The Role of Epigenetics in the Latent Effects of Early Life Exposure to Obesogenic Endocrine Disrupting Chemicals

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Recent research supports a role for exposure to endocrine-disrupting chemicals (EDCs) in the global obesity epidemic. Obesogenic EDCs have the potential to inappropriately stimulate adipogenesis and fat storage, influence metabolism and energy balance and increase susceptibility to obesity. Developmental exposure to obesogenic EDCs is proposed to interfere with epigenetic programming of gene regulation, partly by activation of nuclear receptors, thereby influencing the risk of obesity later in life. The goal of this minireview is to briefly describe the epigenetic mechanisms underlying developmental plasticity and to evaluate the evidence of a mechanistic link between altered epigenetic gene regulation by early life EDC exposure and latent onset of obesity. We summarize the results of recent in vitro, in vivo, and transgenerational studies, which clearly show that the obesogenic effects of EDCs such as tributyltin, brominated diphenyl ether 47, and polycyclic aromatic hydrocarbons are mediated by the activation and associated altered methylation of peroxisome proliferator-activated receptor-γ, the master regulator of adipogenesis, or its target genes. Importantly, studies are emerging that assess the effects of EDCs on the interplay between DNA methylation and histone modifications in altered chromatin structure. These types of studies coupled with genome-wide rather than gene-specific analyses are needed to improve mechanistic understanding of epigenetic changes by EDC exposure. Current advances in the field of epigenomics have led to the first potential epigenetic markers for obesity that can be detected at birth, providing an important basis to determine the effects of developmental exposure to obesogenic EDCs in humans. (*Endocrinology* 156: 3466–3472, 2015)

The current global epidemic of obesity poses a serious threat to human health. The worldwide prevalence of obesity has nearly doubled since 1980 and is currently affecting 11% of men and 15% of women. Obesity and overweight is associated with increased risk of heart disease, strokes, and diabetes and has been estimated to account for 3.4 million deaths in 2010 (1). Although the main contributing factors to this epidemic include high caloric intake, a sedentary lifestyle, and genetic predisposition, recent studies support a role for exposure to environmental obesogens. These are chemicals that alter hormonal pathways that regulate lipid metabolism, and thereby stimulate adipocyte differentiation and a predisposition to obesity and/or increase the susceptibility to obesity and related metabolic disorders (2). In particular, early life exposure to obesogenic endocrine-disrupting chemicals (EDCs) has been linked to latent effects on obesity-related outcomes in epidemiological and animal studies (3, 4). An EDC has been defined by the World Health Organization as “an exogenous substance of mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (5). Developmental exposure to obesogenic EDCs occurs through maternal diet and interaction with products that contain EDCs, such as plastics, personal care products, household and...
other consumer products that can cross the placental barrier and/or transmitted through breast milk (5). The observed sensitivity to EDCs in the period of prenatal and early postnatal development is in line with the Developmental Origin of Health and Disease (DOHaD) paradigm, which proposes that exposure to environmental challenges during this period of developmental plasticity can influence the risk of disease later in life. Altered epigenetic programming of gene regulation is one of the processes that has been implicated to underlie the DOHaD hypothesis by changing gene expression levels in developing tissues (6). In the field of obesogenic EDCs, studies are starting to emerge that demonstrate that alterations in epigenetic programming are associated with obesogenic outcomes.

The goal of this minireview is to briefly describe the epigenetic mechanisms underlying early life developmental plasticity and to evaluate the evidence of a mechanistic link between altered epigenetic gene regulation by EDC exposure early in life, and latent onset of obesity. This review focuses on studies reporting obesity-related outcomes, such as adipocyte differentiation, adiposity, effects on lipid metabolism and body weight increases. A literature survey was performed with Google Scholar, using the search terms “EDC,” “obesogen,” “epigenetic programming,” “developmental exposure,” “adipogenesis,” and “transgenerational.” We included in vitro studies of EDC-induced epigenetic effects in preadipocyte differentiation models because we considered these suitable models for early life adipocyte differentiation, as well as in vivo and transgenerational studies that related early life EDC exposure-induced obesogenic effects to altered epigenetic programming. We did not include studies that assessed the effects of EDCs on nonmetabolic outcomes.

