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Sexually Dimorphic Effects of Early-life Exposures to Endocrine Disruptors: Sex-specific Epigenetic Reprogramming as a Potential Mechanism

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Abstract

Purpose of review—The genetic material of every organism exists within the context of regulatory networks that govern gene expression—collectively called the epigenome. Animal models and human birth cohort studies have revealed key developmental periods that are important for epigenetic programming and vulnerable to environmental insults. Thus, epigenetics represent a potential mechanism through which sexually dimorphic effects of early-life exposures such as endocrine disrupting chemicals (EDCs) manifest.

Recent findings—Several animal studies, and to a lesser extent human studies, have evaluated life-course sexually dimorphic health effects following developmental toxicant exposures; many fewer studies, however, have evaluated epigenetics as a mechanism mediating developmental exposures and later outcomes.

Summary—To evaluate epigenetic reprogramming as a mechanistic link of sexually dimorphic early-life EDCs exposures, the following criteria should be met: 1) well characterized exposure paradigm that includes relevant windows for developmental epigenetic reprogramming; 2) evaluation of sex-specific exposure-related epigenetic change; and 3) observation of a sexually dimorphic phenotype in either childhood, adolescence, or adulthood.

Keywords

lead (Pb); bisphenol A (BPA); epigenetics; sexually dimorphic effects; developmental origins of health and disease (DOHaD)

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Compliance with Ethical Standards

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

Carolyn McCabe, Olivia S. Anderson, Luke Montrose, Kari Neier, and Dana C. Dolinoy declare that they have no conflict of interest.

1. Introduction

Findings from landmark longitudinal birth cohort studies (e.g. Dutch Hunger Studies) have led scientists to postulate that there are key developmental periods important for exposure-related impacts — some of which may persist long after the initial insult [1]. This hypothesis is referred to as the “developmental origins of health and disease” (DOHaD), and it posits that early-life environmental exposures can alter disease risk across the life course into adulthood. Within the scope of the DOHaD hypothesis are adult chronic diseases such as metabolic syndrome, obesity, cancer, and neurodegenerative disorders [2]. Identifying the mechanisms that underlie DOHaD-related conditions will assist in the development of intervention and prevention strategies. For example, in a recent publication of middle-aged adults conceived during the Great Chinese Famine (1959–1961), findings suggest that, similar to European populations, there is an increased risk for hypertension following prenatal under-nutrition [3]. Further, when comparing hypertension rates between rural and urban dwellers prenatally exposed to the Great Chinese Famine, Wu et al. revealed that postnatal diet may be a significant factor in the incidence and a target for prevention of hypertension between these two populations [3].

To fully understand the impact of the prenatal environment and mitigate disease risk, it is critical to identify exposure-specific biomarkers (i.e., biomarkers sensitive to exposure and biomarkers of exposure) and elucidate the mechanisms through which these exposures operate. One potential mechanism is epigenetic chemical modifications (e.g. DNA methylation and histone modification) that result in changes in gene expression that are heritable through mitotic cell division and not the result of a change in the primary DNA sequence [4]. Toxicoepigentic modifications - toxicant exposure-related epigenetic modifications that affect gene expression in labile gene regions without altering the underlying genetic code – that result from perinatal exposures provide a plausible conduit linking early exposure to later disease risk. However, few longitudinal human studies are designed to collect necessary and biologically relevant samples or adequately assess environmental exposures during the perinatal period. Therefore, researchers rely heavily on animal models to supplement these logistical gaps.

Animal models, particularly mouse models, have been essential in understanding the early-life epigenetic programming of two specific types of genes, namely imprinted genes and genes with metastable epialleles [5]. Metastable epialleles display consistent expression across tissues within an individual but display variable expression among genetically identical individuals [6]. Imprinted genes on the other hand are genes expressed in a monoallelic, parent-of-origin fashion [7]. Both varieties can be epigenetically labile, or perturbed by environmental exposure, during early development. Mouse models such as the viable yellow agouti (A^{vy}) mouse, which has the distinct advantage of coat-color change as a visual biosensor, have been used to demonstrate the long-term impacts of perinatal exposures [8][9]. The A^{vy} allele is an example of a metastable epiallele and as such, any perturbation of methylation that occurs in very early development is passed to all resulting daughter cells for the life of the individual. In the case of yellow A^{vy} mice, the early epigenetic perturbation (i.e., hypomethylation of a retrotransposon containing a cryptic agouti promoter) results in increased risk for obesity, diabetes, and tumorigenesis later in life

[10][11]. Toxicoeepigenetic studies have slowly revealed that epigenetic reprogramming occurs in a sexually dimorphic pattern [12]; a phenomenon that has recently gained increasing attention and importance in biomedical research.

Throughout the history of biomedical sciences, a sex bias has prevailed in both human and animal studies. Males have traditionally been favored as study subjects because of the physiological complexities of female reproductive cycles [13]. This sex bias has contributed to difficulties in translating animal findings to humans due to sexually dimorphic biological responses and differences in susceptibilities to adverse effects from treatments and therapies [14]. Despite the recent increase in clinical trials that utilize women, animal research is still lagging behind [13, 14]. To address this, in 2014 the National Institutes of Health (NIH) began to implement policies to encourage the use of both male and female animals and cells in biomedical research to enhance our understanding of sex-specific health outcomes.

