Obesity is a complex and multifactorial disease, which likely comprises multiple subtypes. Emerging data have linked chemical exposures to obesity. As organismal response to environmental exposures includes altered gene expression, identifying the regulatory epigenetic changes involved would be key to understanding the path from exposure to phenotype, and provide new tools for exposure detection and risk assessment. In this report, we summarize published data linking early-life exposure to the heavy metals, cadmium and lead, to obesity. We also discuss potential mechanisms, as well as the need for complete coverage in epigenetic screening to fully identify alterations. The keys to understanding how metal exposure contributes to obesity are improved assessment of exposure and comprehensive establishment of epigenetic profiles that may serve as markers for exposures.

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Keywords: cadmium • DNA methylation • epigenetics • lead • obesity

Approximately 17% of US children and 35% of adults are obese [1], and annual expenditures attributable to obesity and related care exceed US$190 billion [2]. Obese children are more likely to be obese as adults, and its comorbid conditions include Type 2 diabetes, hypertension and cardiovascular disease [3]. Established risk factors are genetic predisposition and energy imbalance, defined as higher caloric intake compared with output. These factors alone, however, do not fully account for the magnitude and rapid increase in the incidence of obesity, especially in early life. A compelling hypothesis receiving consideration posits that increased exposure to epigenetically disruptive chemicals during key developmental stages causes stable epigenetic alterations that may promote obesity. Due to their endocrine-disrupting properties, environmental pollutants including the heavy metals, cadmium and lead, are being investigated as risk factors for obesity. Assessing whether exposure to these chemicals increases obesity risk remains a challenge. Low-level exposure to heavy metals often elicits no immediate symptoms and there is often a long latent period between exposure and obesity outcomes. These exposures may occur as early as the prenatal period while obesity in children may not become evident until middle childhood.

Common heavy metals such as cadmium and lead are ubiquitous environmental pollutants. They frequently co-occur in the environment, and are ranked in the top ten environmental chemicals of concern by environmental health agencies [4]. This concern is driven by well-documented effects of exposure to these heavy metals on neurodevelopmental outcomes. Cadmium or lead exposure increases the risk for both neurodevelopmental disorders [5–8] and lower birth weight [9–12]. Lower birth weight, followed by rapid weight gain is a consistent risk factor for cardiometabolic impairment later in life, such as cardiovascular disease, Type 2 diabetes,
hypothesis, these relationships have been complicated by several methodological shortcomings, including short-term follow-up in contemporary cohorts and the inability to account for competing risk factors for cardiometabolic and neurodevelopmental disorders in older cohorts, complicating causal inference. Given the substantial cost to patients and the healthcare system associated with obesity and its sequelae, including cardiometabolic diseases, it is imperative that biomarkers are found that identify individuals at risk of obesity early in development so it can be more effectively prevented.

Because the etiology of obesity is multifactorial, a potential way of addressing this challenge is to identify epigenetic alterations that occur in response to risk factors such as heavy metal exposure, and to delineate those patterns associated with obesity. Since altering epigenetic gene regulation is a way in which organisms normally respond to environmental change, the identification of these epigenetic modifications has the potential to clarify the etiology of obesity. These epigenetic alterations can also contribute to defining obesity subtypes [16,17] or endotypes that are likely to be responsive to different interventions, if such endotypes exist. To accomplish this, however, requires the gathering of data that demonstrate relationships between epigenetic marks and both obesity and exposure. Alterations in DNA methylation – the most studied epigenetic modification in humans – are proposed to be useful in providing mechanistic insights and identifying stable exposure biomarkers [18–20]. In this report, we discuss the current research examining the epigenetic alterations associated with childhood obesity, developmental exposure to cadmium or lead, potential mechanisms at play and the potential role that cadmium- or lead-induced epigenetic dysregulation has on obesity and cardiometabolic outcomes.

