elements comprise roughly 40% of the human genome [34], it is highly likely that many human genes share the ’epigenetic metastability’ [35] that renders the \(A^{vy}\) locus labile to early nutritional influences on epigenetic regulation.

In another example of early environmental shaping of the mammalian epigenotype, Weaver et al. [36] studied the ’programming’ of stress response by maternal caregiving in the early postnatal period. In an inbred strain of rats, they reported substantial interindividual variation in maternal caregiving behavior; some dams spent a great deal of time licking, grooming, and nursing their pups (high-LGN) whereas others spent much less time on these nurturing behaviors (low-LGN). Weaver et al. [36] discovered that maternal caregiving behavior affects the establishment of CpG methylation in the glucorticoid receptor (GR) promoter region in the hippocampus. Methylation is completely absent at specific CpG sites within the GR promoter before birth, and increases dramatically by 1 day after birth (fig. 3). In the offspring of high-LGN dams, the GR promoter in hippocampal DNA is almost completely demethylated by day 6 postnatally. In offspring of low-LGN dams, however, it remains methylated, and this group difference persists into adulthood. Cross-fostering studies demonstrated that the GR epigenotype is determined by the LGN phenotype of the foster mother, not by the
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Fig. 2. a Phenotypic classes of \( A^{vy}/a \) mice. Yellow mice are hypomethylated at the \( A^{vy} \) allele, whereas pseudoagouti mice are hypermethylated at \( A^{vy} \). b Coat color distribution of all \( A^{vy}/a \) mice born to 9 unsupplemented dams and 10 supplemented dams. The distribution of the supplemented offspring is shifted toward the pseudoagouti phenotype (\( p = 0.008 \)). c Percentage of cells methylated at each of seven CpG sites in the \( A^{vy} \) region of all \( A^{vy}/a \) mice born to 9 unsupplemented dams and 10 supplemented dams. Average \( A^{vy} \) methylation is increased in supplemented offspring (\( p = 0.005 \)). From Waterland and Jirtle [33].

inheritance of subtle genetic or epigenetic differences between high-LGN and low-LGN dams [36]. This elegant study provides a compelling example of a subtle environmental influence during postnatal development affecting the establishment of gene-specific epigenotype.

These two examples demonstrate that environmental influences during embryonic and early postnatal development can affect the establishment of gene-specific DNA methylation. How late into the postnatal period
are mammals susceptible to environmental influences on epigenotype? A continuous supply of methyl donors and cofactors is required to maintain established tissue-specific patterns of CpG methylation through the rapid rounds of cellular proliferation that continues into the postnatal period in many tissues. Maintenance of CpG methylation may therefore be hampered by an inadequate supply of dietary methyl donors and cofactors in the postnatal period, and such affects may be locus-specific [26]. We have been exploring this hypothesis by examining the effects of post-weaning diet on the epigenetic regulation of genomically imprinted genes. Genomic imprinting is an epigenetic phenomenon whereby certain mammalian genes are expressed preferentially from either the allele inherited from the mother or that inherited from the father [37]. The ‘monoallelic’ expression of genomically imprinted genes is regulated by allele-specific CpG methylation that is established differentially in the oocyte and sperm genomes of the parents and transmitted to the next generation. In this manner, CpG methylation appears to serve as the ‘imprint’ that allows the maternal and paternal alleles of imprinted genes to be distinguished. The gene encoding insulin-like growth factor-2 (IGF2) is imprinted in mice and humans, and is

![Fig. 3. Ontogeny of methylation at a critical CpG site within the glucorticoid receptor promoter region in the rat hippocampus. The site is unmethylated before birth (E20) and becomes 80% methylated by 1 day after birth. In pups suckled by mothers with high maternal caregiving (High LG-ABN), methylation at this site returns almost to zero by day 6 postnatally. In rats suckled by less attentive mothers (Low LG/ABN) this developmental demethylation does not occur. This epigenetic response to maternal caregiving persists to adulthood. From Weaver et al. [36].](image-url)
expressed predominantly from the paternal allele. Waterland and Jirtle [38] weaned C57/Castaneus F1 hybrid mice onto either a normal control diet or a synthetic diet lacking methionine, folic acid, vitamin B₁₂ and choline. By 60 days post-weaning, dietary methyl donor deficiency caused a dramatic increase in relative expression of \textit{Igf2} from the maternal allele [38]. Most importantly, after this 60-day diet exposure, when the deficient animals were ‘recuperated’ for an additional 100 days on the control diet, the increment in maternal \textit{Igf2} expression persisted, indicating that the post-weaning dietary deficiency induced an epigenetic alteration that was maintained despite a subsequent return to a replete diet [38].