Exposure to endocrine disrupting chemicals (EDCs) has been strongly associated with sex-specific effects in both animal models and human epidemiological studies [15–18]. EDCs are chemicals that are capable of interfering with hormone signaling and are found ubiquitously in the environment. For example, bisphenol A (BPA), a chemical commonly found in polycarbonate plastics and linings of canned goods, is capable of mimicking estrogen to disrupt estrogen signaling [19]. Phthalates such as diethylhexyl phthalate (DEHP), which are plasticizers found in a wide variety of consumer products including vinyl flooring and children's toys, are anti-androgenic and interfere with androgen signaling [20]. The influence of heavy metals, including lead (Pb) on the endocrine system, is also an emerging area of research in humans and animal models [21]. Hormone signaling is sexually dimorphic in nature, and thus EDCs that disrupt hormonal pathways may elicit sex-specific effects, including altered metabolism, behavior, and cognition [17, 22–24].

Objectives

Although there are many chemicals that have been recognized as EDCs, this review focuses on two chemicals that have been widely studied: bisphenol A (BPA) and lead (Pb). The objective of this review is to summarize recent studies that have evaluated the sex-specific effect of periconceptional/prenatal exposure to the EDCs BPA and Pb in mice and humans, with a specific emphasis on those that follow the exposed individuals and evaluate the epigenetic mechanism of exposure. Figure 1 provides an overview of our proposed mechanism of how developmental exposures to EDCs lead to sexually dimorphic effects. In our review, we included studies that provide evidence for epigenetic reprogramming as a mechanistic link between early-life exposures to BPA and/or Pb and sexually dimorphic effects meeting the following criteria: 1) a well-characterized exposure paradigm that captures the window of developmental programming, 2) measurement of exposure-related epigenetic alterations, and 3) evaluated phenotypic effects in both males and females. In some instances, there were no published studies meeting one of the last two criterion, and we therefore reviewed examples of current literature that evaluated sex-specific phenotypic effects of Pb or BPA exposure (without assessing epigenetics), or evaluated sex-specific epigenetic effects of Pb or BPA exposure (without assessing phenotypic effects) in order to shed light on some of the current evidence for our proposed mechanism.

2. Periconceptional Exposures to BPA

2.1 Relevance

BPA is a chemical produced in high volume for the manufacturing of common consumer goods like metal can linings, medical equipment, receipt paper, and food/beverage containers. Because it is so ubiquitous in the environment, there is a significant risk of exposure to BPA across an individual's life. The most common route of exposure to BPA is through ingestion, but it can also be inhaled and dermally absorbed. In the United States (U.S.), it is reported that greater than 92% of the population has detectable levels of BPA in their urine [25], and epidemiological studies from other countries have reported similar data with the majority of the population having detectable levels of BPA in their urine [26]. BPA exposure studies in animal models and humans have demonstrated an association with a multitude of adverse health effects like metabolic, cardiovascular, and neurological diseases and cancer. Prenatal BPA exposure is of concern, because of its potential to interfere with major fetal developmental events. In 2012, the FDA banned use of BPA in the manufacturing of baby bottles and soon after in the production of baby formula [27]; this led to other industries phasing out BPA in their consumer products, although its use remains prevalent in the lining of aluminum cans and thermal receipt paper.

BPA has the potential to disrupt hormone-signaling pathways that will establish typical sexually dimorphic characteristics. Concurrent with this, sex-specific health outcomes have been observed in response to perinatal exposure to BPA. In this section, we will first review some of the metabolically-related sexually dimorphic findings that followed a periconceptional BPA exposure paradigm in animal models. Additionally, we will summarize the animal literature exploring neurological outcomes following periconceptional BPA exposure. Lastly, we will review sexually dimorphic epigenetic outcomes associated with periconceptional BPA exposure.

2.2 Perinatal BPA Exposure and Associated Phenotypes in Mice

The literature has established a consistent association of BPA exposure with obesity and obesity-related phenotypes in mice. Often referred to as an 'obesogen,' perinatal BPA exposure has been shown to disrupt glucose and insulin homeostasis in a sex-specific manner. Alonso-Magdalena et al. confirmed that male mice offspring developed insulin resistance and glucose intolerance as adults when exposed to 10 ug BPA/kg body weight from gestational day (GD) 9 to 16 [28]. Liu et al. found similar results in male adult mice following exposure to 100 ug/kg/day from GD6 to post-natal day zero (PND0) [29]. A glucose challenge test given at 3 and 6 months of age revealed high levels of serum glucose compared to controls. Liu et al. also demonstrated that a small exposure window of GD6 to 9 resulted in high blood glucose levels at 6 months of age in male offspring. García-Arévalo et al. measured augmented insulin secretion in 30-day old male mice exposed to 10 or 100 ug/g/day from GD9 to 16 and found that increased serum insulin was positively associated to beta-cell mass [30]. Van Esterik et al. exposed female mice two weeks prior to pregnancy through PND21 to food supplemented with 1.8–14 mg BPA/kg [31]. At six weeks of age male offspring showed a dose-dependent increase in weight until 23 weeks of age, while female offspring exhibited an inverse relationship of body weight, muscle mass and fat mass

with BPA exposure level [31]. Van Esterik et al. also found that serum leptin, adiponectin, and lipids were significantly different in adult females perinatally exposed to BPA, but unaffected in males [31]. Finally, male offspring had decreased activity levels, while BPA-exposed females had increased activity following 6.5 and 4.5 days of monitoring, respectively [31].

