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Epigenetic Mechanisms Affecting Regulation of Energy Balance: Many Questions, Few Answers

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Abstract

Extensive human and animal model data show that nutrition and other environmental influences during critical periods of embryonic, fetal, and early postnatal life can affect the development of body weight regulatory pathways, with permanent consequences for risk of obesity. Epigenetic processes are widely viewed as a leading mechanism to explain the lifelong persistence of such "developmental programming" of energy balance. Despite meaningful progress in recent years, however, significant research obstacles impede our ability to test this hypothesis. Accordingly, this review attempts to summarize progress toward answering the following outstanding questions: Is epigenetic dysregulation a major cause of human obesity? In what cells/tissues is epigenetic regulation most important for energy balance? Does developmental programming of human body weight regulation occur via epigenetic mechanisms? Do epigenetic mechanisms have a greater impact on food intake or energy expenditure? Does epigenetic inheritance contribute to transgenerational patterns of obesity? In each case, significant obstacles and suggested approaches to surmounting them are elaborated.

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INTRODUCTION

Although the current obesity epidemic is arguably the single greatest nutrition-related problem in the developed world, its fundamental causes remain poorly understood. Individual genetic variation is certainly important but does not appear to explain the increasing worldwide prevalence of obesity in recent decades. The escalating public health burden of obesity together with the general failure of therapies aimed at achieving long-term weight loss in obese adults are motivating increasing consideration of obesity as a developmental disease (2, 31). Indeed, extensive human epidemiologic and animal model data indicate that environmental influences during critical ontogenic periods affect the developmental establishment of energy balance pathways, determining lifelong susceptibility to obesity (50, 58, 101). Epigenetic processes, which stably regulate gene expression potential and exhibit plasticity to environment during development, are a prime candidate mechanism to explain such "developmental programming" of body weight regulation (101). Accordingly, there has recently been extraordinary interest in the role of epigenetic dysregulation in human obesity (32, 85, 88).

Rather than summarize knowledge in this field, the goals of this review are to highlight outstanding research questions and current progress toward answering them, and elaborate the greatest obstacles and suggested approaches by which they may be overcome. Our long-term goal is to determine if environmental exposures—including nutrition—during critical periods of development can affect epigenetic mechanisms involved in energy balance regulation, thereby modulating a person's lifelong risk of obesity. Hence, rather than survey the accumulating literature illustrating epigenetic correlates of obesity, this review focuses on the potential for early environment to affect developmental epigenetics of body weight regulation.

IS EPIGENETIC DYSREGULATION A MAJOR CAUSE OF HUMAN OBESITY?

Epigenetics is the study of mitotically heritable alterations in gene expression potential that occur without alterations in DNA sequence (43). Epigenetic modifications are a fundamental mechanism of cellular differentiation, in which pluripotent progenitor cells become increasingly committed toward and then morphologically and functionally diverge into diverse cell types during

mammalian development. Several molecular mechanisms regulate epigenetic development, including methylation of cytosines in CpG dinucleotides (i.e., cytosine followed by guanine), various posttranslational modifications of the amino terminal "tails" of the histone proteins that package DNA into nuclear chromatin, autoregulatory transcription factors, and noncoding RNA (for excellent reviews on epigenetic mechanisms, see 14, 43, 75). On the one hand, the inclusion of histone modifications on this list remains controversial. Although they certainly play a role in developmental processes and are widely viewed as an epigenetic mechanism, it remains unclear whether histone modifications have the definitive ability to convey information through mitosis (38, 69). On the other hand, autoregulatory transcription factors that bind to and *trans*-activate their own promoters constitute a classical and bona-fide epigenetic mechanism (92) that is often omitted from recent authoritative reviews on mammalian epigenetics. None of these individual mechanisms functions in isolation; these and potentially other layers of epigenetic modifications are sequentially established during embryonic, fetal, and early postnatal development and function synergistically to maintain cell type–specific regional chromatin conformations that regulate transcriptional competence. Of the known epigenetic mechanisms, DNA methylation stands out; once established during development, cell type–specific patterns of CpG methylation are mitotically inherited via semiconservative replication and are highly stable in differentiated cells and tissues (14). CpG methylation is also stable during tissue collection and storage and can be assayed in minute quantities of DNA. For these reasons, the vast majority of epigenetic studies pertaining to developmental programming of obesity have focused on DNA methylation.

Animal model and human data show clearly that epigenetic dysregulation can cause obesity. The best-characterized animal model is the *agouti viable yellow* (A^{vy}) mouse. The murine *agouti* gene, normally expressed only in hair follicles, encodes a paracrine-signaling molecule that regulates fur pigmentation. The A^{vy} mutation resulted from the spontaneous transposition of a retrotransposon upstream of *agouti*, causing profound epigenetic dysregulation; isogenic A^{vy}/a mice exhibit stochastic and systemic interindividual variation in DNA methylation at A^{vy}, which in turn regulates variable degrees of ectopic *agouti* expression. Owing to its structural similarity to agouti-related protein (Agrp), agouti protein binds antagonistically to the melanocortin 4 receptor, causing hyperphagic obesity (109). Genetically identical A^{vy}/a mice can therefore range from yellow and obese to lean and brown, vividly illustrating epigenetically mediated obesity (99). Mice produced by somatic cell nuclear transfer (cloning) provide another example. Relative to isogenic mice conceived naturally, those produced by cloning are slightly heavier at birth and later develop obesity (90). Cloning requires a somatic cell to be epigenetically reprogrammed to a totipotent state; the adult-onset obesity of cloned mice, therefore, likely results from subtle aberrations in this epigenetic reset. Prader-Willi syndrome is a human neurodevelopmental syndrome characterized by insatiable hyperphagia and obesity, among other symptoms. Although most often caused by a genetic deletion of a specific region of chromosome 15, a subset of "sporadic" Prader-Willi cases is caused by aberrant DNA hypermethylation and epigenetic silencing of the same chromosomal region (34), providing clear evidence that epigenetic dysregulation can cause obesity in humans as in mice.