These animal models demonstrate that epigenetic mechanisms can be affected by environmental influences during development. Clearly, the organism is most susceptible to environmental influences when epigenetic states are undergoing developmental transition. Epigenetic metastability (and nutritional lability) at \textit{Avy} appears to occur only in the very early embryo, and once an individual's \textit{Avy} epigenotype is ‘set’ it is relatively stable throughout life. Developmentally programmed changes in methylation at the \textit{GR} in the hippocampus span an entirely different ontogenic window, during the first few postnatal days, during which maternal caregiving can have a profound and permanent influence on GR methylation and expression. As demonstrated by the studies of post-weaning effects on \textit{Igf2} allelic expression, even after epigenetic states have been established, severe nutritional deficiency during late postnatal development can interfere with their maintenance and thereby cause persistent changes in the epigenetic regulation of certain genes. As nutritionists, we tend to think about inducing alterations in DNA methylation with a ‘supply-side’ approach, i.e. by dietarily producing either an excess or deficiency of the S-adenosylmethionine required for DNA methylation (fig. 1). Importantly, the study by Weaver et al. [36] indicates that simply inducing (or repressing) the transcriptional activity of a promoter region during a critical period of development can cause a persistent alteration in its epigenetic regulation. Early nutritional exposures unrelated to one-carbon metabolism may therefore have a profound effect on developmental epigenetics simply by affecting gene transcription during critical ontogenic periods.

**Epigenetics and Obesity**

There are currently no compelling data indicating that epigenetic mechanisms play a major role in the most common types of human obesity. This evidential void contrasts markedly with the convincing body of evidence that dysregulation of epigenetic mechanisms is an important cause of human cancer [28, 39]. This does not indicate that epigenetic factors do not affect human obesity susceptibility. Rather, establishing links between epigenetics and cancer is relatively straightforward. The ability to compare
tumor tissue with healthy adjacent tissue from the same individual enabled the identification of numerous epigenetic abnormalities associated with carcinogenesis [39]. In obesity, coordinate endocrine dysregulation of multiple organ systems makes it extremely difficult to identify the primary cause of chronic energy imbalance. This complexity will hamper attempts to understand the role of epigenetic dysregulation in the etiology of obesity.

Nevertheless, there are already extensive data indicating that epigenetic dysregulation can lead to obesity. Prader-Willi syndrome is a genetic syndrome that causes hyperphagia, hypogonadism, characteristic facial features, and obesity [40]. The disease results from the lack of expression of genes on a portion of 15q11-q13 that are genomically imprinted and normally expressed from only the paternal allele. Prader-Willi syndrome is most often caused by paternal deletion of 15q11-q13 or uniparental disomy for chromosome 15 [40]. In a small number of 'sporadic' cases, a very small genetic deletion in an imprinting center in the 15q11-q13 region causes the paternally inherited allele to be epigenetically silenced (in addition to the normally silenced maternal allele). Notably, this epigenetic disease initially causes feeding difficulties and failure to thrive in early infancy. The onset of rapid weight gain does not occur until 1–6 years of age [40].

