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Developmental Origins of Common Disease: Epigenetic Contributions to Obesity

Maya Kappil¹, Robert O. Wright^{1,2}, and Alison P. Sanders¹

Alison P. Sanders: alison.sanders@mssm.edu

¹Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029

²Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY 10029

Abstract

The perinatal period is a window of susceptibility for later life disease. Recent epigenetic findings are beginning to increase our understanding of the molecular mechanisms that may contribute to the programming of obesity. This review summarizes recent evidence that supports the role of epigenetically mediated early life programming in the later onset of obesity. Establishing such links between environmental exposures and modifiable molecular changes ultimately holds promise to inform interventional efforts toward alleviating the environmentally mediated onset of obesity.

Keywords

obesity; epigenetics; developmental programming

INTRODUCTION

The prevalence of obesity has been steadily increasing worldwide over the past 50 years (25). Obesity is characterized by a disruption in energy balance regulation that results in an excess accumulation of body fat, and because it is a risk factor for a host of additional chronic health conditions, including type 2 diabetes, hypertension, and cardiovascular disease, its increasing prevalence poses a major public health concern (10, 33, 37). Despite more than \$100 billion invested annually toward treatment-related expenses, obesity remains among the most common, costly, and preventable chronic health conditions worldwide (7).

The discovery of a vast number of key genetic factors has enhanced our etiologic understanding of obesity. However, monogenic mutations are extremely rare, and common variants identified in genome-wide association studies account for only a minor fraction of risk (<10%), indicating that genetics alone cannot explain the rise in obesity prevalence (1, 23). It is also clear that weight gain requires environmental input (i.e., caloric intake) and

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that genetics can affect metabolism or appetite, but such genetic mechanisms can never be independent; they must occur in a particular context of food access and consumption, and this context is not constant across populations or time. Studies have also suggested that additional changes in lifestyle and behavioral factors over time—including a trend toward a sedentary lifestyle, altered reproductive patterns, and a varying microbiome—contribute to the onset of obesity (25). However, not all individuals with predisposing genetic and lifestyle characteristics go on to develop the associated obesity phenotype (14). This interindividual variability in sensitivity to genetic and lifestyle factors may be triggered early in development.

Observations that impaired fetal development (quantified as reduced infant birth weight) is associated with an increased risk for heart disease, hypertension, diabetes, and stroke demonstrated that early life experiences can be important factors in later disease onset (4, 5). This concept was more broadly adapted as the developmental origins of health and disease (DOHaD) hypothesis (9, 13). The DOHaD hypothesis postulates that environmental stimuli during critical windows of susceptibility can elicit structural and functional changes in the organs and tissues of the developing fetus, inducing lifelong alterations in an individual's metabolic profile (3).

Several hypotheses have been proposed that link suboptimal intrauterine conditions with the later onset of common chronic health outcomes, including obesity. Most prominent among these is the predictive adaptive response hypothesis (12), which posits that the intrauterine environment provides cues indicative of the anticipated postnatal environment that then inform the developmental trajectory. If the predicted and actual encountered postnatal environments differ substantially, the developmental trajectory may be maladaptive. This hypothesis most clearly fits the observations linking intrauterine growth restriction (IUGR) and maternal undernutrition to the later onset of obesity (29, 30). Under such circumstances, the nutrient-deficient intrauterine environment signals an impoverished external state and induces metabolic programming in preparation for the anticipated undernourished postnatal environment; if the actual postnatal environment is then energy rich, this adopted metabolic trajectory ultimately increases susceptibility to type 2 diabetes, cardiovascular disease, and obesity. The hypothesized predictive adaptive response is well suited for relating intrauterine nutritional access to obesity, but its applicability to more recent findings linking intrauterine environmental pollutant exposures to obesity is less clear.

The precise mechanism through which developmental programming occurs is not fully understood; however, epigenetic factors are considered to play a critical role (39). Epigenetics, broadly defined as the regulation of gene expression independent of DNA sequence variation, can explain developmental programming. Epigenetic modifications, including methylation at cytosine-phosphate-guanine dinucleotide (CpG) sites, histone modifications, and noncoding RNAs, are mitotically heritable factors that are set early in development to determine cell lineage-specific gene expression patterns, and they have the potential to be modified in response to environmental perturbations. Because of their ability to mediate physiologic changes resulting from gene-environment interactions, epigenetic modifications are commonly measured endpoints in studies assessing perturbations in developmental programming (32, 39).

Here, we review recent evidence suggesting that the early life environment influences the later onset of obesity through epigenetically mediated pathways. We identified 18 studies that examined the epigenetic bases for the developmental origins of obesity; of these studies, 2 were performed in human populations and 16 were animal model in vivo or ex vivo studies. The obesity-related early life environment conditions investigated in the reviewed articles were parental obesity, diet, or dietary/nutritional supplementation (10 studies); IUGR (3 studies); and environmental pollutants (5 studies). We highlight gaps in current research efforts and provide recommendations for future studies.

METHODS

We conducted a literature search to identify recent epidemiologic studies that examined epigenetic factors involved in developmental programming of obesity using the following search terms: (child* OR infan* OR post-natal OR postnatal OR pre-natal OR prenatal OR fetal OR pregnan* OR in utero) AND (epigenetics OR microRNAs OR DNA methylation OR histone modification OR long interspersed nucleotide elements OR chromatin assembly and disassembly) AND (obesity). We used PubMed and Web of Science search engines and restricted the results to peer-reviewed original research published in English between 2010 and October 2015. We excluded studies that did not study a developmental period (for example, those that reported only maternal conditions), report on obesity as the outcome, or measure an epigenetic modification as well as those in which the outcome preceded the epigenetic change. As noted above, a total of 18 studies met our criteria (Table 1).