Other outcomes reported in adult animal models following BPA exposure include altered behavior and anxiety. On PND 60, female mice exposed to 2, 20, and 200 ug BPA/kg/d from GD 0 to 19 were hypoactive with increased anxiety-like behavior, while males were hyperactive. Both outcomes displayed a linear response to dose [32]. Jašarevic et al. exposed female deer mice to 50 and 5 mg BPA/kg of chow/day two weeks prior to pregnancy through lactation and found that the sexually mature male offspring displayed abnormal spatial learning, exploratory behaviors, and anxiety-like effects but no significant difference in female behavior [33].

2.3 A^{vy}/a Mouse Model

Our group has also demonstrated sex-specific phenotypes following maternal dietary BPA exposure in the A^{vy}/a mouse model. Maternal dietary exposure to environmentally relevant levels [34] of BPA during pregnancy and lactation resulted in increased activity levels and energy expenditure in adult female a/a offspring at 3, 6, and 9 months of age [15]. Increased activity levels and energy expenditure led to lower body fat mass, higher lean body mass and improved glucose, insulin, and other adipocytokine profiles compared to control females measured at 10 months of age. Exposed adult male offspring had no significant differences in these metabolic phenotypes compared to their control counterparts.

2.4 Perinatal BPA Exposure and Epigenetic Mechanisms in Mice

As detailed in our objectives and selection criteria above, we are interested in studies that evaluate alterations to the epigenome as the mechanism through which perinatal exposure to BPA elicits sexually dimorphic phenotypic effects. The majority of studies designed to elucidate the effects of environmental exposures on the epigenome utilize a global or candidate gene approach. For example, it is well established that BPA alters DNA methylation at candidate metastable epialleles like A^{vy} and $Cabp^{IAP}$ [34, 35]. Furthermore, utilizing an epigenome-wide platform, one can elucidate regions of altered methylation (RAM) [36]. Using significantly differentially methylated regions, biological pathways can be enriched to discover how, mechanistically, methylation is mediating the sex-specific phenotypes observed. The objective of this section is to detail those studies that evaluated the phenotypic response to perinatal BPA exposure and its associated epigenetic changes.

A study conducted by Mao et al. in 2017 demonstrated that perinatal exposure (GD0 to PND21) to 40 ug/kg-day of BPA resulted in sexually dimorphic metabolic effects that were attributed to differences in epigenetic reprogramming (Table 1) [23]. Both male and female mice perinatally exposed to BPA had increased insulin resistance at 9 weeks of age, but these effects were exaggerated in males. Furthermore, exposed males had decreased islet insulin secretion relative to controls [23]. Mao et al. determined that DNA methylation was increased at the imprinted gene insulin-like growth factor 2 (*Igf2*) in BPA-exposed males,

but not in BPA-exposed females. Overall, this study suggested that male pancreatic islet cells are more sensitive to perinatal BPA exposure than female pancreatic islet cells, and this may be due to differential DNA methylation re-programming at the *Igf2* locus.

Strakovsky et al. examined perinatal exposure to BPA and high fat diets (HFDs) in rats and found that exposure to 100 ug/kg-day BPA and a 45% HFD from GD6 to PND21 resulted in increased fat/lean ratios and hepatic lipid accumulation only in males (Table 1) [22]. Males exposed to a combination of BPA and HFD had increased fat/lean ratios at PND60 and 90, and at PND110, males exposed to HFD, BPA, and a combination of HFD and BPA had increased microvesicular steatosis in the liver [22]. These exposure-related metabolic outcomes were not observed in females. Furthermore, only BPA-exposed males exhibited increased DNA methylation at *Cpt1a*, a gene encoding a key beta oxidation enzyme, in the liver. Thus, the authors concluded that male-specific epigenetic reprogramming at *Cpt1a* contributed to the male-specific BPA-induced metabolic alterations observed in this study.

In 2013, Kundakovic et al. found that perinatal BPA exposure induced sexually dimorphic effects on social and anxiety behaviors in mice (Table 1) [32]. Specifically, BPA appeared to reverse sex differences in activity levels and in anxiety-like behaviors. DNA methylation at the *Esr1* locus and corresponding mRNA expression levels were measured in the hypothalamus and prefrontal cortex to determine whether BPA altered epigenetic programming to result in these sex-specific behavior alterations. The authors found that males exposed to 20 ug/kg-day BPA had increased DNA methylation at the *Esr1* locus in the prefrontal cortex. This effect was not observed in females. Females exposed to 20 ug/kg-day BPA had decreased DNA methylation at the *Esr1* locus in the hypothalamus, but this BPA-induced epigenetic alteration was not observed in males. Taken together, these results indicate that sex-specific behavioral effects of perinatal BPA exposure may be driven by differential DNA methylation at *Esr1* in specific regions of the brain.