Cloned mice provide another demonstration that obesity can be caused by epigenetic dysregulation [41]. When DNA from somatic cells of adult animals is used to create clones, the epigenetic ‘programming’ of the differentiated adult cell must be reset to allow the totipotency required for embryonic development. Our understanding of how to restore epigenetic totipotency remains fairly rudimentary, as suggested by the extremely low success rates for cloning. When mice are successfully cloned, they often have normal birth weights but develop adult-onset obesity [42]. Similar to obese humans, these mice also develop hyperinsulinemia and hyperleptinemia. While we do not yet understand the specific mechanisms that cause obesity in this model, it is clearly an epigenetic, not a genetic, phenomenon. The viable yellow agouti (Avy) mouse model described above also provides an example of epigenetic dysregulation leading to obesity. In Avy mice, hypomethylation in the Avy genomic region enables ectopic expression of agouti protein. When expressed systemically, agouti protein interferes with melanocortin signaling in the hypothalamus, causing hyperphagia and obesity. Notably, the ‘agouti-related peptide’, which shares sequence similarity to the mouse agouti protein, acts on the hypothalamus to stimulate eating behavior in humans.

**Developmental Epigenetics and Obesity: Potential Links**

Obesity is a highly complex disease that can result from dysregulation of literally any physiological system involved in regulating energy intake or the various components of energy expenditure. How can we identify
physiological systems in which early postnatal nutrition may plausibly modify epigenetic mechanisms that will have an impact on later obesity susceptibility? The most compelling insight from the animal models is that epigenetic mechanisms are most labile to environmental influences when they are either first being established or undergoing developmental transition. In this section two physiological systems that contribute to body weight regulation (and its dysregulation in obesity) will be considered from this perspective.

**Neurological Development**

The hypothalamus is a master regulator of eating behavior, as it detects and integrates peripheral signals to maintain energy homeostasis. It is likely that food availability in the early postnatal period acts as a signal to ‘tune’ the sensitivity and responsiveness of hypothalamic regulation of energy intake. Recent data suggest that this developmental maturation occurs via epigenetic mechanisms. Bouret et al. [43] demonstrated that circulating leptin in the early postnatal period acts as a developmental signal that drives the formation of projections from the arcuate nucleus of the hypothalamus (ARH). These projections are severely deficient in leptin-deficient (ob/ob) mice. However, when ob/ob mice received leptin injections during the suckling period (days 4–12 postnatally) they developed ARH projections similar to those of wild-type animals. Unlike untreated ob/ob mice, which are hyperphagic, post-weaning food intake of mice treated postnatally with exogenous leptin was comparable to that of wild-type animals. Importantly, this developmental action of leptin is limited to the early postnatal period. When adult ob/ob mice were treated with leptin for 20 days, there was no effect on the density of ARH projections [43]. This indicates that a developmental maturation of the hypothalamus, likely epigenetic in nature, occurs in the early postnatal period in the mouse.

Several studies have documented profound changes in global [44] and gene-specific [45] CpG methylation in specific regions of mouse brain over the early postnatal period, further validating the hypothesis that early postnatal neurological development occurs via epigenetic mechanisms. The developmental progression of Rett syndrome, an epigenetic neurological disease that is among the most common causes of mental retardation in girls, indicates that postnatal neurological development in humans involves epigenetic mechanisms. Rett syndrome is caused by mutations in the X-linked gene encoding methyl-CpG-binding protein-2 (MeCP2) [46]. MeCP2 is a methylation-dependent DNA-binding protein that binds to methylated DNA regions, helping to stabilize them in a transcriptionally inactive state [46]. Girls with Rett syndrome appear to develop normally for the first 6 months of life, then head growth falters and neurological symptoms manifest. Considering that MeCP2 is normally widely expressed throughout the brain in the prenatal and early postnatal period [46], why
does MeCP2 deficiency not cause symptoms until 6 months after birth? One explanation is that MeCP2 is required for the stabilization of progressive epigenetic silencing that is necessary for normal neurological maturation in late infancy.

Maturation of the Insulin Axis

Peripheral insulin resistance, hyperinsulinemia and hyperglycemia are commonly associated with obesity. While it is widely assumed that obesity leads to secondary insulin resistance which causes compensatory hyperinsulinemia, disentangling the causal pathways linking these comorbidities has perplexed endocrinologists for decades. It therefore remains plausible that primary defects in peripheral insulin sensitivity and/or pancreatic glucose-stimulated insulin secretion cause human obesity. Glucose-stimulated insulin secretion in the endocrine pancreas is blunted in newborn rodents and humans and displays a developmental maturation during early postnatal life [47, 48]. This process can be affected by postnatal nutrition, as demonstrated in rodent models showing persistent effects of suckling-period litter size [16, 18, 19] and high-carbohydrate formula feeding [20]. Waterland and Garza [19] showed that the persistent defect in glucose-stimulated insulin secretion in rats suckled in small litters (compared to those from normal-sized litters) correlated with persistent changes in gene expression within isolated pancreatic islets. The quantitative stability with which these expression differences were maintained from weaning to adulthood suggested that early postnatal overnutrition had affected the establishment of epigenetic gene regulatory mechanisms in islet cells [19].