PARENTAL OBESITY, DIET, AND SUPPLEMENTATION

We identified 10 studies of parental obesity, diet, or dietary/nutritional supplementation, 9 of which examined maternal obesity or nutrition (6, 11, 15, 19, 21, 31, 40, 41, 43) and 1 of which examined paternal obesity (26). We further categorized these studies based on the type of in utero exposure investigated: nonspecified parental obesity, diet-induced parental obesity, reduced-activity-induced parental obesity, and prenatal dietary supplementation. These studies emphasized the maternal rather than the paternal impact on the developmental trajectory of the offspring. This bias likely reflects the inherent disparity in parental investment in fetal development, given that the mother's participation spans the entire gestational period, whereas the father's is limited to fertilization.

Nonspecified Parental Obesity

The correlation between maternal and offspring obesity is well established (28). Although genetic factors likely play a role in transmitting the transgenerational risk of obesity, several studies have also shown increased adiposity of offspring born to mothers before surgical weight loss compared with their siblings born after the surgery (2, 27). This suggests that maternal obesity also has implications for the subsequent onset of offspring obesity as an in utero exposure. This is of particular concern given the increasing prevalence of obesity: Based on a multistate survey of ethnically diverse women conducted in 2002 and 2003, Kim et al. (20) projected that approximately 22% of child-bearing-age women enter pregnancy with a body mass index (BMI) of 30 kg/m² or higher, the World Health Organization definition of obesity.

We identified a single study that assessed epigenetic factors as potential mediators linking non-specified maternal obesity (i.e., obesity that may have resulted from diet and/or reduced activity, but whose exact cause was unknown) to offspring obesity in an epidemiologic setting (31). The Avon Longitudinal Study of Parents and Children, a prospective cohort study of 1,018 mother-child dyads in the United Kingdom, examined the impact of maternal prepregnancy BMI on childhood BMI via CpG methylation. The authors showed that maternal prepregnancy BMI was indeed positively associated with the BMI of the children (mean age of 7.5 years). Epigenome-wide assessment of cord blood methylation using the Illumina 450K BeadChip array revealed two CpG sites [*coiled-coil domain containing 112* (*CCDC112*) and *mucolin 3* (*MCOLN3*)] that were differentially methylated in the offspring of obese mothers compared with those of normal-weight mothers. Interestingly, the magnitude of the association between maternal obesity and cord blood methylation levels was greater than the magnitude of the association between paternal obesity and cord blood methylation levels, highlighting the role of the intrauterine environment in the transmission of obesity. Comparison of offspring methylation at three time points (birth, childhood, and adolescence) showed no overlap in the top hits differentially methylated by maternal adiposity. Furthermore, the level of differential methylation in CpG sites related to maternal prepregnancy BMI identified in cord blood diminished in samples collected at subsequent time points in childhood and adolescence, suggesting postnatal resolution. The authors did observe a trend suggesting a correlation between the methylation status of cord blood CpG sites related to maternal obesity and childhood/adolescent obesity (31).

Diet-Induced Parental Obesity

A single epidemiologic study (15) and six animal model in vivo and ex vivo studies (6, 11, 21, 26, 41, 43) have assessed the effects of diet-induced maternal obesity on offspring obesity. Four studies assessed changes in methylation patterns (6, 11, 15, 21), one additionally assessed histone modifications (43), and two assessed microRNA (miRNA) expression (26, 41).

The Princess Anne Hospital prospective cohort study conducted in the United Kingdom examined the impact of maternal carbohydrate intake on childhood obesity at age 9 (15). The authors assessed methylation at five candidate genes [*retinoid X receptor, alpha* (*RXRA*); *endothelial nitric oxide synthase* (*eNOS*); *superoxide dismutase 1* (*SOD1*); *interleukin 8* (*IL8*); and *phosphoinositide 3-kinase catalytic subunit delta* (*PI3KCD*)], encompassing 68 CpGs from the umbilical cord tissue DNA of 78 healthy neonates. Using adjusted linear regression models, they observed positive associations with child adiposity and obesity for *RXRA* and *eNOS* umbilical cord CpG methylation, and *RXRA* umbilical cord CpG methylation was also inversely related to low to moderate maternal carbohydrate intake. Furthermore, the association between *RXRA* umbilical cord CpG methylation and child adiposity was replicated in an independent cohort. This study demonstrates the feasibility of assessing an epigenetic link between prenatal and postnatal environments in a human population. Moreover, in lieu of access to target tissues, the ability to detect differences in adipogenesis-related genes in surrogate biospecimens, including umbilical cord samples, is particularly relevant in determining the feasibility of assessing this paradigm in human populations.