Despite these compelling examples, and extensive investigations in recent years, it remains unclear whether epigenetic dysregulation contributes meaningfully to the current worldwide obesity epidemic. This ostensibly slow progress can be attributed to several major obstacles. The first is that epigenetic mechanisms are, by nature, largely cell-type specific. In genetic studies of obesity, one can sample an individual's DNA from any easily biopsiable tissue, such as peripheral blood. In most cases, however, it cannot be assumed that epigenetic marks in peripheral blood are correlated with those in other tissues. Hence, epigenetic studies of obesity will in most cases wish to obtain DNA (and potentially other cellular components) from tissues directly implicated

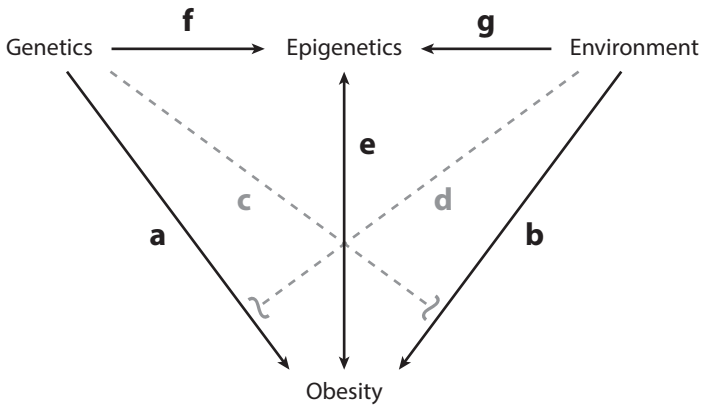

Figure 1

Conceptual framework linking genetics, environment, epigenetics, and obesity. In addition to the direct pathways and interactions by which interindividual genetic variation and environment affect risk of obesity (pathways *a*, *b*, *c*, and *d*), interindividual epigenetic variation can also affect obesity risk (pathway *e*). The strong effect of genetic variation on epigenetic mechanisms (pathway *f*) and the potential for reverse causality (pathway *e*) complicate studies attempting to demonstrate that epigenetic variation causes human obesity. Developmental programming of obesity via epigenetic mechanisms is indicated by pathway *g*-*e*. Adapted from Reference 99 with permission.

in energy balance regulation, requiring more invasive studies. The second major obstacle, which again distinguishes epigenetic from genetic association studies, is that epigenetic marks are inherent malleable. Hence, although epigenetic correlates of obesity may be easily identified, these may represent a consequence rather than a cause of obesity. The third major obstacle is that in outbred populations (such as humans and some animal models) genetic variation is a major determinant of interindividual epigenetic variation (72). One particularly relevant example is the recent report (54) of a "DNA methylation atlas" of two tissues (muscle and fat) in three breeds of pigs; breed (i.e., genetics) was the major driver of methylation differences, which, coincidentally, correlated with differences in adiposity. In another study, interindividual variation in DNA methylation at the human fat mass and obesity-associated (*FTO*) gene was found to be largely determined by local haplotype (6). Also, a recent study of human abdominal subcutaneous adipose tissue (22) identified 149 methylation quantitative trait loci (meQTLs), i.e., methylation variants highly associated with neighboring single-nucleotide polymorphisms (SNPs). Hence, in human populations, interindividual epigenetic variation must be studied in the context of genetic variation. The complexity of studying the contribution of epigenetic dysregulation to human obesity is portrayed in **Figure 1** (98).

Perhaps due to the difficulty of overcoming these obstacles, most of the strongest recent studies fail to provide compelling evidence that epigenetic dysregulation is a common cause of human obesity. For example, recent studies have identified associations between site-specific methylation in peripheral blood DNA and concurrent obesity either at candidate genes (41) or using genome-scale profiling (96). Similarly, methylation of long-interspersed elements (LINE-1) (as a proxy for whole-genome methylation) in visceral adipose tissue of obese individuals was inversely related to concurrent prevalence of metabolic syndrome (94). The reliance on measurements in peripheral blood (in the first two studies), unclear direction of causality of these associations, and potential for genetic confounding, however, makes these results difficult to interpret. Causality has been explored in a recent series of prospective studies by linking responsiveness to supervised weight loss to locus-specific DNA methylation in peripheral blood at baseline (60, 62). The physiological

significance of these predictive methylation marks is unclear, however, since it is unknown whether the variants in peripheral blood indicate systemic epigenetic differences. Also, it is possible that both the methylation variants and responsiveness to the weight loss program are determined by underlying genetic variation. Of course, given the extremely small proportion of overall variance in human obesity that is explained by locus-specific genetic variation (19), one might argue that relatively robust relationships between site-specific DNA methylation and subsequent weight loss could not possibly be mediated by genetics. If we assume, however, that DNA methylation is, like obesity, a complex polygenic trait, then strong associations between locus-specific DNA methylation and various measures of human obesity could indeed be genetically mediated.