Although it is likely that nutrition during postnatal development influences epigenetic mechanisms involved in functional maturation of the endocrine pancreas, surprisingly little is known about the epigenetic regulation of genes that play critical roles in insulin axis function. For example, we know little about DNA methylation of the insulin gene itself, let alone whether it undergoes developmentally programmed changes during the early postnatal period. One study, however, provides precedent for early-postnatal ontogenic changes in gene-specific DNA methylation within the endocrine pancreas. Matsusue et al. [49] showed in rats that pancreatic expression of cholecystokinin type a receptor (Cckar) increases markedly from age 14 to 21 days postnatally. The increase in Cckar expression was associated with concurrent demethylation of Cckar. The dearth of knowledge on the ontogeny of gene-specific epigenetic changes in the endocrine pancreas extends to other tissues important in insulin axis function, including skeletal muscle and adipose tissue. Given that persistent nutritional influences on epigenetic mechanisms are most likely to occur when these mechanisms are undergoing developmental changes, it is critically important for us to gain more information on the developmental epigenetics responsible for functional maturation of the insulin axis.
Conclusions

Clearly, environmental influences during prenatal and early postnatal development can both permanently alter body weight regulation and affect the establishment and maintenance of epigenetic gene regulatory mechanisms. Epigenetic dysregulation can cause obesity, and epigenetic development of physiological systems relevant to energy homeostasis continues into the postnatal period. It is therefore likely that postnatal metabolic imprinting of epigenetic gene regulatory mechanisms plays a role in determining individual susceptibility to obesity [8]. Improving our understanding of the biologic mechanisms whereby early nutrition influences developmental epigenetics may eventually enable the formulation of early postnatal nutritional interventions aimed at decreasing individual obesity susceptibility.

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References

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Discussion

**Dr. Woltil:** What is known about body composition determinants and the development of epigenetic regulation disturbances? Preterm infants and small-for-gestational age babies grow best during their hospital stay, there is evidence for that. There are now formulas available for this group of infants. The effects of these formulas have been shown, not only in weight gain but also the gain of fat-free mass, on absolute fat mass but not relative fat mass. Is it known which of these parameters affect the epigenetic regulatory mechanisms, and if not can you speculate on that?

**Dr. Waterland:** There is very little known about how nutrition influences epigenetic gene regulation in humans and we are just now starting to use animal models to get a better understanding of the specific genomic characteristics of gene regions that are epigenetically labile to early environmental influences. As far as speculating on what types of genes might be involved in regulating body composition in humans, there too it is almost an open question. I gave one example regarding maturation of the hypothalamus because it is clear that the nutritional transitions that occur at birth, and also at weaning, are important developmental windows. These might also be critical periods when development of epigenetic processes is occurring, but that remains to be seen.

**Dr. Schmitz:** I would like to ask two questions. The first one is: what is the evidence that this modification in the epigenetic mechanism can extend in the first or second year of life? The second question is: do you think that the quantitatively small variations in feeding during infancy might be able to modify these very important mechanisms and the one you are showing?

**Dr. Waterland:** The first question is whether these types of induced modifications of epigenetic mechanisms occur in the postnatal period in humans. There are no good data available in humans yet, but what we have to do to start with is to really understand comparative genetics and comparative genomics and determine whether these processes occur the same way in mice as in humans. Regarding your second question, I think that our study in mice is really relevant, the supplemented diet was only about 3-fold higher on average in those various nutrients relative to the unsupplemented diet, so that wasn’t an extreme mega supplementation or anything. When you consider the various practices in terms of maternal vitamin intake and supplementation of children, I think that in terms of weaning foods and weaning practices there is a very broad variation in food intake during at least late infancy. So I do think that there is a potential for even these relatively subtle differences in diet to have a big impact down the road.
Dr. Verloove: I think it has been shown that the behavior patterns of the parents can reduce stress hormone levels in early childhood and infancy. There are changes in the hormonal axis. I don't know if any of you is more familiar with the literature than I am, but it has been proven in humans from the behavioral and the hormonal side.