Given the complexity of studying human populations, the remaining studies that examined maternal diet were conducted in animal models. In a study using a rat model, Borengasser et al. (6) fed dams either normal chow (NC) or a high-fat diet (HFD) for 3 weeks prior to mating to examine methylation and expression level differences in adipose tissue. Obesity-exposed male offspring had higher body weight and fat mass at weaning as well as elevated levels of insulin, leptin, and resistin. White adipose tissue (WAT) collected from the offspring showed differential expression of lipogenic genes, indicating heightened adipogenic potential of obesity-exposed male offspring. The authors also identified obesity-associated differentially methylated regions (DMRs) in CpG island shores (rather than promoters) at key adipogenic transcription factor genes in WAT, including *CCAAT/enhancer binding protein β* (*C/ebp- β*), *zinc-finger protein 423* (*Zfp423*), and *peroxisome proliferator-activated receptor γ* (*Ppar γ*). They observed concordant hypomethylation and increased mRNA expression of *Zfp423* in an ex vivo culture of WAT-derived stromal vascular cells. Additional interrogation of molecules in the Wnt signaling pathway indicated that maternal obesity may regulate offspring adipogenesis via a pathway in which enhancer of zeste homolog 2 (*Ezh2*) represses Wnt signaling by downregulating Wnt1-inducible signaling pathway protein 2 (*Wisp2*) (6). This study did not evaluate female offspring.

A similar study used a mouse model to examine the role of *Zfp423*, which encodes a zinc-finger protein involved in adipocyte differentiation (17), as a potential mediator between diet-induced maternal obesity and adipogenic potential during fetal development (43). Yang et al. (43) fed dams either NC or HFD for 8 weeks prior to mating, then collected fetal tissue at gestational day 14.5 to examine *Zfp423*-related expression, methylation, and histone modifications. Preconception maternal HFD resulted in enhanced *Zfp423* expression, decreased methylation at a *Zfp423*-associated CpG shore, and alterations in *Zfp423*-related histone modifications in offspring. Corresponding with differential expression of *Zfp423*, greater adipogenic differentiation was observed in cell cultures derived from HFD-exposed fetal tissue. Furthermore, knockdown of *Zfp423* reduced the adipogenic potential of HFD-exposed cells, and overexpression of *Zfp423* enhanced the adipogenic potential of unexposed cells. The observed differences in preadipocyte commitment and adipogenic differentiation in offspring differentially exposed to HFD at such early stages of development highlights the potency of this exposure in programming obesity (43).

Using a sheep model, Yan et al. (41) examined the impact of in utero HFD exposure (60 days prior to conception to gestational day 75) on miRNA content in fetal skeletal muscle. Out of 155 expressed miRNAs in the sheep fetal muscle, let-7g and miR-376 were downregulated and miR-381 was upregulated in the HFD-exposed fetuses. The use of a quantitative real-time polymerase chain reaction (qPCR) confirmed that the putative let-7g mRNA targets *tumor necrosis factor receptor superfamily member 4* (*Tnfrsf4*) and *follistatin* (*Fst*) and the inflammatory response genes *tumor necrosis factor α* (*Tnfa*), *Il6*, and *Toll-like receptor 4* (*Tlr4*) were upregulated in the skeletal muscle of HFD-exposed fetuses. Induced overexpression of let-7g in a mesenchymal stem cell line led to the downregulation of the let-7g target *Fst* and the inflammatory response gene *Tnfa*, reduced cell proliferation, and reduced expression of the adipogenes *Ppar γ* and *C/ebp- α* concomitant with reduced formation of adipocytes. The authors posited that because both muscle cells and adipocytes develop from mesenchymal stem cells, in utero exposure to obesity may enhance

adipogenesis at the expense of myogenesis in the cells, and that this process may be partially mediated through let-7g-induced reduction of *Fst*.

A mouse model study examined the impact of multigenerational HFD-induced maternal obesity exposure, analyzing gene expression and DNA methylation in adipose tissue of HFD-exposed second filial generation (F2) female mouse offspring. In this study, Ding et al. (11) fed F0 dams either NC or HFD for 1 month after weaning and then mated them with NC-fed males. They then continued to feed the F1 female offspring the selected maternal diet and mated them with NC-fed males 2 months after weaning. HFD-exposed female offspring had higher body weight, visceral adipose tissue mass, serum insulin, and leptin levels than unexposed offspring. Histologic analysis of adipose tissue from exposed offspring also had elevated levels of inflammatory response markers. These changes in the metabolic and adipogenic profile were particularly notable in the F2 generation. Pathway analysis of differentially expressed genes based on transcriptome-wide analysis of exposed and unexposed offspring underscored the role of genes involved in inflammatory pathways and lipoprotein response. On the basis of microarray analysis, the authors selected three candidate genes [*Tlr1*, *Tlr2*, and *linker for activation of T cells (Lat)*] for further methylation analysis. The *Tlr1* and *Tlr2* promoters were hypomethylated in the HFD-exposed F2 group compared with the unexposed F2 group. Additionally, the methylation levels of these promoters decreased across the three generations within the exposed group but remained stable across the three generations within the unexposed group. Finally, expression of *DNA (cytosine-5)-methyltransferase 1 (Dnmt1)* was higher in the exposed group compared with the unexposed group across all three generations. The expression levels of *Dnmt3a* exhibited a similar trend. The authors posited that the elevation in methyltransferase activity suggests that continuous HFD exposure induces genome-wide hypermethylation in addition to the observed locus-specific hypomethylation; however, global methylation levels were not measured to verify this hypothesis. Interestingly, at least one other study has reported elevation in methyltransferase activity following ancestral environmental exposure (22).