**Childhood obesity & epigenetics**

The evidence is mounting that DNA methylation alterations at regulatory regions contribute to early onset obesity [21–24]. DNA methylation in the promoter region of genes has been studied extensively because increased methylation of regions leads to transcriptional silencing [25,26]. We conducted a literature search in PubMed using the keywords ‘child, obesity, and epigenetics and/or methylation’. The search generated 168 results primarily of reviews and earlier reports on Prader–Willi syndrome, a genetic disorder associated with hyperphagia and obesity, and 24, for the purposes of our review, were relevant original research articles. Summaries of these articles are included in reverse chronological order in Table 1.

In targeted analyses using bisulfite-sequenced DNA, promoter regions of genes known to be involved in obesity or its correlates, such as dyslipidemia or hyperglycemia [29,33,36–39,41,46] and regulatory regions of imprinted genes [40,42,45,51] were among the first epigenomic regions to be interrogated. Regulatory regions of genomically imprinted genes are characterized by parent-of-origin methylation that controls gene expression. Using DNA methylation measurements, imprint control regions associated with obesity include *ZAC1* (*PLAGL1/HYMA1*), a putative nodal regulator of a large network of growth effector genes [52], and *IGF2/H19*: these genes are involved in growth regulation, lipid distribution and early obesity [42,45,53,54]. Data from targeted analyses also support that obesity in children is associated with differential DNA methylation in the regulatory regions of multiple genes, some not imprinted. These include *POMC, FAIM2, BDNF, HIF3A* and the *IGF2/H19* imprinted domain [29,32,33,36,37,41,42,45,46]. One study utilized a combination of *in vitro* and *in vivo* experimental approaches to evaluate the role of SOX6 in adipogenesis. The authors reported that SOX6 was an enhancer of adipogenesis through its regulation of adipogenic genes such as *MEST, PPARy, C/EBPα* and FABP4. SOX6 expression was higher in adipocytes from small for gestational age (SGA) neonates. CpGs adjacent to putative SOX6-binding sites in the *MEST* promoter were hypomethylated in SGA-differentiated adipocytes with increased expression of *MEST*. SGA has been shown to be a risk factor for obesity. In mice, SOX6 was also shown to regulate lipid metabolism where *Sos6* knockdown reduced serum and liver triglycerides and serum cholesterol levels. Loss of Sox6 in zebrafish larvae also resulted in reduced adipogenesis [55]. These data support the role of epigenetics in the genesis of obesity; however, the regions interrogated thus far remain limited.