To eliminate genetic confounding, several groups are studying pairs of monozygotic (MZ) twins discordant for obesity. One study (83) measured methylation at 11 differentially methylated regions of imprinted genes in salivary DNA from 16 pairs of adult MZ twins discordant for body mass index (BMI) but found no correlations between intrapair BMI differences and intrapair differences in DNA methylation. Another study (112) did find significant positive correlations between twin-twin differences in adiposity and DNA methylation at the promoter of the gene encoding serotonin transporter (*SLC6A4*) in peripheral blood leukocytes. Although these within-twin-pair correlations cannot be explained by genetics, as the authors noted, the direction of causality and the physiological relevance of *SLC6A4* methylation in peripheral blood DNA remain to be determined.

One promising approach to gain insights into the importance of epigenetic dysregulation in human obesity is to study human metastable epialleles (MEs). At MEs, establishment of DNA methylation occurs stochastically in the very early embryo, resulting in interindividual variation in epigenetic regulation (70) that is neither genetically mediated nor tissue specific (100, 102). One of the best-characterized MEs is the murine A^{vy} allele described above. At birth, isogenic A^{vy}/a mice with differential DNA methylation at A^{vy} appear indistinguishable (**Figure 2*a***); the effects of differential epigenetic regulation at A^{vy} are not fully apparent until adulthood (**Figure 2*b***). Nonetheless, one could have taken a drop of blood from each of these newborn mice and, by measuring DNA methylation at the A^{vy} locus, predicted with absolute certainty which one would become yellow and obese. Although the adult-onset obesity of A^{vy}/a mice is caused by paracrine effects of aberrant *agouti* expression in the hypothalamus (109), the epigenetic lesion causing this misexpression is detectable in peripheral blood DNA at birth! Analogously,

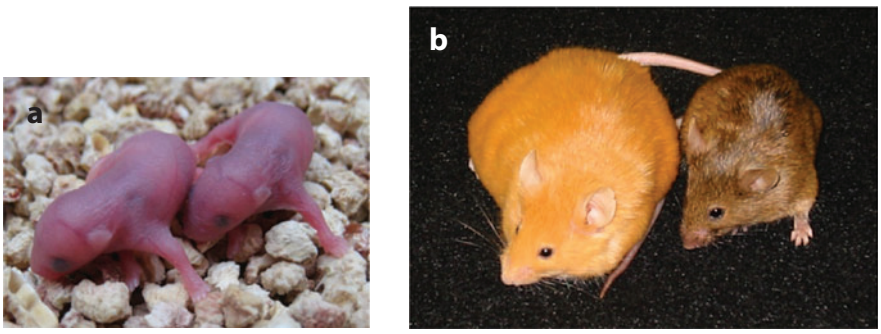

Figure 2

The agouti viable yellow (A^{vy}) mouse as a paradigm for minimally invasive prospective detection of obesity-promoting epigenetic dysregulation. Genetically identical A^{vy}/a sisters appear indistinguishable at birth (*a*), but by adulthood (*b*) manifest the effects of differential epigenetic regulation at the A^{vy} metastable epiallele. (**Figure 2*b*** is adapted from Reference 97 with permission.)

Table 1 Two classes of interindividual epigenetic variation*

	Metastable epialleles	Cell type–specific differentially methylated regions
Tissue specificity	Little/none	Tissue- or cell type–specific
Developmental establishment	Early embryo	Fetal and early postnatal
Studying association with obesity	Relatively simple	Difficult, especially in humans
Genomic prevalence	Rare	Widespread
Likely role in human obesity	Occasional	Extensive

*Adapted from Reference 99.

discovery of human MEs that affect the regulation of genes involved in body weight regulation may someday enable prospective studies to test the hypothesis that interindividual epigenetic variation causes human obesity. Using a genome-wide screen for systemic interindividual variation in DNA methylation, a small set of candidate human MEs was recently identified (103); work is currently underway to identify MEs associated with human disease, including obesity. Interestingly, the limited data available suggest that a CpG island (CpG-dense region) in the 3' end of the human proopiomelanocortin (*POMC*) gene may be an ME (48).

IN WHAT CELLS/TISSUES IS EPIGENETIC DYSREGULATION MOST IMPORTANT FOR ENERGY BALANCE?

Although human MEs may be discovered that explain some interindividual variation in body weight regulation, the cell type–specific nature of most epigenetic regulation means that, in most cases, studies in easily accessible tissues such as peripheral blood will not be sufficient to test causal hypotheses about the epigenetic etiology of obesity (**Table 1**). Hence, rigorous studies in appropriate animal models offer some of the best opportunities to advance our understanding of epigenetic mechanisms in body weight regulation. The first obvious question is: What cell or tissue types should be studied? Most studies thus far have focused on white adipose tissue (WAT) (because of its ability to act as an endocrine organ regulating energy balance) and hypothalamus (given its central role in energy balance regulation) (for review, see 99). For example, several studies have characterized cell type–specific epigenetic regulation of the leptin (*LEP*) gene in adipocytes compared to other cell types (57, 67, 84). Also (as discussed below), animal model studies of developmental programming of body weight regulation have focused on epigenetic alterations induced in either hypothalamus or adipose tissue by early nutritional exposures. Even in these tissues, however, major questions remain unanswered. For example, most studies of epigenetic regulation in the hypothalamus have studied whole hypothalamus. This approach has limitations, considering its complex cell type–specific and region-specific epigenetic specialization. The two principal cell types in neural tissue are neurons and glia, each comprising approximately half of the cells in most adult brain regions. Previous studies have documented important epigenetic differences between neuronal and nonneuronal brain cells (42, 55, 81). In addition to the gross dichotomy of neurons and glia, another level of hypothalamic heterogeneity is its distinct functional regions, termed hypothalamic nuclei. In particular, the arcuate nucleus of the hypothalamus (ARH) integrates peripheral signals such as leptin and insulin and, via diverse neuronal projections, relays anabolic/orexigenic or catabolic/anorexigenic signals to other hypothalamic nuclei including the paraventricular, ventromedial, and lateral hypothalamus (27, 80). The specialized functions and gene expression patterns of the hypothalamic nuclei are maintained by epigenetic mechanisms