Two studies evaluated physical activity interventions and their potential to alleviate obesity-induced adverse phenotypes in offspring, primarily via markers of metabolic dysfunction (21, 26). In a mouse model study, Laker et al. (21) exposed dams to diet-activity interventions to assess the impact on the methylation and expression status of *peroxisome proliferator-activated receptor gamma coactivator 1a (Pgc-1a)*, a gene previously shown to be hypermethylated in the skeletal tissue of patients with type 2 diabetes and in the skeletal muscle and liver tissue of offspring. The chosen diet-activity interventions included NC with sedentary activity, HFD with sedentary activity, and HFD with exercise (voluntary wheel running); the interventions were implemented starting 6 weeks before mating and lasted throughout gestation. Maternal exercise attenuated maternal diet-induced metabolic dysfunction (glucose intolerance and insulin resistance) in the offspring assessed at 6 months; however, this attenuation did not persist into adulthood. Maternal exercise attenuated maternal HFD-induced hypermethylation of the *Pgc-1a* promoter in the skeletal muscle but not the liver tissue of the offspring, and these differences were sustained until adulthood (12 months). Although *Pgc-1a* expression levels were not correlated with expression at birth, a trend toward correlation was observable in adulthood. Interestingly, maternal exercise alone did not affect either *Pgc-1a* promoter methylation levels or glucose

tolerance, suggesting that the benefits of maternal exercise are specific to the condition of maternal HFD.

The second exercise intervention mouse model study utilized diet-activity interventions to determine the potential role of paternal programming in metabolic dysfunction by assessing the miRNA content in sperm (26). In this study, McPherson et al. (26) fed male mice either NC or HFD for an initial 9-week period. For another 9-week period, they then continued the NC-fed mice on the same diet and allocated the HFD-fed male mice to one of four intervention groups: HFD with no exercise, NC with no exercise, HFD with exercise (swimming), and NC with exercise (swimming). In addition to having a higher body weight at weaning, female offspring of HFD-fed male mice presented with features of metabolic syndrome, including increased triglycerides and insulin resistance. Both the diet and exercise interventions attenuated this difference, but the exercise intervention had the greatest effect. Out of the selected candidate X-linked miRNAs, the authors observed differential abundances of four miRNAs (miR-503, miR-456b-5p, miR-542-3p, and miR-652) in the sperm of HFD-fed fathers compared with those of NC-fed fathers. Again, although both interventions restored sperm miRNA abundance to a certain extent, the exercise intervention group showed the greatest normalization. These findings imply that the paternal diet, and potentially other exposures, can be transmitted to offspring, even though the paternal influence on fetus formation is restricted to genetic and epigenetic information conveyed at fertilization.

Reduced-Activity-Induced Parental Obesity

In addition to overnutrition, a variety of other factors can drive parental obesity, including reduced physical activity. In an exercise intervention mouse model study, Wei et al. (40) exposed dams to standard laboratory conditions (mimicking a sedentary human lifestyle) or to short-term (2 weeks) or long-term (4 weeks) voluntary-activity-promoting enriched environments (a large space with toys and wheels) in order to assess the impact of these environments on the methylation profiles of maternal oocytes, blastocysts at embryonic day 3.5, and offspring liver tissue. Long-term exposure to an enriched environment resulted in maternal weight loss and an overall improvement in the offspring's metabolic profiles, including reduced fat accumulation, reduced lipid levels, and improved glucose tolerance and insulin sensitivity. Pathway analysis of offspring gene expression from liver tissue indicated downregulation of genes related to lipid and cholesterol biosynthesis among offspring exposed to the enriched environment compared with unexposed offspring. Accordingly, these genes also showed promoter hypermethylation, and promoter hypermethylation of *3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr)* persisted in the F2 generation. Recapitulation of these phenotypes through embryo transplantation experiments of oocytes derived from animals either exposed or not exposed to an enriched environment indicated that the maternal metabolic profile may influence the offspring through alterations detectable in oocytes. Furthermore, the authors observed hypermethylation of the promoters of *Hmgcr* and *lanosterol synthase (Lss)* in F1 oocytes, which is consistent with the hypermethylation of these promoters observed in F2 livers and suggests maternal transmission. These differences were not present after short-term exposure to the enriched environment.

Prenatal Dietary Supplementation

Aspects of maternal nutrition other than high energy consumption, including dietary supplementation during pregnancy, may contribute to offspring metabolic dysregulation. Although periconceptional folic acid supplementation lowers the risk of birth defects, excess levels of folate (a participant in one-carbon metabolism) may exert adverse effects on the developing fetus through aberrant DNA methylation. We identified a single mouse model study that compared varying levels of pregestational folate supplementation followed by postweaning HFD in order to determine the effect on global methylation patterns in male offspring WAT (19). In this study, Huang et al. (19) fed dams either a control (2 mg/kg), recommended (5 mg/kg), or high (40 mg/kg) dose of folic acid starting 2 weeks before conception and continuing throughout the pregnancy, and fed the resulting male offspring HFD from postnatal week 7 until week 15. Offspring exposed to either recommended or high levels of folic acid showed no differences in body weight or body composition at baseline compared with controls. Following 8 weeks of HFD feeding, offspring exposed to high levels of folic acid had marginally greater body weight and adiposity index, greater glucose intolerance, and greater insulin resistance than unexposed offspring. Interestingly, before the start of the HFD diet, the group exposed to the high dose exhibited a decrease in WAT expression and amount of adiponectin (a protein encoded by *Adipoq*, which has been implicated in insulin sensitivity) compared with the unexposed group. Finally, the group exposed to the high dose also exhibited an increase in global methylation levels compared with the unexposed group, although the implications of this difference are unclear.