Agnostic experimental approaches, primarily using array technology of preselected CpG dinucleotides, have also identified regions associated with obesity in children. These studies utilized the 450K methylation array [28,30–32] or 385K methylation array [35], and alternative and older methods: the 27K methylation array, GoldenGate, MassARRAY [34,47,48,50,56] and global methylation [44,49]. Consistent relationships have been found between *LINE-1* hypomethylation and obesity [44,49]. Gene-specific methylation associated with obesity that were identified using these agnostic approaches include *CORO7* [34], *FZD7, PRLHR, EXOSC4* and *EIF6* [35], as well as *TAOK3, PIWIL4* and *FYN* [30]. Furthermore, differential DNA methylation of miRNA-coding regions in obese compared with nonobese children were identified [28] as were differences in the distribution of differentially meth-
| Author (year) | PMID   | Study location | Sample size/characteristics | Measured outcomes | Tissue source and assay type | Results | Ref. |
|--------------|--------|----------------|-----------------------------|-------------------|------------------------------|---------|------|
| Dalgaard (2016) | 26824653 | Germany | n = 18 obese and n = 22 nonobese children ages 2–15 years (prepubertal) | Mice: glucose tolerance, basal metabolic rate, levels of fasting plasma hormones, fatty acids, adipokines, adipocyte histology, size, and number | Mice: perigonadal white adipose tissue. Humans: subcutaneous white adipose tissue; qRT-PCR, RNA-seq, and reduced representation bisulfite sequencing | Trim28 dependent network can trigger obesity in an on/off manner. An obesity 'on' position is associated with the reduced expression of Nnat, Peg3, Cdkn1c, and Plagl1. Humans cluster into Trim28 associated subpopulations | [27] |
| Mansego (2016) | 26780939 | Spain | n = 12 obese and n = 12 nonobese children and n = 95 in validation sample | BMI | Peripheral blood leukocytes; 450K and validation through MassARRAY Epityper | 16 differentially methylated CpGs identified between obese and nonobese children. Three miRNAs, mir-1203, 412, and 216A were associated with BMI. KEGG pathway analysis identified 19 obesity related biological pathways | [28] |
| Wang (2015) | 26717317 | China | n = 110 severely obese and n = 110 nonobese children ages 7–17 years, age and sex matched | Height, weight, hip and waist circumference, fasting levels of glucose, total cholesterol, triglycerides, HDL-C, LDL-C, ALT | Peripheral blood leukocytes; MassARRAY Epityper on HIF3A | HIF3A methylation is associated with childhood obesity and is positively associated with ALT levels independent of BMI | [29] |
| Huang (2015) | 26646899 | Australia | n = 54 severely obese and n = 54 nonobese children (each group pooled for methylation analysis). For validation, n = 78 obese and n = 71 nonobese children with mean age: 12–13 years (which includes the discovery set) | BMI, fasting insulin and glucose, blood pressure, cholesterol, LDL, HDL, triglycerides | Pooled DNA from whole blood; 450K and pyrosequencing for validation on individual samples | 129 differentially methylated CpG loci in 81 genes with >10% difference in methylation. Candidate genes validated and identified include FYN (hypermethylated), PIWI4, TAOK3 (hypomethylated) | [30] |
| Cao-Lei (2015) | 26098974 | Canada | n = 31 (19 male and 12 female adolescents at mean age 13.3 years) | Height, weight, waist circumference | T cells from blood; 450K | Prenatal maternal stress is associated with BMI and central adiposity and is mediated by DNA methylation of genes in Type 1 and 2 diabetes pathways with a potentially protective role | [31] |

ALT: Alanine aminotransferase; ASMM: Allele-specific methylated multiplex; DMC: Differentially methylated CpG site; DMR: Differentially methylated region; DVC: Differentially variable CpG site; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MS: Methylation specific; PAH: Polycyclic aromatic hydrocarbon; qRT: Quantitative reverse transcriptase; RTQ: Real-time quantitative.
Table 1. Relationship between childhood obesity and its correlates and epigenetic alterations (cont.).

| Author (year) | PMID         | Study location | Sample size/characteristics | Measured outcomes | Tissue source and assay type | Results                                                                 | Ref. |
|---------------|--------------|----------------|------------------------------|-------------------|-----------------------------|--------------------------------------------------------------------------|------|
| Pan (2015)    | 26011824     | Singapore      | n = 991 infants (weight and subscapular and triceps skinfolds measured between birth and 24 months) | Weight, length, and subscapular and triceps skinfold | Umbilical cord tissue; 450K | Reported positive association between HIF3A methylation, birth weight, and adiposity | [32] |
| Wu (2015)     | 25922107     | China          | n = 59 obese and n = 39 nonobese children ages 8–18 years | BMI, glucose, total cholesterol, triglycerides, HDL, LDL. Questionnaire about sedentary behavior and physical activity | Peripheral blood leukocytes; MassARRAY Epityper on FAIM2 promoter | Associations between FAIM2 promoter methylation, sedentary behavior, and physical activity in obese children compared to nonobese children | [33] |
| Eriksson (2015) | 25887538    | Greece         | n = 24 obese and n = 23 nonobese pre-adolescent females; n = 11 obese and n = 11 nonobese pre-adolescent males ages 9–13 years | BMI | Peripheral whole blood; 27K | Genome wide DNA methylation reveals lower CORO7 methylation in obese children. In mice Coro7 is expressed in the brain in regions involved with appetite and regulation of energy homeostasis. Studies in drosophila identified increased resistance to starvation with knockdown of pod1 (a homolog of CORO7) and increased expression of pod1 when fed a protein and sugar rich diet | [34] |
| Ding (2015)   | 25871514     | China          | n = 32 obese and n = 32 nonobese children sex and age matched ages 3–6 years | BMI | Peripheral blood leukocytes; 385K and validation of select genes using pyrosequencing | 251 promoters and 575 CpG islands demethylated in obese compared to nonobese children and 141 promoters and 277 CpG islands hypermethylated and a chromosomal imbalance of demethylated promoters and CpG islands on chromosomes 3, 16, 17, and 19 and more differentially methylated promoters and CpG islands on chromosome X over Y. Validated differentially methylated promoters of FZD7, PRLHR, EXOSC4 and EIF6 | [35] |