(40). By integrating genome-scale DNA methylation data in the mouse hypothalamus at both the cell type–specific and region-specific levels, it was recently shown that early-postnatal increases in DNA methylation in neurons within the ARH are strongly associated with genes regulating neuronal differentiation (53), providing the first insights into epigenetic regulation within specific cell types within a specific hypothalamic nucleus. Although this represents important progress toward disentangling the epigenetic complexity of the hypothalamus, we still have far to go. For example, even within one hypothalamic nucleus (such as the ARH) there are various types of neurons (and various types of glia), each likely to be epigenetically specialized.

Regarding epigenetic studies in adipose tissue, an important but often neglected point is that WAT likewise constitutes a heterogeneous mixture of cell types. Many studies seem to assume that when genomic DNA is isolated from WAT, this represents predominantly adipocyte DNA. Indeed, it is sometimes stated that WAT is primarily composed of adipocytes (16). Given their large size, adipocytes do take up most of the volume in WAT, but in epigenetic studies the number of cells (i.e., the contribution to the total pool of DNA) is what matters. Widely various estimates of the cellular composition of WAT have been published over the past few decades. Just several years ago, however, two studies by the same group—using completely different methods—arrived at remarkably consistent estimates. In human WAT isolated surgically or by aspiration, adipocytes constituted only about 20% of the cells, quantitated either by multicolor flow cytometry following collagenase digestion (86) or by whole mount histology and triple-fluorescence staining (26). According to these solid data, stromal-vascular cells [composed mainly of mural cells, fibroblasts, and leukocytes (26)] are actually the predominant cell type in human WAT. Hence, to understand how epigenetic regulation of adipocytes (relative to their role as endocrine cells) contributes to energy balance regulation, it is inappropriate to study whole WAT [as many studies have done (see 99)].

Other than hypothalamus and WAT (and, as described below, the liver), a variety of tissues with important roles in body weight regulation remain largely unexplored from an epigenetic perspective. In addition to the widely recognized metabolic control of energy balance centered in the hypothalamus (27), it has been argued that cortico-limbic systems orchestrating cognition and reward may in fact contribute more importantly to obesity predisposition in the current environment of food abundance (8, 49). The involvement of some of these brain regions (including the neocortex, hippocampus, amygdala, nucleus accumbens, and ventral tegmental area) in energy balance is only recently being elaborated; future studies should consider the potential for individual epigenetic variation in these regions to affect cognitive control of food intake. Humans are born with a substantial depot of brown adipose tissue (30), which can expend excess calories to produce heat. Although most is lost by mid childhood, there is a recent resurgence of interest in the potential for residual brown adipose tissue to play a meaningful role in adult energy balance (37). Epigenetic mechanisms are likely involved in the process of shutting down the production of brown adipose tissue during childhood; understanding these may lead to effective interventions to prevent or treat human obesity. Comprising the largest single component of adult resting metabolic rate (108) and indispensable for physical activity, skeletal muscle plays a central role in energy balance. Interestingly, some of the earliest data supporting a role for DNA methylation in development was the demonstration that in vitro treatment of various (non-muscle) cells with the DNA-demethylating agent 5-azacytidine can induce differentiation toward contractile muscle cells (91). The classical transcription factor cascade that establishes myogenic fate is reinforced by epigenetic mechanisms at multiple levels, including DNA methylation, histone modifications, and noncoding RNA (79). Surprisingly few investigations, however, have considered whether epigenetic regulation (and dysregulation) in skeletal muscle contributes to obesity. Given its roles in endocrine regulation of satiety (e.g., ghrelin and glucagon-like peptide 1) and mediation of

nutrient uptake, the gut plays a central role in energy balance. However, despite a long-standing recognition of the likely importance of epigenetic mechanisms in gastrointestinal development (97), this remains a relatively understudied area. One interesting review (61) recently proposed the novel hypothesis that early diet affects the establishment of the gut microbiome, which in turn produces metabolic by-products (including folate and butyrate) that induce permanent changes in epigenetic regulation in the developing gut. Clearly, we have a great deal to learn about how epigenetic dysregulation in various organ systems contributes to body weight regulation.

DOES DEVELOPMENTAL PROGRAMMING OF HUMAN BODY WEIGHT REGULATION OCCUR VIA EPIGENETIC MECHANISMS?

Developmental programming of energy balance occurs when nutritional exposures during critical ontogenic periods affect the establishment of body weight regulatory mechanisms, with permanent consequences for obesity risk (31). Classic studies of survivors of the Dutch famine at the end of World War II (74) showed that famine exposure during prenatal or early postnatal development has opposing effects on risk of obesity in adulthood. More recently, comparisons of siblings born before and after maternal bariatric surgery have provided compelling evidence that maternal obesity before and during pregnancy promotes obesity in offspring (47, 82). These observations in humans are supported by vast experimental data in diverse animal models (64, 101). Induced alterations of epigenetic gene regulation are now widely viewed as the leading candidate mechanism to explain developmental programming of obesity (32).