INTRAUTERINE GROWTH RESTRICTION

The relationship between maternal nutritional status and childhood obesity has been posited to follow a U-shaped curve, whereby both prenatal undernutrition and overnutrition trigger metabolic dysregulation of the offspring. In the case of the former, metabolic dysregulation of the offspring may result from a mismatch between a thrifty phenotype programmed in the undernourished intrauterine environment and an affluent postnatal environment. Restricted fetal access to substrates may result from maternal undernutrition or abnormal utero-placental function. Although food restriction is the most extensively studied mode of IUGR, utero-placental insufficiency is likely to be a more prevalent route underlying IUGR in industrialized countries. We identified three studies that examined epigenetic shifts associated with the IUGR and subsequent obesity (8, 16, 44).

Using a rat model of protein-restriction-induced IUGR, the most extensively studied type of IUGR, Zeng et al. (44) assessed *Pgc-1α* and *glucose transporter type 4 (Glut4)* methylation levels in the skeletal muscle of female offspring. The authors fed dams either a normal (20% protein) or low-protein (8% protein) diet throughout pregnancy to induce IUGR. Although the birth weights of IUGR offspring were considerably lower than those of control offspring, the IUGR offspring showed accelerated weight gain. At 12 weeks of age, the IUGR rats were significantly more obese and more prone to display markers indicative of insulin resistance compared with control offspring. The authors examined the expression of selected insulin resistance-related candidate genes [*insulin receptor (Ir)*, *insulin receptor substrate 1 (Irs-1)*, *Pgc-1α*, and *Glut4*] and found that *Pgc-1α* and *Glut4* expression was

downregulated. Further methylation analysis of these loci indicated elevated *Pgc-1a* promoter methylation levels in the skeletal muscle of IUGR offspring.

Claycombe et al. (8) examined the combinatorial impact of in utero undernourishment and postweaning HFD on the methylation and expression of *insulin-like growth factor 2 (Igf2)*, an imprinted gene involved in growth promotion, in the adipose tissue of obesity-prone male rat offspring. The authors fed dams either a normal (20% protein) or low-protein (8% protein) diet from 3 weeks before conception through lactation, then fed the postweaning offspring either NC or HFD for 12 weeks. Male offspring exposed to postweaning HFD following undernourishment exhibited increased *Igf2/H19* imprint control region methylation and *Igf2* expression, enhanced adipose tissue growth, and reduced insulin sensitivity compared with male rats exposed only to postweaning HFD. However, they did not observe any interaction, as the combined effect did not exceed the main effect of prenatal undernourishment or postnatal HFD (8).

Because the germline of the developing fetus is also exposed to the intrauterine environment, the programming induced by in utero exposures can extend into the F2 generation. To examine the multigenerational impact of IUGR and possible recovery through dietary supplementation, Goodspeed et al. (16) used a utero-placental insufficiency-induced IUGR rat model to examine the promoter methylation levels of *Igf1*, a driver of gestational and postnatal growth, in the livers of F2 offspring. The authors performed bilateral uterine ligations in pregnant female F0 rats on embryonic day 19, then fed the F1 offspring either NC or an enriched-chow diet. Both NC and enriched-chow F1 offspring were then mated to generate the following mating pairs (where the first of each pair is maternal and the second is paternal): non-IUGR $\times$ non-IUGR, non-IUGR $\times$ IUGR, IUGR $\times$ non-IUGR, and IUGR $\times$ IUGR. Similarly to F1 offspring, F2 rats were underweight at birth and obese by adulthood. F2 rats also had increased fat mass, elevated levels of serum triglycerides and fatty acids, and insulin resistance. Additionally, promoter methylation of *Igf1* was elevated in the liver of IUGR-exposed F2 rats. Following essential-nutrient methyl-donor dietary supplementation from postweaning F1 through postweaning F2 offspring, the higher body weight and fat mass and dyslipidemia evident among F2 offspring fed an unsupplemented diet were reduced, with a more pronounced effect in male offspring. However, most of these findings reflect an F2 lineage derived from parents that were both exposed to IUGR.

EFFECT OF ENVIRONMENTAL POLLUTANTS ON OBESITY

An increasing number of studies are examining the role of environmental pollutants in the programming of later life disease. We identified five studies that examined the effects of in utero exposure to polycyclic aromatic hydrocarbons (PAHs) (42), bisphenol A (BPA) (35), dichlorodiphenyltrichloroethane (DDT) (34), methoxychlor (24), or the jet fuel JP-8 (36) on obesity phenotypes. Each of the studies provides preliminary evidence of the adipogenic potential of xenobiotic exposures to alter the epigenome in utero and contribute to later life obesity.