ALT: Alanine aminotransferase; ASMM: Allele-specific methylated multiplex; DMC: Differentially methylated CpG site; DMR: Differentially methylated region; DVC: Differentially variable CpG site; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MS: Methylation specific; PAH: Polycyclic aromatic hydrocarbon; qRT: Quantitative reverse transcriptase; RTQ: Real-time quantitative.
| Author          | PMID  | Study Location  | n: obese and n: nonobese | Sample size/characteristics | Measured outcomes                              | Tissue source and assay type                                                                 | Results                                                                                                                                                                                                 |
|-----------------|-------|-----------------|--------------------------|----------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Gardner (2015)  | 25779370 | USA             | 32 and 32               | BMI, percent body fat, food and satiety responsiveness | Measured outcomes                              | Tissue source and assay type                                                                 | Results                                                                                                                                                                                                 |
| Wu (2015)       | 25696115 | China           | 59 and 39               | BMI, height, and full metabolic panel | Measured outcomes                              | Tissue source and assay type                                                                 | Results                                                                                                                                                                                                 |
| Yan (2014)      | 25347678 | In vivo (mouse) | 66 and 40 adolescents   | BMI, fasting glucose, cholesterol, triglycerides, leptin, total adiponectin | Measured outcomes                              | Tissue source and assay type                                                                 | Results                                                                                                                                                                                                 |
| Garcia-Cardona (2014) | 24549138 | Mexico          | 106 and 40 female adolescents | BMI, fasting glucose, cholesterol, triglycerides, leptin, total adiponectin | Measured outcomes                              | Tissue source and assay type                                                                 | Results                                                                                                                                                                                                 |
| Azzi (2014)     | 24222450 | South Korea     | 90 mother-infant pairs  | Height, weight, waist circumference, glucose, triglycerides, cholesterol, HDL cholesterol | Measured outcomes                              | Tissue source and assay type                                                                 | Results                                                                                                                                                                                                 |

**Table 1. Relationship between childhood obesity and its correlates and epigenetic alterations (cont.).**

**Abbreviations:** ALT: Alanine aminotransferase; ASMM: Allele-specific methylated multiplex; DMAC: Differentially methylated CpG site; DMR: Differentially methylated region; RTQ: Real-time quantitative
### Table 1. Relationship between childhood obesity and its correlates and epigenetic alterations (cont.)