Despite an accumulation of loosely supportive evidence, however, the data substantiating this hypothesis remain somewhat sparse. Various factors, including the complexity of the causal pathway, the long time period between initial exposure and adult outcomes, and the potential for reverse causality conspire to make it particularly challenging to test for mediation via epigenetic alterations. According to the mechanistic construct of metabolic imprinting proposed 15 years ago (101), if induced epigenetic alterations indeed serve as a primary imprint that mediates the persistent effects of early nutrition on later risk of obesity, they should (*a*) be present directly after the imprinting period and in adulthood and (*b*) be measurable in vitro (ideally, at the level of specific cell types). Additionally, in human observational studies, the potential for genetic confounding must be considered.

Several recent human studies have tested whether maternal obesity before and during pregnancy affects establishment of DNA methylation in the offspring. A study of 319 newborns (59) reported no correlations between maternal prepregnancy BMI and methylation at LINE-1 repetitive elements in either placenta or cord blood DNA. In a smaller study of 57 mother/infant dyads (29), maternal prepregnancy BMI was positively correlated with DNA methylation at the promoter of the peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PPARGC1A*) gene in umbilical cord of the infant. (Together, these studies suggest that such associations are likely to be locus specific.) More recently (25), DNA methylation was measured at several candidate genes in cord blood and chorionic villus of infants born to mothers with or without gestational diabetes mellitus (GDM). Interestingly, infants of mothers with GDM showed decreased methylation in both tissues at the imprinted mesoderm specific transcript (*MEST*) gene and the gene encoding glucocorticoid receptor [nuclear receptor subfamily 3, group C, member 1 (*NR3C1*)]. Although these positive findings are generally consistent with the hypothesis that maternal obesity (and/or GDM) affects epigenetic development in the human fetus, there are several caveats; epigenetic changes in placenta clearly cannot serve as a mechanism to sustain physiological changes in the soma, the persistence of the methylation changes in peripheral blood was not evaluated, and it is unclear if these indicate systemic effects that could plausibly affect body weight regulation. One

recent study indirectly examined the issue of effect persistence by prospectively relating neonatal DNA methylation in umbilical cord to measures of adiposity at age 9 (33). Methylation at a specific CpG site in the retinoid X receptor alpha (*RXRA*) gene was both inversely correlated with maternal carbohydrate intake in early pregnancy and positively correlated with the children's adiposity at age 9. Most impressively, similar results were obtained in a second independent cohort. Although these findings do suggest a causal pathway from maternal diet to offspring DNA methylation to offspring adiposity, it will be important to determine if *RXRA* methylation in umbilical cord at birth indeed predicts that in physiologically relevant somatic tissues later in life.

Given the ability to conduct controlled experiments and obtain tissues of greatest interest, animal model studies provide an important complement to the human data. Several years ago, in A^{vy} mice it was shown that maternal obesity causes transgenerational amplification of obesity (106); supplementation with a promethylation dietary supplement prevented this effect, suggesting potential mediation via induced alterations in DNA methylation. The protective effect of methyl supplementation was recently corroborated in a different model of maternal obesity. Adult offspring of dams fed a high-fat diet before and during pregnancy exhibited higher body weight, behavioral changes, and altered gene expression and DNA methylation in specific brain regions (12). Many of these changes were prevented if the mothers received a dietary methyl supplement in addition to the high-fat diet.

The rodent small litter (SL) model is a classic paradigm for studying persistent effects of early postnatal overnutrition (3); rats and mice suckled in SLs remain heavier and fatter throughout life. This model was recently used to test the hypothesis that the persistent effects of early postnatal overnutrition are mediated in part via induced DNA methylation changes in skeletal muscle (56). As an appropriate test for a primary imprint (101), mice were studied both directly after weaning on postnatal day 21 (P21) and in adulthood (P140). Skeletal muscle of SL mice had lower insulin receptor substrate 1 (*Irs1*) expression at both ages, and higher methylation at specific CpG sites in the *Irs1* promoter at P140 (56). However, there were no group differences in *Irs1* promoter methylation at P21; clearly, detecting epigenetic changes in adulthood does not necessarily indicate that they were induced directly by and persisted since the exposure. Another recent study of SL mice (52) tested whether their persistently altered body weight regulation is mediated by changes in DNA methylation in the hypothalamus. DNA methylation was measured at 24 candidate loci, both shortly after weaning (P25) and in adulthood (P180). Subtle but consistent alterations in DNA methylation were found at four loci at both ages, providing the first evidence that early postnatal overnutrition induces persistent epigenetic alterations in the hypothalamus. Since whole hypothalamus was studied, however, it is impossible to rule out that these changes reflect shifts in the cellular makeup of the hypothalamus rather than persistent differences within specific subpopulations of cells.

Recent studies suggest that maternal obesity during pregnancy may induce epigenetic changes affecting adipogenic potential in the offspring. In one study (9), offspring of overfed rat dams were cross-fostered at birth (so exposure to maternal obesity was limited to fetal development only). Both at P21 and P100, the stromal-vascular fraction from WAT of rats born to overfed dams exhibited increased adipogenic potential, and alterations in DNA methylation were detected in whole WAT at one age. Most interestingly, enhanced expression and reduced DNA methylation at the gene encoding the adipogenic transcription factor zinc finger protein 423 (*Zfp423*) (9) were independently corroborated in fetal offspring of mouse dams fed a high-fat diet (111), suggesting a potential causal pathway for how maternal obesity during pregnancy might enhance adipogenesis in the offspring.