Using an epigenome-wide platform, we observed enriched biological pathways within RAMs [36]. Candidate genes related to the activity and insulin signaling phenotypes were identified within the top enriched biological pathways. DNA methylation of these specific candidate genes in the functional pathways was measured as a mediator for BPA exposure and the resulting phenotypes. For example, the methylation levels of two candidate genes, *Janus kinase-2 (Jak-2)*, and *Retinoid X receptor (Rxr)* attenuated energy expenditure in BPA exposed females.

3. Periconceptional Exposures to Lead (Pb)

3.1 Relevance

The heavy metal lead (Pb) is a persistent environmental toxicant affecting human populations in developed and developing countries around the world. After a sharp increase in environmental Pb levels at the end of the 20th century, stringent United States (U.S.) policies were established to limit the use of Pb in gasoline and paint. While these efforts reduced the risk of exposure to both adults and children [37], early-life exposure to Pb remains a major public health concern. The U.S. Centers for Disease Control and Prevention (CDC) estimates there to be at least 4 million US households where children are exposed to

high levels of Pb, primarily through the ingestion of paint [38]. In addition, there are mounting concerns about the risk of exposure to Pb due to the aging U.S. water infrastructure. Maternal intake of contaminated drinking water can lead to fetal exposure, because Pb can pass both the placental (Chen et al. 2014) and blood-brain barrier (Stein et al. 2002) of most mammals including humans. Further, these early-life Pb exposures may disproportionately affect susceptible populations, including low socio-economic communities and persons of color. In such populations, co-exposures to stress or poor nutrition may exacerbate negative health responses related to Pb. To mitigate the risk of periconceptual Pb exposure, a comprehensive research strategy including well designed animal and human studies must be employed.

Pb toxicity research has traditionally focused on neurological-related outcomes [39], however recent findings suggest that Pb may also act as an endocrine disrupting chemical [40]. Brain-specific effects have been observed following early and late Pb exposures. Here, we will first review some of the brain-related sex-dependent findings following a periconceptual exposure paradigm. Relative to the number of studies investigating neurological outcomes, there is a paucity of studies aiming to identify links between metabolic-related outcomes and Pb. We will next review the three studies that we identified which met the overall criteria of this review, which found sex-dependent Pb-induced metabolic findings including two studies conducted in our research group. Lastly, we will review three studies that met the overall criteria of this review, which included epigenetic measurements, investigated periconception Pb effects, and observed sex-dependent results.

3.2 Periconceptual Exposures to Pb in rodents

The brain is a known target of Pb toxicity, with negative health outcomes that include spatial learning and memory impairments, behavior changes, and cognitive performance deficits. In a series of studies, Anderson et al. investigated the effect of perinatal Pb exposure on learning and memory in rats and examined whether rearing condition was protective. Long Evans dams were fed relatively high levels (0, 250, 750, 1500 ppm) of Pb in their chow from 10 days prior to mating through PND21. Anderson et al. observed that in 55-day-old rat offspring, rearing condition (e.g. including enrichment in the cage or not) confers an advantage to low and medium level Pb-exposed male rats whereas any amount of Pb exposure blunted the beneficial effect of enrichment for the females [41]. This group then investigated the impact of Pb on cognition-related gene expression in 55-day-old rat hippocampal tissue following the same dosing regimen described above and found dose and sex-dependent differences [42]. To more precisely determine the developmental stage when Pb exposure is most important, Anderson et al. exposed rats perinatally or postnatally to Pb (0, 150, 375, 750 ppm) and found sex-dependent differences in learning and memory. For example, in a test of memory recall, females were affected more by postnatal exposure to Pb, while males were affected by perinatal exposure [37]. These results may be reflective of the sex-dependent differences in hippocampal transcriptomics this group observed previously [43]. Yang et al. also found evidence that males' spatial memory development may be more susceptible during the prenatal exposure period compared to females [44].

Another group of researchers investigated the effect of developmental exposure to Pb with and without stress in a rat model. In these experiments, Long Evans dams were exposed to Pb (1st experiment was 0 or 150 ppm, 2nd experiment was 0 or 50 ppm) through their water from 2 months prior to mating through lactation and half the control and exposed groups were exposed to stress (manual restraint for 45 min 3 × day on GSD 16 and 17). Cory-Slechta et al. showed that Pb alone in males and Pb plus stress in females permanently impacted corticosteroid levels, which were measured at 55 days [45]. Weston et al. showed that when exposed to a more modest dose of Pb during development, male rats are more susceptible to Pb plus stress resulting in changes in neurochemicals and protein production (e.g. BDNF) [46]. Together these studies reviewed here indicate that exposure responses can vary by Pb dose, stress, and sex. Thus, along with manipulations of exposure paradigms, studies should also include both sexes when conducting risk assessments.