| Author (year) | PMID      | Study location | Sample size/characteristics | Measured outcomes                                                                 | Tissue source and assay type                  | Results                                                                 | Ref. |
|---------------|-----------|----------------|------------------------------|-----------------------------------------------------------------------------------|---------------------------------------------|------------------------------------------------------------------------|------|
| Deodati (2013) | 23774180  | Italy          | n = 85 obese children age ~11 years | Oral glucose tolerance, blood levels of C-peptide, insulin, and glucose, blood pressure, body composition (DXA scan), height, weight, birth weight, triglycerides, total cholesterol, HDL, LDL, adiponectin and leptin | Blood lymphocytes; Methyl-Profiler DNA Methylation qPCR Assay for IGF2 methylation | Association between the degree of IGF2 methylation and lipid profile in obese children | [42] |
| Xu (2013)     | 23644594  | USA            | n = 48 obese (24 females, 24 males) and n = 48 (sex and age-matched) nonobese African–American youth ages 14–20 years | BMI                                                                                      | Peripheral blood leukocytes; 450K          | Both DMCs and DVCs can predict obesity status                          | [43] |
| Perng (2013)  | 23638120  | Colombia        | n = 553 children ages 5–12 years | BMI-for-age Z-score, waist circumference Z-score, skinfold thickness ratio (subscapular to triceps) Z-score, height-for-age Z-score | Peripheral blood leukocytes; pyrosequencing LINE-1 | Lower LINE-1 methylation associated with adiposity development in male children (BMI and skinfold thickness) | [44] |
| St-Pierre (2012) | 22907587  | Canada         | n = 50 mother–infant pairs      | Birth and placenta weight, height, head and thorax circumferences                  | Maternal and umbilical cord blood and placental tissue biopsy (maternal and fetal sides) intervillous tissue and chorionic villi and fetal villous tissue; pyrosequencing of IGF2–DMR and H19–DMR | Placental DNA methylation changes of IGF2/H19 locus associated with fetal developmental and birth weight | [45] |
| Kuehnen (2012) | 22438814  | Germany        | n = 91 females and n = 80 males obese average age 11 years and n = 55 females and n = 35 males nonobese average age 17.9 years and n = 21 from longitudinal birth cohort study with peripheral blood DNA at ages 5 or 13 years (normal weight) and at 13 or 20 years (obese), and newborn screening cards (peripheral blood DNA from Guthrie spots) | BMI                                                                                      | Peripheral blood; bisulfite sequencing of POMC | DNA hypermethylation variant at intron 2-exon 3 boundary in POMC associated with obesity. POMC exon 3 hypermethylation shown to interfere with the binding of P300, a transcription enhancer leading to a reduction in POMC transcript expression | [46] |

ALT: Alanine aminotransferase; ASMM: Allele-specific methylated multiplex; DMC: Differentially methylated CpG site; DMR: Differentially methylated region; DVC: Differentially variable CpG site; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MS: Methylation specific; PAH: Polycyclic aromatic hydrocarbon; qRT: Quantitative reverse transcriptase; RTQ: Real-time quantitative.
Table 1. Relationship between childhood obesity and its correlates and epigenetic alterations (cont.).

| Author (year) | PMID    | Study location | Sample size/characteristics | Measured outcomes | Tissue source and assay type | Results                                                                 | Ref. |
|---------------|---------|----------------|----------------------------|-------------------|-----------------------------|-------------------------------------------------------------------------|------|
| Relton (2012) | 22431966 | UK             | Two birth cohorts. n = 24 (11–13 years) for gene expression analysis and n = 178 (~9 years) for DNA methylation analysis | BMI, birth weight, and body composition–fat and lean mass (DXA scan) | Peripheral blood and umbilical cord blood; sodium bisulfite pyrosequencing and GoldenGate assay | DNA methylation in umbilical cord blood has some association with altered gene expression, body size and composition in childhood | [47] |
| Almen (2012)  | 22234326 | Greece         | n = 23 obese and n = 24 nonobese pre-adolescent females ages ~10–12 years | Height and weight | Peripheral whole blood; 27K | Methylation level differences in five sites (six genes) between homozygous carriers of normal allele and obesity risk allele of FTO. The authors also identified 20 differentially methylated genes in obese pre-adolescent females | [48] |
| Michels (2011) | 21980406 | USA            | n = 319 mother–infant pairs | Birth weight, gestational age, birth weight/placenta weight ratio, height | Umbilical cord blood; LINE-1 pyrosequencing | Lower LINE-1 methylation levels in infants born with low or high birth weight or born prematurely | [49] |
| Godfrey (2011) | 21471513 | UK             | Two cohorts. n = 78 infants then as 9 year olds and n = 239 infants then as 6 year olds | Adiposity (measured by DXA scan), birth weight | Umbilical cord tissue; MassARRAY EpiTYPER of five candidate genes RXRA, eNOS, SOD1, IL8, and PI3KCD | Higher methylation of RXRA chr9:136355885+ and eNOS chr7:150315553+ associated with childhood fat mass and % fat mass in the first cohort In the second cohort, no association between eNOS + methylation but associations between RXRA + methylation, fat mass and % fat mass | [50] |