Given the central role of the liver in lipid and carbohydrate metabolism, many investigators have examined whether induced epigenetic alterations in the liver may contribute to developmental

programming of obesity. In offspring of rats fed a high-fat diet during pregnancy and lactation, methylation of the cyclin-dependent kinase inhibitor 1A (*Cdkn1a*) promoter region was reduced in hepatic DNA at P2 but not at P27 (23). Another study compared wild-type (*a*/*a*) offspring born to either obese yellow or lean pseudoagouti A^{vy}/a dams (51). The male offspring of obese mothers had elevated body weight and deranged hepatic lipid metabolism into adulthood. Additionally, they exhibited changes in hepatic expression of some genes and DNA methylation changes at others. Given the lack of coordination between the methylation and expression changes, and that methylation was not also assessed at weaning, these data do not provide strong evidence that hepatic DNA methylation changes act as a primary imprint to perpetuate persistent effects of exposure to maternal obesity. Interestingly, however, among the most strongly upregulated genes (51) were the major urinary proteins, in which, more than 20 years ago, long-term consequences of early environment on epigenetic regulation were demonstrated in liver of mice derived from nucleocytoplasmic hybrids (76). A related study in a sheep model employed embryo transfer at seven days of gestation (E7) to study the persistent effects of embryonic exposure to maternal obesity during the periconceptional period only (66). Although there were widespread alterations in hepatic gene expression in the offspring at P90, no associated changes in DNA methylation were detected.

These studies (most of which were published in just the past few years) provide many meaningful insights. A conceptual weakness affecting them all, however is that there seems to be a tendency to neglect the fact that epigenetics is just one of several interrelated developmental mechanisms that potentially underlie developmental programming (101). In the murine hypothalamus, for example, the suckling period is a critical time for the establishment of DNA methylation patterns that distinguish diverse hypothalamic cell types (53). This same postnatal epoch, however, is also a critical period for formation of the neuronal projections from the ARH to other hypothalamic nuclei, establishing the "wiring" that is so critical for lifelong integrative regulation of energy balance (10). Any early postnatal nutritional exposure that can affect epigenetic development within specific cell types in the hypothalamus, therefore, will likely also cause permanent alterations to its neuroanatomic architecture, with consequent effects on intercellular signaling, hypothalamic function, and gene expression. One could postulate analogous interdependence pertaining to almost any organ system relevant to body weight regulation (**Figure 3**). For example, when examining epigenetic changes in adipocytes in response to maternal obesity, we must ask

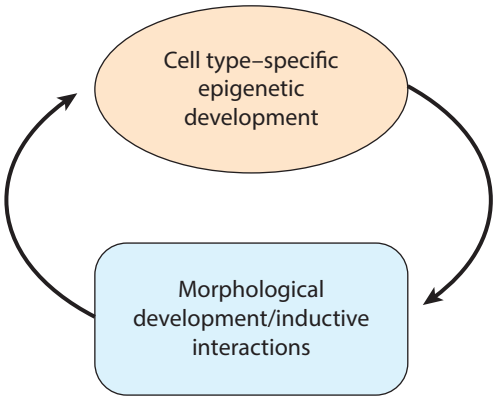

Figure 3

The interdependency of epigenetic and morphological development means that these processes must be studied in an integrated fashion.

whether maternal obesity also affects the establishment of the vascular network in WAT. Similarly, studies examining the effects of early postnatal overnutrition on epigenetic development in myocytes should consider whether the exposure coordinately affects myofiber formation and skeletal muscle innervation. These examples raise fascinating research questions. For example, is intracellular epigenetic development guided by inductive intercellular interactions or vice versa? Is cell type–specific epigenetic development instructive to or permissive of associated morphological development? Answering these complex questions will in many cases require collaborative interdisciplinary efforts. But without a doubt, understanding environmental effects on development will require epigenetic outcomes to be studied in the context of other developmental processes that are being coordinately impacted.

Another outstanding question pertaining to developmental programming of obesity is, what are the specific inputs that lead to alterations in epigenetic regulation? The dietary methyl donor supplementation paradigm (12, 17, 102) assumes that providing an excess supply of methyl groups leads to DNA hypermethylation at specific loci simply by mass action. In most of the epidemiologic and animal model studies relevant to developmental programming of obesity, however, the exposures are not obviously related to one-carbon metabolism, and inputs at the molecular level are largely unexplored. It has been proposed that the obesogenic effect of maternal obesity during fetal development is mediated by hyperglycemia (68). In a prospective study of women with and without GDM, however, the greatest predictor of high BMI in the offspring at age 9 was high maternal prepregnancy BMI; no effect of maternal GDM was found (13). Animal models afford opportunities to improve our understanding of exactly how effects of maternal obesity during pregnancy and early postnatal overnutrition are transduced to affect developmental epigenetics, but these must be carefully characterized to determine their appropriateness. For example, a recent study showed that offspring born to obese A^{vy}/a dams are growth restricted in utero (4); since infants born to obese women tend to have elevated birth weight (15), these data indicate that the A^{vy} mouse is not generally an apt model in which to understand programming effects of maternal obesity in humans.

Inextricably linked to the question of exactly how early environment affects developmental epigenetics is the question of when these effects occur. Metabolic imprinting occurs only during a specific ontogenic period of susceptibility—the critical window (101); defining the critical window for candidate phenomena will help narrow the list of potential underlying mechanisms. Ontogenic periods when epigenetic mechanisms are undergoing establishment or maturation comprise potential critical windows when environment can affect these processes (104). Various critical windows are implicated in developmental programming of body weight regulation, including early embryonic (66), fetal (4, 47), and early postnatal development (3, 87). Detailed understanding of the developmental changes in epigenetic regulation occurring in specific cell and tissue types during these epochs will help identify candidate genes and gene regions most susceptible to induced alterations in epigenetic regulation. Performing such studies in both animal models and humans, when possible, will be necessary to determine if critical windows for epigenetically mediated metabolic imprinting are conserved across species. For example, early postnatal life is a critical window for cell type–specific epigenetic development in the murine hypothalamus (52, 53). Based on neuroanatomic developmental milestones, this period in the mouse is generally believed to be comparable with late fetal development in the human brain (36). It is currently unknown, however, when cell type–specific epigenetic regulation is established in the human hypothalamus.