Epigenetic Regulation of Gene Expression

Epigenetics can be described as the different mitotically inheritable mechanisms that govern chromatin conformation and gene expression, including DNA methylation, histone modifications, and RNA interference. Together these epigenetic marks can alter the 3-dimensional structure of DNA and thereby inhibit or promote gene transcription (7). DNA methylation of cytosines that are located next to guanines in the genome (CpG sites) affects chromatin structure because it mediates the binding of proteins in the major groove of the DNA-helix, making hypermethylated regions less transcriptionally active (8). Aberrant hypo- or hypermethylation can result in effects on genome stability and transcriptional gene silencing or activation, respectively (9). Cytosine methylation is relatively stable, which means that DNA methylation that is laid out early in life is not likely to change thereafter, making early development a particular sensitive period for toxicant-induced epigenetic alterations (9–11). Recently also hydroxymethylation has been associated with the establishment of gene regulation during development (12). Histone modifications, on the other hand, are more dynamic epigenetic marks. Histones are organized in protein complexes that coil DNA, either forming more densely packaged chromatin that is less transcriptionally active or loosely packaged chromatin that is more active. Histone modifications that have been related to epigenetic programming include methylation of the fourth lysine on the H3 core histone (H3K4me), as well as H3K9me and H3K27me (11). Recently, also RNA interference has been associated with epigenetic gene regulation, because stretches of noncoding RNA, like small interfering RNA and long noncoding RNA, can orchestrate processes like transcriptional gene silencing and altering of chromatin states (13, 14).

The Role of Epigenetics in Development

During early embryogenesis several major global epigenetic events occur in the genome that regulate the timely expression or repression of pluripotency- and development-associated genes (11). Parental epigenetic marks are first removed after fertilization, which allows for the re-establishment of epigenetic marks, partly under the influence of environmental triggers (11). The epigenetic marks of parentally imprinted genes, however, are not removed during this period, but allow for non-Mendelian transgenerational inheritance of gene expression patterns (15). The process of epigenetic programming is thought to determine the developmental plasticity regulating adaptation to environmental challenges described in the DOHaD hypothesis (16, 17). This implies that adverse health effects might arise when early life stressors do not properly reflect circumstances of the adult environment. For example, investigation of the epigenome of people perinatally exposed to extreme famine during the Dutch Hunger winter showed altered imprinting of IGF-2 (18), which correlated with significantly higher fat deposition in women born during this winter (19). This indicates that obesity-related symptoms can arise when a mismatch occurs between early life established gene expression and the adult environment. Exposure to toxicants during early development has been found to induce similar responses (20).
EDC Mechanisms of Action in Obesity

The proposed mechanism by which early life EDC exposure can affect epigenetic programming of obesity is through their ability to bind nuclear receptors and other transcription factors, thereby influencing consequent gene expression. Nuclear receptors, such as steroid receptors, are a family of transcription factors that, after activation by a ligand or multiple ligands, can bind directly to hormone-response elements in the DNA. These nuclear receptors can recruit chromatin-modifying complexes that include methyl- and acetyltransferases, directly altering epigenetic marks that regulate the expression of the target genes (21). By binding, activating or inhibiting nuclear receptors and other transcription factors, EDCs can modify local chromatin states as well as expression of histone and DNA modulators such as DNA or histone methyltransferases (22).

The peroxisome proliferator-activated receptor (PPAR)γ is an important nuclear receptor involved in regulating the expression of metabolic genes during differentiation and is considered the master regulator of adipogenesis (3). During early development, the relative expression of PPARγ-induced genes determines whether mesenchymal stem cells differentiate into osteocytes or adipocytes, potentially predisposing the body to fat accumulation (23). As described in more detail below, EDCs, such as tributyltin (TBT), brominated diphenyl ether 47 (BDE-47), and polycyclic aromatic hydrocarbons (PAHs), act as ligands for the PPARγ nuclear receptor and have been shown to cause obesogenic effects accompanied by altered methylation of PPARγ or PPARγ target genes.

The estrogen receptor (ER)α is another important transcription factor related to the latent onset of obesity (24). Several estrogenic EDCs, including genistein and bisphenol A (BPA), can activate ERα (21, 25) and also induce transcription of ERs (26–28). For BPA, it has been reported that activated ERα can associate to estrogen-responsive elements that are present in the promoter of the histone modifying methyltransferase EZH2. This binding of ERα to estrogen-responsive elements attracts other coregulators, such as the histone modifying enzymes that acetylate and methylate histone tails, both resulting in an activated chromatin state and eventual expression of EZH2 that can then result in elevated levels of H3K27 trimethylation, potentially affecting global epigenetic gene regulation (29, 30). Thus, although most of the present environmental obesogen research focuses on PPARγ as the main target of obesogens, it is likely that other mechanisms, including the activation of other steroid hormone receptors, are involved as well. The impact of EDCs directly on epigenetic machinery such as the expression of DNA methyltransferases and the availability of methyl donors can also not be ruled out (31, 32).