3.3 Perinatal Exposure to Pb and Metabolic Findings in Rodents

Pb-induced metabolic alterations are a relatively novel area of research, however sex-dependent effects have been observed in a limited number of studies. Leasure et al. investigated the effect of gestational Pb exposure (0, 27, 55, 109 ppm) in mice by measuring body weight, motor function and dopamine neurochemistry in year-old mice. To determine the timing of Pb effects, these researchers exposed mice from 2 weeks prior to mating through PND10 or from PND0- 21. Similar to many of the rat studies reviewed above, this group found a non-monotonic dose response to Pb, with the largest Pb-induced alterations appearing in the low-dose mice [47]. Further, Leasure et al. found that male mice exhibited late-onset obesity, decreased motor activity, and altered dopamine levels. Interestingly, these male-specific deficits were only true of the gestationally-exposed mice and not the postnatal-only exposed male mice. In a similarly designed study, we exposed mice perinatally from 2 weeks prior to mating through lactation to 0, 2.1, 16, or 32 ppm lead in the dams' water and then measured body weight and insulin response at 10 months. We observed that male mice exposed to Pb displayed increased weight gain and increased insulin response [16]. Further, the altered insulin response was non-monotonic in relation to exposure. Using the same cohort of mice, the gut microbiota was compared between control mice and the high Pb exposure group of 10-month-old mice, finding that perinatal Pb exposure induced changes in gut microbiota in adult male and female mice, but microbiota changes were correlated to increases in body weight in male mice only [48].

3.4 Periconceptional Exposures to Pb in Rodents with Epigenetic Measurements

A very small number of studies have investigated potential epigenetic mechanisms linking health outcomes and periconceptional exposure to Pb in rodent models (Table 1). Sanchez-Martin et al. exposed mice perinatally from 2 weeks prior to mating through lactation to 0, 3, or 30 ppm Pb in the dams' water. Brain cortex and hippocampus were collected from offspring at PND60 and evaluated using epigenome-wide DNA methylation and RT qPCR for targeted gene expression. In both exposure groups compared to controls, females but not males displayed hypermethylated regions of the hippocampus [49]. Further, in the low exposure group there was a trend for females only toward a correlation between the observed three differentially methylation regions and changes in gene expression levels of the sixty genes selected for validation.

After exposing mice perinatally to three increasing levels of Pb, Faulk et al. measured body weight and DNA methylation of two retrotransposons and two imprinted genes. Developmental Pb exposure of any level was associated with an increase in weight for males when compared to control mice [50]. Further, there was a modest cubic trend for an association between Pb exposure and DNA methylation at the Insulin-like growth factor 2 (*Igf2*) loci, a candidate gene, which is known to play a role in growth. As a follow up to this study, we further evaluated the epigenetic lability of a class of retrotransposons called Intracisternal A Particles (IAPs) in the context of perinatal Pb exposure. Using brain and kidney tissue collected from 10-month-old mice from the same cohort of mice exposed to 0, 2.1, 16, or 32 ppm Pb during development, we evaluated DNA methylation profiles of four candidate IAPs which were found to exhibit variable methylation in a previous experiment [51]. Montrose et al. found that the methylation profiles of the selected IAPs were both dose and sex-dependent (Montrose et al. 2017). These findings add to a body of work supporting an overall hypothesis that epigenetic perturbations provide a viable mechanism for a developmental origin of an adult disease and that sexually dimorphic alterations may confer sex-dependent disease susceptibility.

4. Prenatal Exposure to Pb and BPA in Humans

The animal work reviewed in **Sections 2 and 3** established the basic understanding of the impact of early-life exposure to BPA and Pb. Not only have these animal studies been crucial in elucidating the sex-specific phenotypic and metabolic effects of these exposures, but they have also revealed the role of the epigenome as a mechanistic link between periconceptual exposure and developmental outcomes. This section will detail studies completed in human birth cohorts that evaluate the epigenetic impact of prenatal exposure to Pb and BPA. We will discuss one study that evaluated prenatal BPA exposure and three studies that evaluated prenatal Pb exposure.

4.1 Prenatal Exposure to BPA

Human epidemiological studies have found sex-specific associations with maternal BPA exposure and human clinical disorders in offspring during childhood such as attention-deficit and hyperactivity disorder, depression, and aggression [52–55]. The exhibition of behavioral outcomes in males and females vary according to the timing of measurement. Harley et al. found that higher levels of urinary BPA in mothers during pregnancy were associated with lower BMI status in their 9-year old female offspring [56]. Another group found a similar inverse association between prenatal BPA exposure and BMI status in female children 1–4 years of age, but also found a positive association between prenatal BPA and BMI status in male offspring [57].

Of currently published reports, the 2016 study conducted by Goodrich et al., fits our proposed model of evaluating early-life environmental exposures and epigenetic effects (Figure 1). Goodrich et al. evaluated prenatal exposure to BPA and its association with DNA methylation in peri-adolescence [58]. Maternal BPA (*in utero*) exposure was measured in 3rd trimester (T3) urine samples, and peri-adolescence exposure was measured in participants between the ages of 7–14. The authors examined associations of BPA exposure with DNA

methylation in tertiles, because 31 and 15% of maternal and child BPA concentrations fell below the limit of quantification. Compared with the lowest tertile of maternal third trimester (T3) urinary BPA, peri-adolescent children in the second and highest tertiles had higher (1.26 and 1.81%, respectively) *IGF2* DNA methylation levels. Concurrent BPA tertiles were not significantly associated with peri-adolescent DNA methylation. Despite the detection of differences in DNA methylation, this investigation did not reveal any sexually dimorphic effects.