ALT: Alanine aminotransferase; ASMM: Allele-specific methylated multiplex; DMC: Differentially methylated CpG site; DMR: Differentially methylated region; DVC: Differentially variable CpG site; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MS: Methylation specific; PAH: Polycyclic aromatic hydrocarbon; qRT: Quantitative reverse transcriptase; RTQ: Real-time quantitative.
lated regions between chromosomes where specific chromosomes were over-represented for demethylation of promoters and CpG islands in obese versus nonobese children [35]. A combination of methylation array and genome-wide genetic variant analysis showed an enrichment for obesity-related genes [43]. The strong relationships between DNA methylation and RNA expression supports the functional significance of many differentially methylated regions identified [57].

While the number of studies is growing, replicating the multiple CpGs identified has remained a challenge. First, these earlier human studies were conducted in DNA obtained from accessible specimens, such as saliva and peripheral blood leukocytes, which may not have direct relevance to obesity, as methylation marks are cell specific. Second, these studies are often underpowered with the majority of reviewed studies interrogating epigenetic marks in <200 individuals. Third, the scope of CpGs investigated thus far using existing array technology is also relatively small compared with the >28 million present in the human genome [58]. Coverage is based on annotated genes, promoters and CpG islands, which excludes most of the genome, including most intergenic regions and large portions of intragenic regions. Also, some imprint control regions are not covered partly due to their distance from genes as well as their low CpG content. Furthermore, comparisons of available data are also complicated by differences in the obesity indicators to which the CpGs are evaluated in different studies, with varying use of overall weight with or without adjusting for height, age or sex, waist circumference or skinfold thickness, and other indicators of early truncal fat accrual. Thus, it is still unclear which CpG dinucleotides are associated with patterns of childhood obesity. Although the identification of epigenomic regions related to childhood obesity has provided clues about the potential pathways leading to obesity in children, a comprehensive analysis tool that captures the entirety of the epigenome, and relating these to specific obesity outcomes, is needed.

As the cost of treating obesity and its comorbidities increases with age, it is critical to identify epigenetic perturbations that occur during early development and use these data to better focus early intervention efforts for obesity endotypes based on epigenetic biomarkers. Identification of such biomarkers will require comprehensive and unbiased screening, with tools such as whole-genome bisulfite sequencing at sufficient depth to measure DNA methylation at most cytosines, including atypical non-CpG sites, to identify obesity related regions. For clinical utility, it will also be important to demonstrate that biomarkers identified in surrogate cell types, accessible without invasive sampling from otherwise healthy humans, are relevant to cell types targeted by the exposure. Only then can methylation marks identified from agnostic approaches be useful in identifying the endotypes of obesity. Recently, this endotyping approach was employed to identify epigenetically labile regions in the peripheral blood of obese asthmatic children [59]. Overlapping genes and pathways identified in these obesity studies could potentially provide patterns of epigenetically dysregulated genes that characterize the obesity endotypes.