Polycyclic Aromatic Hydrocarbons

A mouse model study evaluated the impact of prenatal PAH exposure, a family of air pollutants produced from incomplete combustion of organic matter, on promoter methylation levels of *Pparγ* in offspring WAT. Yan et al. (42) exposed pregnant dams to aerosolized PAHs at a final concentration of 7.29 ng/m³ from gestational days 1–3 through gestational days 19–21 or until delivery. Exposed F1 and F2 offspring showed accelerated weight gain compared with controls. These offspring also showed an increase in WAT fat mass and a larger adipocyte size, albeit in a sex- and tissue-specific manner. WAT and brown adipose tissue of exposed offspring also showed differential expression of adipocyte differentiation-promoting genes, including *Pparγ*, *Clebp-α*, *Adipoq*, *cyclooxygenase 2* (*Cox-2*), and *Fas cell surface death receptor* (*Fas*). Exposed F1 and F2 offspring exhibited reduced WAT methylation of *Pparγ* promoter CpG sites, and the methylation status of these sites was negatively correlated with *Pparγ* gene expression and body weight.

Bisphenol A

A mouse model study evaluated the multigenerational effects of prenatal exposure to low and high doses of BPA on the methylation status of the *Igf2* DMR in F1 and F2 embryos. Susiarjo et al. (35) exposed dams to either NC, a low dose of BPA (10 µg/kg per day), or a high dose of BPA (10 mg/kg per day) from 2 weeks before conception through lactation. F1 offspring exposed to low or high BPA doses had higher body weight and fat mass, greater glucose intolerance, and altered insulin secretion compared with unexposed offspring. However, this effect was restricted to male offspring. F2 male offspring exposed to high BPA doses also had different metabolic phenotypes than unexposed male offspring. The authors also analyzed *Igf2* expression and methylation in embryos derived from a cross between BPA-exposed F2 females and unexposed males. Embryos exposed to high BPA doses showed elevated *Igf2* expression and site-specific methylation compared with unexposed F2 embryos. Finally, male mice engineered to overexpress *Igf2* showed increased body weight, increased body fat content, and glucose intolerance compared with wild-type male mice, linking *Igf2* overexpression to metabolic dysregulation.

Dichlorodiphenyltrichloroethane

The use of DDT as an insecticide has been banned in the United States since the 1970s; however, it is unclear whether ancestral exposure to this and other pollutants may continue to exert a transgenerational effect. In a transgenerational rat model study, Skinner et al. (34) administered F0 dams dimethyl sulfoxide (vehicle control), a lower dose of DDT (25 mg/kg per day), or a higher dose of DDT (50 mg/kg per day) during gestational days 8–14 to examine the impact of in utero DDT exposure on the methylation profile of F3 sperm. Offspring exposed to the lower or higher dose showed an increase in obesity compared with unexposed offspring, but this difference was observable only in the F3 generation. To determine the presence of parent-specific transmission, the authors assessed metabolic outcomes in F4 offspring derived from either outcrosses of wild-type females with F3 males exposed to the lower DDT dose or outcrosses of wild-type males with F3 females exposed to the lower DDT dose. Transmission of the DDT-associated metabolic outcomes was observed in male offspring derived from F3 exposed females, but the transmission in female offspring

was less clear. Methylation analysis comparing F3 control sperm with F3 sperm exposed to the lower DDT dose revealed 39 DMRs. Further analysis of 3 of these DMRs confirmed the differentially methylated state of *tubulin beta class 3 (Tubb3)*, *coactivator-associated arginine methyltransferase 1 (Carm1)*, and *solute carrier family 4 (Slc4a4)* (34).

Methoxychlor

Following the ban on DDT, other pesticides entered the market to take its place, including methoxychlor. Methoxychlor is a synthetic organochlorine used primarily on crops and livestock to control pests; it was banned in the United States in 2003, and its latent effects are still unknown. In a transgenerational rat model study, Manikkam et al. (24) administered F0 dams either a vehicle or methoxychlor (200 mg/kg per day) during gestational days 8–14 in order to examine the impact of in utero methoxychlor exposure on the methylation profile of F3 sperm. F3 females showed an increased incidence of obesity. F3 outcross experiments indicated that male obesity was transmitted through the female germline, whereas female obesity did not appear to be transmitted by either germline alone. Analysis of methylation profiles in F3 sperm from the methoxychlor-exposed lineage compared with those of unexposed rats revealed 37 methoxychlor-related DMRs. The functional categories of the DMR-containing genes included metabolic pathways, ribosome, transcription, and human T cell leukemia virus type 1 infection.

JP-8 Jet Fuel

The effects of environmental exposure to hydrocarbons on human health have elicited heightened interest following the *Deepwater Horizon* oil rig contamination of the Gulf of Mexico in 2010. Tracey et al. (36) evaluated the potential transgenerational effect of in utero JP-8 exposure in F1–F3 rat offspring. In this rat model study, the authors administered F0 dams either a vehicle or JP-8 (500 mg/kg per day) during gestational days 8–14 in order to examine the impact of in utero JP-8 exposure on the methylation profile of F3 sperm. Similar to the reported transgenerational effects of DDT and methoxychlor, rats from the JP-8-exposed lineage presented with greater body weight and excess fat mass compared with unexposed offspring; however, this effect was observable only in the F3 generation. A comparison of F3 control sperm with F3 sperm from the JP-8-exposed lineage revealed 33 DMRs. The functional categories of DMR-containing genes included signaling, metabolism and transport, transcription, and development.

DISCUSSION AND RECOMMENDATIONS FOR FUTURE STUDIES

The studies reviewed above suggest that epigenetic alterations play an influential role during development in the onset of obesity. However, much work remains to be done, including analyzing a broader scope of epigenetic factors, formally assessing epigenetic marks as mediators, determining the importance of exposure timing in developmental origins (including the role of paternal influence), identifying unifying molecular pathways, and validating important findings generated in animal studies in prospective longitudinal studies in human populations.