4.2 Prenatal Exposure to Pb

As discussed, prenatal exposure to Pb in humans is associated with preterm birth [59] and poorer cognitive function in childhood and adolescence [60]. We were unable to find studies that met all three of our criteria; thus the three studies included in this section did not evaluate phenotype in a sexually dimorphic fashion. Sen et al. utilized a nested case-control study in the ELEMENT Cohort to study the impact of the highest and lowest quartiles of prenatal Pb exposure on 5-hydroxy-methylated cytosine (5hmc) and 5-methylated cytosine (5mc) [61]. This group found that sex was significantly associated with 5hmc and 5mc epigenetic profiles such that, as detailed in Table 2, differentially hydroxy-methylated and methylated regions (DhMRs and DMRs, respectively) were either conserved between both males and females, male-specific or female-specific. Furthermore, sex had a greater effect on differential 5mc epigenetic profiles as compared to 5hmc epigenetic profiles. The most striking effect of prenatal Pb exposure was the significant lower levels of 5hmc in the conserved genes *GSTM1* (13%) and *GSTM5* (21%). These genes are highly involved in the detoxification of electrophilic compounds like carcinogens, therapeutic drugs and environmental toxicants [62]. Also of interest, the gene ontology of the genes implicated in the DMRs of 5mc in males fell mainly into the functional categories of telencephalon development and glial cell differentiation, while females fell mostly into the regulation of cellular division. Nye et al. utilized the NEST cohort study to evaluate the impact of prenatal Pb exposure on imprinted genes *H19*, *MEG3*, *PEG3*, and *PLAGL1* in infant cord blood [63]. Ultimately, they found that the highest tertile of maternal Pb exposure was associated with higher DNA methylation at *MEG3*, but not at any of the other imprinted genes.

Goodrich et al. also studied the ELEMENT cohort. Maternal Pb exposure was evaluated using three biospecimens, and childhood Pb exposure was evaluated across three age ranges (1–4, 6–12 and 8–12) to evaluate the impact of Pb exposure on repetitive elements and environmentally responsive genes [58]. Average maternal blood Pb during pregnancy was associated with LINE-1 hypomethylation. Early childhood Pb exposure was associated with lower H19 DNA methylation, while increasing IQR was associated with higher H19 DNA methylation. As expected, males and females responded differently to prenatal Pb exposure. Females who were exposed to increasing amounts of Pb during pregnancy demonstrated lower LINE-1 DNA methylation, while males exposed to the low categories of Pb exposure and the highest category demonstrated higher LINE-1 DNA methylation.

5. Conclusion

Epigenetics have taken center stage in the study of diseases such as cancer, obesity, and neurodegeneration; however, its integration into toxicology and sex effects research is in its infancy. Animal model and human birth cohort studies have revealed key developmental periods that are important for epigenetic programming and are vulnerable to environmental insults. Thus, epigenetic modifications represent a potential mechanism through which sexually dimorphic effects of early-life exposures manifest. As reviewed here, several animal studies, and to a lesser extent human studies, have evaluated life-course sexually dimorphic health effects following developmental exposures to two represented toxicants, BPA and/or Pb; many fewer studies, however, have evaluated epigenetics as a mechanism mediating developmental exposures and later outcomes.

A vast majority of the studies included in this review utilized a target-gene approach in order to investigate epigenetic mechanisms of sex-specific effects, likely due to the high expense or lack of access to platforms for whole-genome analyses. However, analyzing only a few select regions in the genome may result in missing important epigenomic modifications that contribute to sex-specific effects. For example, there are many interactions between sex hormones and metabolic hormones, and analyzing only one or two hormone receptors will not reveal the interactions that may be important as a mechanism of sex-specific effects on metabolism. Additionally, all of the animal studies focused on measuring epigenetic markers in target tissues (e.g., liver) and did not perform epigenetic analyses in biologically available tissues (e.g., blood), making it difficult to bridge findings between animal and human studies. The Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) consortium, a program founded by the National Institutes of Environmental Health Sciences (NIEHS), is aiming to address some of these issues. Consortium members are currently carrying out epigenome-wide analyses in several target tissues and biologically available tissues from male and female mice exposed to a wide variety of EDCs, including Pb and BPA, with the goal of generating a large publicly available database for all researchers to use.

Future studies examining epigenetic reprogramming as a mechanistic link between early-life chemical exposures and sexually dimorphic effects should attempt to meet the following criteria: 1) a well characterized developmental exposure and exposure period that includes relevant windows of epigenetic reprogramming; 2) detection of sex-specific exposure-related epigenetic change; and 3) observation of a sexually dimorphic phenotype in either childhood, adolescence, or adulthood.

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•• Of major importance

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Figure 1. Conceptual model of sex-specific epigenetic reprogramming as a mechanism of sexually dimorphic effects

The perinatal period (including periconception and gestation) is particularly susceptible to endocrine disrupting chemical (EDC) exposures; a factor contributing to this susceptibility is the dynamic epigenetic reprogramming that takes place during development. We propose that sexually dimorphic effects (e.g., obesity) following perinatal exposure to EDCs is due to sex-specific alterations of epigenetic marks (e.g., DNA methylation at a specific gene loci) during epigenetic reprogramming that persist into childhood, adolescence, and/or adulthood to influence health effects (e.g., obesity).