Overall, although substantial progress has been made in recent years, more sophisticated studies will be required to rigorously test the hypothesis that developmental programming of body weight regulation occurs via induced epigenetic alterations. In human studies, if epigenetic marks will be measured in peripheral blood (or other easily accessible tissues), correlational analyses should

initially be conducted (using cadaver tissues, for example) to determine if the epigenotype in peripheral tissues is correlated with that in tissues in which the gene is expressed and thought to regulate energy balance. In both human and animal model studies, quantitative measurement of epigenetic marks (such as DNA methylation) both directly after the exposure and later in life is critical to document a persistent epigenetic change (which may serve as a primary imprint) (101). To the extent possible, determining whether epigenetic changes within a tissue reflect induced alterations in intracellular epigenetic regulation or induced shifts in cellular composition will require studying separately isolated cellular subpopulations. Finally, programming mechanisms should be studied in a holistic framework integrating epigenetic and anatomic development.

DO EPIGENETIC MECHANISMS HAVE A GREATER IMPACT ON FOOD INTAKE OR ENERGY EXPENDITURE?

Positive energy balance requires excess energy intake and/or deficient energy expenditure. Although the debate has raged for some time as to which component is more important in human obesity, a compelling argument was recently put forth that, in the current environment of high food availability, increasing energy expenditure (via physical activity) is likely to be more effective at combatting obesity than reducing energy intake (39). For decades, studies of developmental programming of body weight regulation have focused almost exclusively on satiety and mechanisms regulating food intake (21, 44). Except for a handful of animal model studies (7, 45, 95), the potential for early environment to affect development of pathways that regulate physical activity has been largely neglected. Now, in two completely different animal models, studies published in the past year (4, 52) provide strong evidence that developmental programming of obesity may be mediated primarily via induced alterations in spontaneous physical activity. In the SL mouse model of early postnatal overnutrition, the persistently elevated weight and adiposity of SL mice were associated not with increased food intake but rather with decreased spontaneous physical activity (52). Likewise, the obesogenic effect in offspring of obese A^{vy}/a mice (106) was found to be mediated by a persistent decrease in spontaneous physical activity (4). Interestingly, in both models the effect on physical activity was specific to female offspring.

These results suggest a completely new area of research: exploring epigenetic and other developmental mechanisms involved in establishing individual lifelong propensity for physical activity. More than 15 years ago, Rowland proposed that each person has an "activity-stat," a physiological set point that regulates the individual's level of spontaneous physical activity (78). Unfortunately, compared to our understanding of central regulation of food intake, we still know relatively little about the neurobiology of voluntary physical activity (28). In support of the activity-stat construct, recent twin and association studies have documented a considerable genetic component to human physical activity (5, 20), with heritability estimates ranging from ~0.3 to 0.8. Because genetic factors clearly contribute to regulation of physical activity, it is likely that interindividual epigenetic variation does also, suggesting a testable causal pathway in which early environment affects epigenetic mechanisms involved in the establishment of the activity-stat, with lifelong consequences for risk of obesity. Whereas a few candidate genes have been identified in human genetic studies (20), this hypothesis initially will be most easily tested in animal models. Physical activity is typically dichotomized as either voluntary exercise or spontaneous physical activity (SPA). In rodents, running wheel activity is the universal model for voluntary exercise (28), and home cage activity is viewed as indicative of SPA (46). Although there are neurobiological differences in the regulation of SPA and voluntary exercise, common neural pathways contribute to both (46). SPA appears to be primarily regulated in the hypothalamus (by key molecular players including orexins, agouti-related peptide, and neuromedin U) (46). The lateral hypothalamus likewise plays a pivotal role in

the regulation of voluntary exercise (77), but dopaminergic signaling (particularly in the nucleus accumbens and hippocampus) is also important (28). It will be fascinating to determine whether early environmental influences on the epigenetic regulation of candidate genes within specialized brain regions leads to permanent alterations of the activity-stat in experimental animal models.

DOES EPIGENETIC INHERITANCE CONTRIBUTE TO TRANSGENERATIONAL PATTERNS OF OBESITY?

In addition to their definitive mitotic heritability, epigenetic marks can in some cases be meiotically heritable and hence conveyed from one generation to the next. Whereas transgenerational epigenetic inheritance is well documented in plants, fungi, and worms (35), evidence in mammals is relatively sparse. However, the phenomenon of genomic imprinting (in which alleles are epigenetically marked and expressed in a parent-of-origin-specific manner) (35) and transposon-associated epialleles such as A^{vy} (63) and $Axin^{Fused}$ (71) do provide clear examples of transgenerational epigenetic inheritance in mammals. [The reader is referred to several excellent reviews on this topic (18, 35, 73).] Accordingly, there has been growing interest in the possibility that transgenerational epigenetic inheritance is contributing to the epidemic of obesity and metabolic syndrome in developed countries (1, 110). Two points, however, merit special emphasis here. The first is that not all transgenerational phenomena are epigenetically mediated. Transgenerational epigenetic inheritance requires the transmission of epigenetic information across generations. Just as the gene was initially postulated as the fundamental basis for genetic inheritance, the sine qua non of epigenetic inheritance is a specific epigenetic mark that is present both in the parental germ line and in the offspring (**Figure 4*a***). Given the multiple biological safeguards against this (18), transgenerational effects of environmental exposures (particularly through the maternal lineage) can in many cases more parsimoniously be explained by recapitulation (i.e., the reoccurrence of an established pattern or characteristic). The most intuitive example is transgenerational recapitulation of language (**Figure 4*b***); we generally speak the same language as our parents not because of