Dr. Waterland: But still it is difficult to disentangle that completely from the potential genetic contributions to those epigenetic mechanisms.

Dr. Verloove: As far as I understood they came quite a long way in proving that.

Dr. Singhal: I would like to make a comment on the evidence for epigenetic factors in humans in terms of postnatal programming. There is huge interest in imprinted genes in which the effect of the gene depends on whether it is inherited from the mother or the father. These epigenetic phenomena are likely to influence early postnatal growth which itself is likely to influence early appetite, a key area of interest. Although we don't have much data, there is some evidence to show for example that growth in the first 2, 3 days of life (which is nutritionally dependent) affects your IgF-1 levels permanently [1]. So the critical window is very early on, and is likely to involve imprinted mechanisms. I think human data are beginning to emerge to support this.

Dr. Waterland: In terms of the relevance of the mouse studies to humans, the imprinted genes actually give a nice example because in a lot of mouse studies it has been shown that different stimuli during early embryonic development can cause persistent changes in epigenetic regulation at genomically imprinted genes. And as far as humans go, very recently we are starting to get data on various epigenetic diseases in humans born by assisted reproductive technologies and there appears to be a higher prevalence of some of these epigenetic diseases such as Angelman's syndrome and Beckwith-Wiedemann syndrome in individuals who are produced by these artificial technologies that require incubating an early embryo in vitro for a couple of days before implantation. So we can see how this provides another indication of the similarities in the way these things work between mice and humans, and it is a very scary thought when you think of how many children are being produced by these techniques now. We think we have a great understanding of everything and can control nature but now we are finding out that even though we can successfully produce an infant with these techniques, we might actually be causing some problems as well.

Dr. Singhal: How do you explain nutritional programming or metabolic imprinting in identical twins, a model in which the mothers have the same diet but you still see the same programming phenomena? This is one area in which I don't understand how the epigenetic mechanisms work.

Dr. Waterland: That is actually an excellent example because in a lot of cases of identical twins you actually have very different placenta, so it is very common to have one twin whose birth weight is significantly lower than that of the co-twin. When you think that these differences in birth weight are actually based on different nutrient supply through the placenta, then it is very easy to understand how this could cause epigenetic differences between the twins.

Dr. Heymans: A prospective study in humans is not feasible so we have to stick to things that happened. We are still investigating a population that was exposed to famine in Amsterdam in 1944. There is a huge difference between those who were exposed in the beginning of the pregnancy and those who were exposed at the end [2]. Wouldn't that be an interesting group to try and research in your project, and see what the differences in methylation are, for instance?

Dr. Waterland: I would be very interested in following up with that cohort eventually. That early paper by Ravelli et al. [3] looking at obesity as an outcome in those individuals was the very first paper that sparked my interest in this whole field. My goal is to be able to use these animal models to really refine our specific hypothesis of what we want to test in these human populations because there are several cohorts like that.
where we have documented differences in early nutritional exposures, but the question is, before we try to follow up on these individuals, if we are going to be asking for blood samples and that sort of thing, I would like to have a really good idea about exactly where in the genome we expect to find epigenetic differences caused by these early exposures.

Dr. Verloove: For your information, both the Amsterdam cohort and the new cohorts from other places in Holland where we are trying to study these effects, all the blood samples are frozen. So if you have thought about what you would like to know, you can ask and we will see what we can do.

Dr. Kleinman: Have we evolved any mechanisms to protect ourselves against imprinting? For example, once methylation occurs, is it a permanent phenomenon or is there a repair mechanism that is available to protect against ‘mistakes’ that might work to the disadvantage of the animal or human?