Table 1
Studies Characterizing Sexually-Dimorphic Epigenetic Effects of Perinatal Exposure to BPA and Lead in Rodents

Toxicant	Authors	Objective	Model Organism	Study design	Outcome timepoint	Epigenetic Finding
BPA	(Mao et al., 2017)	To investigate whether perinatal exposure to BPA impairs pancreas function in adulthood, and whether folate supplements could prevent BPA-induced pancreatic dysfunction. Epigenetic mechanisms were examined by measuring DNA methylation at <i>Igf2</i> in islets.	Animal study on Sprague Dawley rats investigating islet cells in the pancreas. N=10 per group per sex	Oral dose of 40 ug/kg-day BPA from GD0 to PND21	Islets isolated from 9-week-old BPA-exposed males had reduced insulin secretion relative to unexposed control males. Islets isolated from females exposed to BPA did not exhibit this effect.	↓DNA methylation at <i>Igf2</i> in BPA-exposed male islets.
	(Strakovsky et al., 2015)	To evaluate whether perinatal exposure to BPA contributes to NAFLD development through altered epigenetic marks and expression of <i>Cpt1a</i> in the liver.	Animal study on Sprague Dawley rats investigating the liver. N=6 per group per sex	Oral dose of 100ug/kg-day BPA from GD6 to PND21, with or without a postnatal HFD (45% fat) challenge	BPA exposure, with or without postnatal HFD challenge resulted in increased hepatic lipid accumulation at PND110 in males. BPA exposure also resulted in increased fat/lean ratio in males between PND60 and PND90, and resulted in increased hepatic triglycerides and FFAs in males at PND1.	In BPA-exposed males: ↑DNA methylation at <i>Cpt1a</i> ↓mRNA expression of <i>Cpt1a</i> ↓H3Ac at <i>Cpt1a</i> ↑H4Ac at <i>Cpt1a</i> ↓H3Me2K4 at <i>Cpt1a</i> ↓H3Me3K36 at <i>Cpt1a</i>
	(Kundakovic et al., 2013)	To examine behavioral outcomes of perinatal BPA exposure and to characterize BPA-related DNA methylation changes at <i>Esr1</i> in the brain.	Animal study on BALB/c mice investigating the brain. N=6 per group per sex for gene expression and DNA methylation analyses N=8–12 for behavioral analyses	Oral dose of 2, 20, and 200 ug/kg-day BPA from GD0 to GD19	BPA exposure reversed sexually dimorphic play behaviors, hyperactivity, and anxiety-like behaviors between PND30 and PND70.	Prefrontal cortex: ↑DNA methylation at <i>Esr1</i> in males dosed with 20 ug/kg-day BPA Hypothalamus: ↓DNA methylation at <i>Esr1</i> in females dosed with 20 ug/kg-day BPA
	(Anderson et al., 2017)	To elucidate epigenome-wide alterations following perinatal BPA exposure and to identify candidate genes in DMRs to assess DNA methylation following a biological pathway analysis. To measure DNA	Animal study on Viable yellow agouti mice (non-agouti offspring) investigating the liver.	Dietary exposure of 50 ng, 50 ug, and 50 mg BPA/kg diet from 2 weeks prior to GD0 through PND 21.	10 months	BPA exposed females: ↑DNA methylation Jak-2 and Rxr

Toxicant	Authors	Objective	Model Organism	Study design	Outcome timepoint	Epigenetic Finding
		methylation at candidate genes as a mediating factor of previously measured metabolic phenotypes	N=5–6 per group per sex			DNA methylation as a mediator in females; methylation status associated with ↓ energy expenditure
Pb	(Montrose et al. 2017)	Determine the epigenetic profile of 4 selected mouse IAP retrotransposon elements by pyrosequencing in the adult (10 months of age) mouse brain and kidney following perinatal lead exposure	A ^{vy} Mice	Maternal exposure of 0 (n=22), 2.1 (n=19), 16 (n=26), or 32 (n=26) ppm lead in water from 2 weeks prior to mating through lactation	10 months	Sex-specific methylation profiles in some but not all IAP elements measured in brain and kidney
	(Sanchez-Martin et al. 2015)	To determine if perinatal lead causes persistent DNA methylation changes in Brain cortex and hippocampus of 60 day old mice	C57BL/6 Mice	Maternal exposure of 0 (n=16), 3 (n=16), or 30 (n=16)ppm lead in water from 2 months prior to mating through lactation	PND60	Comparing exposed (both 3 and 30 ppm) to control females but not males displayed hypermethylated regions of the hippocampus Trend towards negative correlation between methylation and mRNA expression in the hippocampus of female mice in the 3ppm exposure group
	(Faulk et al. 2013)	Evaluate the effect of perinatal lead exposure on bodyweight and epigenetic patterning by pyrosequencing at PND22	A ^{vy} Mice	Maternal exposure of 0 (n=39), 2.1 (n=41), 16 (n=48), or 32 (n=42) ppm lead in water from 2 weeks prior to mating through lactation	PND22	Males trended toward exposure-related weight increase Modest cubic trend for IgF2 methylation in males but not females

Table 2
Studies Characterizing Sexually-Dimorphic Epigenetic Effects of Perinatal Exposure to BPA and Lead in Humans