Beyond DNA Methylation: Assessing Multiple Epigenetic Factors

Although various epigenetic processes are hypothesized to contribute to alterations in developmental programming, 15 of the 18 reviewed studies exclusively assessed DNA methylation, and two studies evaluated posttranscriptional regulation via miRNA expression levels. Among the reviewed articles, only a single study evaluated more than one epigenetic marker (43). As shown by the findings reported in this study, the resultant gene expression levels reflect an interplay among multiple layers of repressive and active epigenetic marks [i.e., methylation of histone H3 on lysine 27 (H3K27me), methylation of histone H3 on lysine 4 (H3K4me), and CpG methylation]. Because developmental plasticity is likely determined through the combined effect of various epigenetic alterations, studies should continue to address the possible joint or integrated effect of multiple epigenetic phenomena.

Applying Formal Tests for Mediation

The vulnerability of epigenetic changes to environmental perturbations early in development and their role in determining lineage-specific gene expression patterns suggest that epigenetic mechanisms likely play a pivotal role in the pathway between adverse intrauterine conditions and chronic disorders later in life. Indeed, many of the studies reviewed above include associations between the intrauterine condition and epigenetic changes in addition to correlations between epigenetic marker levels and indicators of obesity. However, few studies have formally tested the mediation (38) of epigenetic marks in the association between the intrauterine condition and onset of obesity. Further in-depth exploration of the data that include such mediation analysis will help further clarify the role of epigenetics in the pathophysiologic pathway between intrauterine exposure and obesity onset later in life.

Addressing Timing of Exposure and Developmental Windows of Susceptibility

In addition to identifying specific elements in the intrauterine environment that contribute to the development of obesity, the potential impact of the timing of exposure needs to be evaluated, as the effect elicited likely varies across key developmental stages. Studies examining the role of developmental plasticity in later onset of obesity span both exposures restricted to the intrauterine period, as shown by Borengasser et al. (6), and multigenerational exposures, as shown by Ding et al. (11). The exposure window specified in each of these studies answers different questions: Exposures restricted to the intrauterine period enhance our understanding of developmental windows of susceptibility, whereas multigenerational exposures more closely reflect real-world conditions. Hence, additional studies spanning these various windows will be necessary to coalesce the findings into meaningful interpretations.

Additionally, an increasing amount of evidence indicates that exposures can exert long-lasting effects beyond the fetus itself. Because the gametes of the F1 offspring are developing during the intrauterine stage, exposures during this period directly affect both F1 and F2 generations, and additional evidence is accumulating in support of a transgenerational effect that extends even to the unexposed F3 generation. Although a few studies have formally examined transgenerational transmission of obesity, most have focused on the role of environmental pollutants (24, 34, 36). Additional intrauterine

conditions, including the transmission of the effects of parental obesity, are worth exploring in future studies.

Beyond the Maternal Environment: Assessing Developmental Plasticity in Response to Paternal Influences

Further complicating epidemiologic studies, emerging findings suggest that in addition to maternal contributions to the intrauterine environment, paternal exposures may be transmitted transgenerationally (26). Intriguingly, as shown by McPherson et al. (26), metabolic syndrome may be transmitted to offspring through altered miRNA content in the sperm of HFD-fed paternal mice. Additional focus on the possible transmission of paternal exposure histories and associated adverse offspring health effects is warranted.

Identifying Unifying Molecular Pathways

Critical to identifying potential interventional endpoints is to delineate the molecular pathways underlying the exposure-outcome paradigm. A survey of the reported findings indicates a few candidate loci of interest. Methylation of CpG sites that correlated with the expression of *Zfp423*, *C/ebp-β*, *Pparγ*, and *Pgc-1α* was dysregulated in several studies that linked maternal HFD to offspring obesity. Interestingly, these studies spanned assessments in different tissues [fat (6) and muscle (41)] and biological models [rat (6) and sheep (41)]. Additionally, studies have demonstrated that perturbations of certain loci are relevant in the onset of offspring obesity across different exposures, including *C/ebp-β* [HFD (6, 41) and PAHs (42)], *Pparγ* [HFD (6, 41) and PAHs (42)], and *Igf2* [IUGR (8) and BPA (35)]. The repeated observations of the involvement of these loci could point to the relevance of these genes in the molecular pathway leading to offspring obesity. Alternatively, these observations may reflect the specific focus of current studies on a select handful of loci. The implementation of a more hypothesis-free, epigenome-wide approach may substantiate these previously identified loci and widen the net of consideration through the identification of novel epigenetic marks. Finally, as additional loci emerge, formal testing of the interrelationship among the identified loci should be pursued in order to coalesce individual findings into overriding perturbed pathways.

Validating Findings in Human Populations

Currently, the literature that supports an epigenetically mediated link between developmental conditions and later onset of obesity is derived largely from in vivo animal studies. Unlike human observational studies, the controlled experimental settings of these studies are well suited to identifying environmental agents and windows of susceptibility and elucidating disease etiology. However, the extent to which these findings translate to human populations in more complex exposure settings remains unclear because few epidemiologic studies with sufficient follow-up time to capture the onset of obesity later in life have been conducted to address this question. As in vivo and ex vivo studies continue to refine our understanding of the epigenetic bases underlying the link between suboptimal intrauterine conditions and later onset of obesity, there will be a need for additional parallel human birth cohort studies.