Dr. Waterland: That is an interesting question. As far as evolving mechanisms to fix these things, the flip-side of that is why we might have this type of environmental susceptibility in the first place. So really if you think about it, having some kind of plasticity built into these epigenetic processes might actually have given an evolutionary advantage, allowing a newborn infant to adapt to the specific nutritional conditions which were prevalent at that time. But now the same types of developmental plasticity can be detrimental for example in developing countries where the infant might have made adjustments to one environment and now 30, 40 years later the nutritional environment is quite different. But as far as the ability for these things to change during later life, with age there is a gradual loss of epigenetic information throughout the genome. For example the overall level of genomic methylation declines with age in most tissues, suggesting that a gradual loss of this information, as some people have postulated, might be implicated in the aging process itself. But as far as repair mechanisms are concerned, I am not aware of too much work in that area.

Dr. Kleinman: If you think about this from an evolutionary perspective we have evolved a number of mechanisms to protect ourselves against our nutritional environment, excessive intake of iron for example or inadequate consumption of carbohydrate, and certainly 500 years ago there were much wider swings in nutritional intake from day to day likely, and certainly season to season. So in a sense this imprinting would seem to be counterproductive if it leads to an inability to respond to a changing environment. At least if you look at it from a long evolutionary perspective, our diets have become much more consistent over the past 50 years than they were over the past previous 900 years, so it is a little bit difficult to understand the benefit of imprinting in the context of being able to adapt to a changing environment.

Dr. Waterland: We could do a lot of hand-waving about the exact evolutionary perspective, but I think it is really unknown exactly how well nutrition in infancy provides a mechanism for the individual to sense its environment to some extent. I don’t know what would be the appropriate nutrition in infancy to prepare one to be ‘super-sized’ later in life, so the food environment that we have in the US, and increasingly in other countries, is a problem and it will be important to understand how these processes interact with the environment.

Dr. Hernell: We have been discussing very extensively during the last decades metabolic imprinting or programming and it seems clear that early events do have lasting effects. Still there are many environmental factors that affect for instance development of obesity in adulthood. Would you even care to speculate how much could these early events, including epigenetic effects, contribute to the total risk panorama during a lifetime?

Dr. Waterland: I know that one problem a lot of people have with this whole early origins hypothesis is that people may just give up, they say, ‘My birth weight was only
2.3 kg, I am doomed, so there is no sense exercising or eating a healthy diet’. Now I entirely disagree with that. I still believe that the largest part of staying in good health is taking care of yourself and eating properly and exercise and all that sort of thing, but I don’t want to deny the potential importance of even a minor effect because if it is influencing the entire population it is going to have a pretty big overall effect on health. But in terms of obesity I want also to mention that some epigenetic alterations are actually meiotically as well as mitotically heritable, so there is the potential for epigenetic inheritance to occur. This might actually allow trans-generational influences of early nutritional exposures, and that is another area where imprinted genes are especially relevant because genomically imprinted genes have essentially evolved to propagate epigenetic information trans-generationally. We know that the methylation patterns at imprinted genes are established during late gestation and the early postnatal period. So if nutrition during these periods can influence methylation in these critical imprinted gene regions then those changes could actually be transferred to the next generation. One could imagine a feed-forward trans-generational effect of increasing levels of obesity, by which even a small effect could actually accumulate over successive generations.

Dr. Verloove: This reminds me of our discussion yesterday about allergy, food allergy and that the such. Do you think there might be a link there?

Dr. Waterland: I think we are going to find more and more that epigenetic processes are fundamentally involved in almost everything.

Mr. Turini: In clinical settings antibiotics together with anti-inflammatory drugs are often administered to children. Anti-inflammatory drugs can affect the methylation of histones, for instance, and it is also known that some aspects of anti-inflammatory processes could limit the development of tolerance as suggested in a paper published in Nature Medicine. Do you think giving aspirin to young children is a risk for allergy development, especially during the weaning period?

Dr. Waterland: It is important to consider that, even though I focused my research on nutritional influences, as I demonstrated with that example on maternal care-giving behavior, many other types of environmental influences can impact the establishment and maintenance of epigenetic processes during development. Pharmacological exposures could certainly cause epigenetic changes just as nutritional exposures.

References