Obesogenic EDC Exposure and Epigenetic Effects

We reviewed publications that demonstrated that developmental exposure to EDCs resulted in adipogenic or obesity-related effects and concomitantly measured epigenetic gene regulation. Only a limited number of such studies have been published up to now and are summarized in Table 1.

The organotin pesticide TBT is a known obesogenic EDC that has been associated with body weight increases in rodents after developmental exposure (33). Experimental evidence exists in both in vitro and in vivo studies indicating that the obesogenic effects of TBT are at least in part mediated by changes in methylation of PPARγ itself or PPARγ target gene methylation. TBT exposure in vitro in 3T3-L1 mice preadipocytes resulted in increased adipocyte differentiation that was accompanied by decreased global DNA methylation levels (34). Also adipose-derived stromal cells (ADSCs) isolated from mice exposed perinatally to TBT showed an increase in differentiation towards the adipogenic lineage, at the cost of decreased osteogenesis (33). TBT exposure in ADSC-derived adipocytes was associated with increased expression of adipogenesis marker genes, including the PPARγ target gene Fabp4, in which decreased methylation levels were found in the promoter/enhancer region. Although increases in Pparγ mRNA levels were found, no effects on DNA methylation levels of its promoter/enhancer region were identified (33). An explanation for this lack of epigenetic regulation through DNA methylation might be that PPARγ is under the control of H3K27me3, which causes the gene to be promptly up-regulated after histone demethylation during differentiation processes and possibly EDC exposure (3). Importantly, prenatal exposure to TBT has been recently shown to cause transgenerational inheritance of adiposity (35). It remains to be determined whether these transgenerational effects are related to permanent changes in DNA methylation profiles or other epigenetic processes.

Exposure to the flame retardant BDE-47 results in dose-dependent adipocyte differentiation in vitro 3T3-L1 mice preadipocytes, with a modest decrease in global DNA methylation levels (34). BDE-47 binds and activates human PPARγ in vitro (36). In 3T3-L1 cells, BDE-47 increased in Pparγ2 expression levels, which was associated with a decrease in DNA methylation of the Pparγ2 promoter. Also increased levels of several adipogenic genes were found after BDE-47 exposure, including Lep, but no
 Exposure to the obesogenic effects of BDE-47 in vivo has not resulted in increased body weight in rodents (37), to our found (36). Though perinatal exposure to BDE-47 re-

Table 1. Studies Relating Developmental EDC Exposure, Adipogenesis, and Obesity-Related Outcomes and Epigenetic Effects

<table>
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<tr>
<th>Chemical, dose and timing of exposure</th>
<th>Experimental design</th>
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<td>In vitro studies</td>
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<td>BDE-47 (0.003 μg/kg–25 μg/kg)</td>
<td>Mouse 3T3-L1 preadipocytes, 8-d exposure</td>
<td>Dose-related increase in percentage of differentiated adipocytes for BDE-47 and BPA exposure; increase in differentiation by BDE-47</td>
<td>Global hypermethylation by BPA (80 μg/kg); decreased global methylation by TBT; overall inverse correlation between methylation level and adipocyte differentiation</td>
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<td>BPA (100 μg/kg BW maternal gavage)</td>
<td>Perinatally exposed (GD6–PND22) Sprague-Dawley rats</td>
<td>Increased adipocyte differentiation; weak activation of PPARγ but not of RXRα, increased mRNA expression of Pparγ2, Cebpa, Cebpb, Cebpα, Smadfla, Lpl, Siglec4, Fabp4, Adipoq, G6pc, Lept, Igf1, and Inr and decreased expression of Igf1r</td>
<td>In PND1 males near Cpt1a TSS, increased hepatic methylation, alterations in histone modifications H3K4me2, H3K36me3, H3Ac, H4Ac, and decreased binding of Pol II, C/EBPβ and SREBP1; in females, no DNA methylation effects or binding of chromatin factors but slight decrease in H3K36me3</td>
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<td>Genistein (50–100 mg/kg BW neonatal gavage)</td>
<td>Neonatally exposed (PND1–PND22) Sprague-Dawley rats</td>
<td>Increased (PND60–PND90) bodyweight, fat/lean mass ratio, and fat mass as well as increased adipocyte size and density in females but not in males; in PND22 female WAT, increase in mRNA of Cebpa, Cebpb, and Ppar and decrease of Wnt10b; increase of only Wnt10b in male WAT</td>
<td>In females, increased methylation downstream of promoter and near and within exon 4 of Wnt10b gene; in males, decreased methylation near and within exon 4</td>
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<td>Mixture of PAHs (7.29 ng/m³ or delivery) BALB/cByj mice (F1) and unexposed F2-offspring</td>
<td>Prenatally exposed (GD1–GD21) or delivery</td>
<td>No effects on mean body weight in F1 but adult onset increase in obese individuals amongst males and females in F3, transgenetic inheritance of male obesity through female, and of female obesity through male or of both parental germlines</td>
<td>Transgenerational sperm epimutations in F3 regulated by methyloxychlor exposure found in 39 DMRs; highly interconnected genes with obesity-associated genes containing DMRs with epimutations in F3 were Tubα3, Capn1, and 5c4d4</td>
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<td>Methoxychlor (200 mg/kg BW maternal injection)</td>
<td>1-y-old prenatally exposed (GD8–GD14) Sprague-Dawley rats (F1) and unexposed F3-offspring</td>
<td>No effects on mean weight in F1 but increase in obese individuals in F3 females; transgenetic inheritance of male obesity through female germline</td>
<td>Transgenerational sperm epimutations in F3 regulated by methyloxychlor exposure found in 37 DMRs; the associated pathways included metabolic, fatty acid elongation, and Wnt signaling pathways</td>
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| DEHP, diethyl hexyl phthalate; DBP, dibutyl phthalate; RXRα, retinoic X receptor.