Moreover, a growing body of evidence indicates that subclinical measures in childhood and adolescence may provide valuable prognostic information for the development of adult

disease, including obesity (18). Longitudinal analysis of biological samples in childhood and adolescence may provide meaningful evidence of risk factors for the development of chronic disease in later life. This is of particular relevance because the potential epigenetic basis underlying the developmental route to obesity onset (Figure 1) suggests that the maladapted changes may be reversible, providing potentially modifiable endpoints for intervention.

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Glossary

DOHaD	developmental origins of health and disease
IUGR	intrauterine growth restriction
CpG	cytosinephosphate-guanine dinucleotide
BMI	body mass index
miRNA	microRNA
NC	normal chow
HFD	high-fat diet
WAT	white adipose tissue
DMR	differentially methylated region
F1, F2, and F3	first, second, and third filial generation, respectively
PAH	polycyclic aromatic hydrocarbon
BPA	bisphenol A
DDT	dichlorodiphenyltrichloroethane

Figure 1.

Potential epigenetic bases underlying the developmental origins of obesity. We propose that environmental epigenetic interactions that program obesity are dependent in part on exposure timing: Preconceptional insults may alter epigenetic marks in parental gametes that are passed on to the offspring, periconceptional insults may disturb epigenetic programming of embryonic cells when epigenetic marks are laid that are propagated to all cells, and early life insults following cell lineage differentiation may elicit cell type-specific alterations that are tissue specific. Delineating the true landscape of epigenetic programming for obesity will require comprehensive assessment of epigenetic modifications (including histone modifications, DNA methylation, and noncoding RNAs) as well as the interactions among them. Longitudinal studies designed to capture the timing-dependent programmed trajectory of obesity via epigenetics will require assessment of obesity indicators [e.g., body mass index (BMI), body fat composition, glucose intolerance, and insulin resistance] measured prior to adult disease onset. Images printed with permission from ©Mount Sinai Health System.

Table 1
Recent studies of epigenetic mechanisms linking intrauterine environment to later onset of obesity

Intrauterine environmental agent	Epigenetic locus	Epigenetic assessment	Biospecimen	Outcome measure	Model	Reference
Parental obesity	<i>CCDC112, MCOLN3</i>	DNA methylation	Cord blood	Child BMI	Human	31
	<i>RXRα, eNOS, SOD1</i>	DNA methylation	Cord blood	Adiposity at age 9	Human	15
	<i>Zip423, Cebp-β, Pparγ</i>	DNA methylation and expression	Fat	Body weight and fat mass	Rat	6
	<i>Zip423</i>	DNA methylation, expression, and histone modifications	Fat	Adipogenic potential of fetal tissue	Mouse	43
	<i>let-7g, miR-381, miR-376, Tnfrsf4, Fst, Tnfa, Il6, Tlr4, Pparγ, C/ebp-α</i>	miRNA and mRNA	Muscle and mesenchymal stem cell line	Adipogenic potential of fetal tissue	Sheep	41
	<i>Tlr1, Tlr2, Lat, Dnm1l, Dnm13a/b</i>	DNA methylation	Fat	F1-F2 body weight, adipocyte size, and metabolic dysfunction	Mouse	11
	<i>Pgc-1α</i>	DNA methylation and expression	Muscle	Fat mass	Mouse	21
	<i>miR-503, miR-456b-5p, miR-542-3p, miR-652</i>	miRNA	Sperm	Metabolic dysfunction (glucose intolerance and insulin sensitivity)	Mouse	26
	<i>Hmgcr, Lss</i>	DNA methylation	Liver and oocytes	F1-F2 body weight, WAT weight, and metabolic dysfunction	Mouse	40
	Global methylation	DNA methylation	Fat	Body weight and fat mass	Mouse	19
Dietary supplement	<i>Pgc-1α</i>	DNA methylation and expression	Muscle	Fat mass and metabolic dysfunction	Rat	44
	<i>Igf2</i>	DNA methylation and expression	Fat	Fat mass and metabolic dysfunction	Rat	8
	<i>Igf1</i>	DNA methylation	Liver	F1-F2 body weight, fat mass, and metabolic dysfunction	Rat	16
	<i>Pparγ, C/ebp-β, Cox-2, Fas, Adipoq</i>	DNA methylation and expression	Fat	F1-F2 weight gain and fat mass	Mouse	42
PAHs	<i>Igf2</i>	DNA methylation and expression	F2 embryos	F1-F2 weight gain, fat mass, and metabolic dysfunction	Mouse	35
BPA	<i>Tubb3, Carm1, Slc4a4</i>	DNA methylation	Sperm	F3 body weight and fat mass	Rat	34
DDT	37 DMRs	DNA methylation	Sperm	F3 obesity incidence	Rat	24
Methoxychlor	33 DMRs	DNA methylation	Sperm	F3 body weight and fat mass	Rat	36

Abbreviations: BMI, body mass index; BPA, bisphenol A; DDT, dichlorodiphenyltrichloroethane; DMR, differentially methylated region; F1, F2, and F3, first, second, and third filial generation, respectively; IUGR, intrauterine growth restriction; miRNA, microRNA; PAH, polycyclic aromatic hydrocarbon; WAT, white adipose tissue.