Toxicant	Authors	Objective	Study Type	Exposure	Outcome	Epigenetic Finding
BPA	(Goodrich et al., 2016)	To determine the epigenetic impact of prenatal and childhood exposure to BPA.	Cohort Study: ELEMENT BPA metabolites measured in 3 rd trimester maternal urine samples. Participants followed up between ages of 7–14. N=247	BPA exposure, 31% of maternal and 15% of child urinary BPA concentrations fell below LOQ. Samples divided into tertiles.	DNA methylation of repetitive elements and environmentally responsive genes.	Since 31 and 15% of maternal and child urinary BPA concentrations fell below the LOQ, associations of tertiles of BPA exposure with DNA methylation were examined. Compared with the lowest tertile of maternal T3 urinary BPA, children in the second and highest tertiles had ↑ (1.26 and 1.81%) <i>IGF2</i> methylation.
Pb	(Sen et al., 2015)	To investigate the relationship between Pb exposure and 5mc and 5hmc modifications during early development.	(1) <i>In vitro</i> human embryonic stem cell model of Pb exposure. (2) Cord blood from 48 mother-infant pairs in ELEMENT. Nested case-control of highest and lowest quartiles of Pb exposure $n=24$ 1 st $n=24$ 4 th	(1) hESCs exposed to either 0.8 μM (16 μg/dL) and 1.5 μM (32 μg/dL). Distilled water was vehicle control. (2) Prenatal lead exposure, measured at birth.	5mc and 5hmc changes.	<p>5mc Conserved ↑ (3%) PIK3R1</p> <p>5mc Male ↓ (4%) GLI2 ↓ (3%) FGF20 ↑ (4%) SLITRK5 ↓ (2%) TOP1MT</p> <p>5mc Female ↓ (6%) MBP ↑ (1%) SLC2A1 ↑ (4%) GJB3</p> <p>5hmc Conserved ↓ (13%) GSTM1 ↓ (21%) GSTM5</p> <p>5hmc Male ↓ (11%) PEG10/SGCA ↑ (3.8%) GOLPH3</p> <p>5hmc Female ↓ (11%) MEST ↑ (3%) DDAH2</p>
	(Nye et al., 2016)	To investigate whether prenatal Pb exposure alters DNA methylation of imprinted genes resulting in lower birth weight and rapid growth.	Cohort study: Newborn Epigenetic Study (NEST) Maternal peripheral blood, collected at ~12 wks gestation. Infant cord blood collected at birth. N= 321.	Prenatal Pb exposure.	DNA methylation at <i>H19</i> (4 CpGs), <i>MEG3</i> (8 CpGs), <i>PEG3</i> (10 CpGs), and <i>PLAGL1</i> (6 CpGs).	Highest tertile maternal Pb exposure, ↑ DNA methylation <i>MEG3</i> ($\beta= 1.57$, se= 0.82, p= 0.06).

Toxicant	Authors	Objective	Study Type	Exposure	Outcome	Epigenetic Finding
	(Li et al., 2016)	To determine whether maternal, postnatal, and early childhood lead exposure can alter the differentially methylated regions (DMRs) that control the monoallelic expression of imprinted genes involved in metabolism, growth, and development.	Cohort Study: Cincinnati Lead Study Serial blood levels collected from birth to 78 months. Blood collected in adulthood in same subjects. N=105 (64 males, 41 females).	Prenatal/early childhood lead exposure.	DNA methylation at differentially methylated regions of 22 human imprinted genes.	Mean blood Pb concentration from birth to 78 months: ↓ PEG3 DMR methylation, stronger in males than females. ↑ mean childhood blood Pb, ↓ IGF2/H19 DMR methylation, primarily in females. ↑ blood Pb conc. during neonatal period, ↑ PLAGL1/HYMAI DMR methylation.
	(Goodrich et al., 2016)	To determine the epigenetic impact of prenatal and childhood exposure to lead	Cohort Study: ELEMENT: Participants followed up between ages of 7–14. N=230	<i>In utero</i> Pb exposure: (1) avg. maternal blood, at least two trimesters (2) maternal tibia 1 month post-partum (3) maternal patella 1 month post-partum. Childhood Pb exposure: (1) early childhood (1–4 years of age) (2) late childhood (6–12 years of age) (3) peri-adolescence (7–14 years of age).	DNA methylation of repetitive elements and environmentally responsive genes.	High Pb at all three windows of exposure, ↑ (1.32%) H19 . Females with ↑ Pb exposure during pregnancy (HHL, HLH, or HLL), ↓ LINE-1 DNA methylation. Males in the low pregnancy Pb category (LHH, LHL, or LLH) and the high category (HHH), ↑ LINE-1 DNA methylation compared with LLL males. Average maternal blood Pb during pregnancy, ↓ LINE-1 DNA methylation. IQR ↑ maternal blood Pb, ↓ (0.29%) LINE-1 methylation ($P = 0.04$). Early childhood Pb exposure, ↓ H19 DNA methylation. IQR ↑ early childhood blood Pb, ↑ (0.62%) H19 methylation. LINE-1 methylation ↓ with ↑ maternal tibia Pb ($P = 0.03$) but ↑ with early childhood blood Pb ($P = 0.006$). HSD11B2 hypermethylation associated with maternal patella Pb among males ($P = 0.016$).