changes in DNA methylation of the leptin promoter were found (36). Though perinatal exposure to BDE-47 resulted in increased body weight in rodents (37), to our knowledge, epigenetic analysis linking developmental exposure to the obesogenic effects of BDE-47 in vivo has not been performed.

Sex-specific obesogenic effects have been demonstrated after neonatal exposure of rats to the phytoestrogen genistein at levels mimicking human infants fed soy formula, with increased fat/lean mass ratio, fat mass, adipocyte size and number in adult female mice that persisted to adulthood (38). The obesogenic effects of genistein were associated with an increase in mRNA expression levels of Pparγ and related adipogenic genes in white adipose tissue (WAT). Interestingly, expression of Wingless-related Mouse mammary tumor virus integration site 10b

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(Wnt10b), a critical regulator of adipogenic cell fate determination, was decreased in female neonates, and was associated with hypermethylation of the promoter of this gene. The authors hypothesize that active methylation of the Wnt10b promoter by genistein likely inhibits transcription factor binding (38), a plausible proposition given the methyl donor properties of genistein (39). A provocative alternative hypothesis that warrants further investigation is that genistein modifies the activity of the histone H3K27 methyltransferase EZH2, which is known to repress Wnt genes to facilitate adipogenesis (40).

Numerous animal studies have demonstrated the obesogenic and metabolic disruptive effects of developmental exposure to the plastic monomer BPA (41), although it should be noted that contradictory effects have been found in animal studies, indicating that effects are dependent on sex, dose, strain, duration and route of exposure (42). Studies examining links between epigenetic alterations and the obesogenic outcomes of BPA exposure are just starting to emerge. BPA induces the differentiation of 3T3-L1 mice preadipocytes in vitro at low nanomolar concentrations (43), which has been associated with increased global DNA methylation at higher concentrations (34). In an elegant study that is one of the first to combine analysis of DNA methylation and histone modifications after EDC exposure, Strakovsky et al (44) show that early life BPA exposure increased fat/lean mass and exacerbated high-fat diet induced hepatic steatosis in adult males only. On a molecular level, BPA exposure was associated with decreased expression of genes in males early in life that are involved in triglyceride synthesis and mitochondrial β-oxidation. Epigenetic analysis of the CPT1A gene, the key β-oxidation enzyme, showed increased DNA methylation and altered H3K4me2, H3K36me3, H3Ac, and H4Ac in its transcription start site, revealing a novel regulatory mechanism for the transcription of CPT1A after BPA exposure. This study shows that both DNA methylation and histone modifications play an important role in EDC toxicity by influencing chromatin conformation and subsequent binding of transcription factors (44).