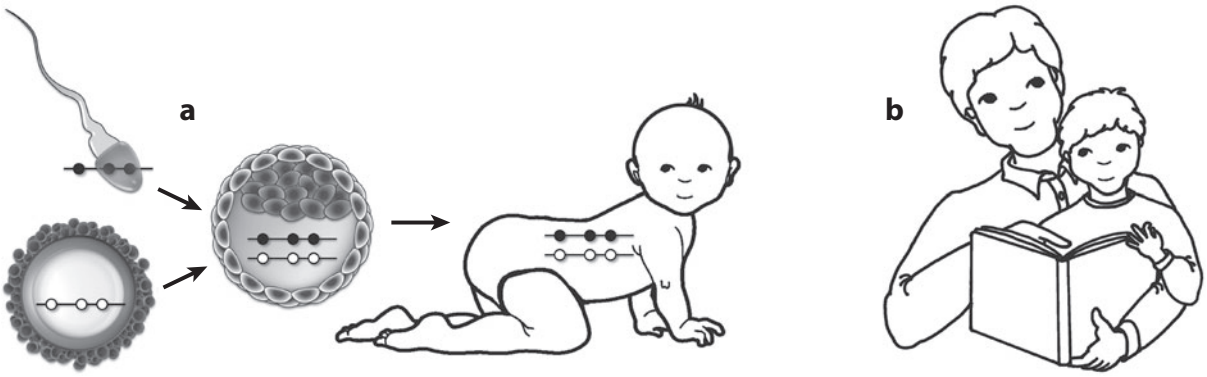

Figure 4

Transgenerational epigenetic inheritance must be distinguished from recapitulation. (*a*) Documenting epigenetic inheritance requires evidence that a specific epigenetic mark is actually inherited from the parental germ line to the offspring. Here, hypermethylation (*black*) of specific CpG sites in the haploid sperm genome is present both in the early embryo and subsequently in somatic tissues of the offspring. (In this example, the sperm hypermethylation is intended to indicate an environmentally induced epigenetic change, not the result of genomic imprinting or allele-specific methylation.) (*b*) Recapitulation (for example, the early acquisition of language) describes transgenerational reoccurrence of phenotypic traits and is distinct from transgenerational epigenetic inheritance. [Adapted from Elementary CORE Academy Clip Art (available in the public domain: **http://www.usu.edu/coreacademy/Materials/ClipArt**)].

genetic or epigenetic inheritance, but rather because of recapitulation. In rats, maternal caregiving behavior affects offspring postnatal development in a nongenetic fashion; female offspring suckled by more nurturing mothers (i.e., more licking, grooming, and nursing) become nurturing mothers themselves (107). Interestingly, this is associated with epigenetic changes in the hippocampus of the offspring, suggesting epigenetically mediated transgenerational recapitulation of behavior but not epigenetic inheritance. In another relevant example, transgenerational amplification of obesity occurs when A^{vy} is passed through the female germ line (106). This does not occur via epigenetic inheritance at A^{vy} (105); rather, female offspring of A^{vy} dams are "programmed" in utero for reduced physical activity and elevated adiposity (4). Hence, although potentially involving epigenetic mechanisms within each generation, this feed-forward transgenerational cycle does not constitute epigenetic inheritance.

Transgenerational effects transmitted through the male germ line are more often viewed as providing evidence of epigenetic inheritance. Indeed, several molecular mechanisms including DNA methylation, various proteins (including prions), and diverse classes of RNA molecules could plausibly mediate male germ-line epigenetic inheritance (35). However, the second point deserving emphasis is that in outbred populations (such as humans) it is possible that paternal environment selects for specific haploid sperm genomes (73). The altered physiological milieu of an obese father, for example, could drive clonal selection during sperm development (or even favor specific sperm genotypes in terms of motility or other factors affecting fertility). In this regard, it is noteworthy that many animal models purporting paternal transgenerational epigenetic inheritance of metabolic phenotypes have utilized either outbred (11, 65, 93) or hybrid strains (24). It will be important to test whether these effects are also observed in inbred populations of animals. Moreover, the widespread availability of genome-wide sequencing now makes it possible to directly test the sperm selection hypothesis. Given the complexity of these issues, a recent expert opinion piece (35) concluded, "there are not yet any clear-cut studies that would unambiguously demonstrate the transgenerational epigenetic inheritance of environmentally induced effects" (p. 232). Hence, although plausible, much work will be required to convincingly demonstrate that epigenetic inheritance plays an important role in human obesity.

CONCLUSIONS

The exquisite system for regulation of energy balance is established just once in each individual's life. In addition to the instructions laid down in the genetic blueprint, environmental influences during critical ontogenic periods determine the outcome of this process, with permanent consequences for body weight regulation. Given their importance in orchestrating and stabilizing cellular differentiation, epigenetic mechanisms must play a central role in maintaining the various physiological set points that conspire to promote obesity in our current environment of food surfeit. Despite the complexity of the compounded research obstacles elaborated here, there is considerable reason for optimism. Advances in epigenetic reprogramming (89), for example, illustrate the enormous potential to manipulate epigenetic processes to shape and potentially reshape developmental outcome. Although the challenges are daunting, developing a thorough understanding of the epigenetic mechanisms underlying energy balance regulation should eventually offer outstanding opportunities to devise effective approaches for the prevention and treatment of human obesity.

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