To our knowledge, one of the first studies to examine the effects of mixtures of EDCs on obesity-related outcomes and epigenetic gene regulation was recently published, in which pregnant dams were exposed to an airborne mixture of PAHs in aerosol form that was designed to replicate the proportions of individual PAH measured in pregnant women (45). Perinatal airborne PAH exposure was related to the development of adiposity in the F1 and F2 generation, with persistent changes in body weight, fat mass, and adipocyte size. Expression of the genes encoding PPARγ and related adipogenic genes was increased and accompanied by decreased DNA methylation in the promoter of Pparγ (45). The authors of this study associate the obesogenic effects of the PAH mixture to direct activation of Pparγ, although it is conceivable that other mechanisms are involved, given the multiple endocrine disrupting and carcinogenic properties of PAHs and their metabolites. Considering the increasing number of epidemiological studies demonstrating a relationship between early life PAH exposure (either directly or indirectly through air pollution) and altered DNA methylation (46, 47), it would be evident to examine body weight and adiposity measures in these cohorts.

**Obesogenic EDCs and Transgenerational Effects**

In a series of studies published by the Skinner Lab at Washington State University, EDCs, including the organochlorine pesticides DDT and methoxychlor, have been shown to induce transgenerational obesogenic effects. Both of these compounds were associated with unaffected body weight in F1 prenatally exposed individuals, but increased incidence of obesity in the F3 offspring. The F3 offspring, however, was never directly exposed, indicating that effects on body weight were induced by epimutations in the germline that were introduced in the F1 (48). This was indeed confirmed with genome-wide DNA methylation analysis, which revealed epimutations in differentially methylated regions of genes that can be associated with obesity (49, 50). A similar effect on transgenerational inherited obesity and related sperm epimutations was found for a mixture of the EDCs BPA and phthalate plasticizers diethyl hexyl phthalate and dibutyl phthalate (51). Interestingly, even though a similar obese phenotype was observed and correlated with epimutations in genes involved in obesity-related metabolic pathways, each EDC or mixture tested also promoted a unique signature or pattern of epimutations in the F3 generation sperm, allowing for the possibility of developing unique biomarkers for ancestral exposure and future disease susceptibility (48, 51), but also highlighting the challenge of developing more general epigenetic biomarkers that can relate developmental exposure to the complex mixture of EDCs in our everyday lives to latent onset of obesity. It should be noted that the concentrations tested in the transgenerational studies performed by Skinner et al (49) and Skinner and coworkers (50, 51), are high in comparison with human environmental exposure levels and these high concentrations might mask more subtle epigenetic changes present at relevant concentration levels.
Conclusion and Outlook

Although some of the tested EDC concentrations were not relevant to human exposure levels and the number of studies is limited, the studies outlined here provide the first irrefutable evidence that environmental exposures during early life can induce persistent alterations in the epigenome, which may lead to increased adipogenesis, adiposity and body weight, even across multiple generations. Understandably, the studies published so far tend to focus on PPARγ, the master regulator of adipogenesis, but this provides an undeniably narrow interpretation of the multiple mechanisms that could be altered when considering a complex disorder as obesity. Genome-wide epigenetic analysis is clearly the way forward to provide a more unbiased identification of regulated genes and sites of epigenetic modification. It is also clear that the complex interplay of DNA methylation, histone modifications, and noncoding RNA has not been studied yet in the context of obesogenic EDCs, because most studies in this young field have focused on DNA methylation. The study of Strakovsky et al (44) provides an elegant “proof of principle” that a combination of changes in histone marks and DNA methylation to key genes involved in mitochondrial metabolism underlie the effects of perinatal BPA exposure and latent onset of steatosis. More studies of this type are needed to better understand chromatin conformational changes by EDCs.

Although the field of environmental obesogens is still young, with the first seminal paper by Grün and Blumberg published in 2006 (2), the field of epigenetics and obesity is also still developing. The first potential epigenetic markers for obesity that can be detected at birth have only recently been identified (reviewed in Ref. 52). These markers include retinoic X receptor (a dimerization partner of PPARγ) promoter methylation in umbilical cord tissue, which could explain up to 26% of the variation in childhood adiposity (53), and could be useful targets for examining effects of EDC exposure. In a recent European study examining the role of developmental exposures to EDCs in latent onset of obesity (54), preliminary analyses indicated a positive relation between cord blood concentrations of dichlorodiphenyldichloroethylene the major metabolite of DDT, and global DNA methylation (S. Remy, unpublished results). In turn, prenatal dichlorodiphenyldichloroethylene has been associated with accelerated growth and body mass index in children from the same cohorts (55). Epidemiological studies which link EDC exposure, epigenetic gene regulation, and obesity outcomes are needed to understand the effects of developmental exposure to EDCs and to identify epigenetic biomarkers of latent onset of obesity in humans.

Acknowledgments

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