**Exposure to cadmium or lead & obesity**

An example of the application of epigenetic endotyping is in addressing the emerging question of whether epigenetic mechanisms mediate, at least in part, observed associations between early exposure to heavy metals and obesity risk in children. Cadmium or lead exposure during the prenatal period has long been associated with lower birth weight and SGA [9–12,60]. Low birth weight, which is often followed by rapid adiposity gain is a consistent risk factor for cardiovascular and metabolic impairment later in life [13]. Some but not all [61–63] human observational studies demonstrate a positive association between lead or cadmium exposure and obesity [64,65] as well as cardiovascular disease or metabolic syndrome [66,67]. In support of these human observations, animal studies of perinatal lead exposure show increased fat mass, body weight or food intake in adulthood [68–71]. Early-life cadmium exposure has also been shown to increase fat mass in male mice. This study utilized the transplantation of fecal microbiota from cadmium-exposed male mice to recipient controls and they exhibited increased fat mass and body fat percentage compared with recipient controls from unexposed control donors [72]. Cadmium exposure has also been associated with altered adipocyte differentiation [73].

Thus far, there are limited data available to demonstrate associations between prenatal or early postnatal exposure to these compounds and subclinical markers of cardiometabolic impairment during childhood. Given the evidence linking cadmium and lead exposure to low birth weight and data linking low birth weight to rapid weight gain and obesity in childhood, it is important to determine if these heavy metals alter the epigenome early in development. If informative epigenetic marks are identified, these marks could serve as a predictive tool for identifying children at risk for obesity or cardiovascular diseases in later life.

A PubMed literature search using keywords ‘child, epigenetics or methylation, and lead exposure or cadmium’ generated 35 results of which five were primary research articles. This was further augmented with a
Table 2. Relationships between cadmium or lead exposure and obesity and its correlates.

| Author (year)       | PMID       | Study location | Sample size/characteristics | Exposure                      | Measured outcomes                                                                 | Results                                                                                                                                                                                                 | Ref. |
|---------------------|------------|----------------|-----------------------------|-------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Ba (2016)           | 27634282   | In vivo (mice) | Early-life cadmium exposure | Adiposity (body fat, lean mass, and total mass), plasma TC, LDL, VLDL, HDL, plasma and liver TG, plasma free fatty acids, plasma leptin, gut microbiota, and hepatic gene expression | In male mice, LDC exposure led to fat accumulation and increased levels of plasma TC, TG, and free fatty acids, and liver TG, alterations in gut microbiota, and hepatic gene expression related to fatty-acid and lipid metabolism was enhanced. Transplant of fecal microbiota from LDC exposed male mice into unexposed male controls led to increased mass and percent body fat in these recipients | [72] |
| Wu (2016)           | 26962054   | In vivo (mice) | Early-life lead exposure    | Gut microbiota composition and body weight | Increased adult body weight in male mice. Decrease of aerobes and increase of anaerobes in lead exposed mice. Changes in gut microbiota and body weight in male mice |                                                                                                                                                                                                               | [70] |
| Cassidy-Bushrow (2016) | 26358768  | USA n = 299 children (ages 2–3 years) | Early-life lead exposure | BMI | Having detectable blood lead levels associated with smaller body size at 2–3 years of age |                                                                                                                                                                                                               | [61] |
| Faulk (2014)        | 25105421   | In vivo (mice) | Early-life lead exposure    | Energy expenditure, spontaneous activity, food intake, body weight and composition and glucose tolerance | Increases in food intake at differing ages for females and males. Increased body fat, body weight, and insulin response in males |                                                                                                                                                                                                               | [68] |
| Delvaux (2014)      | 24742724   | Belgium n = 114 children ages 7–9 years (n = 57 females and 57 males) | Prenatal cadmium exposure | BMI, abdominal fat (waist circumference) and subcutaneous fat (skinfolds) | Inverse association between prenatal cadmium exposure and body weight, BMI, abdominal fat and subcutaneous fat in females |                                                                                                                                                                                                               | [62] |
| Scinicariello (2013) | 24099784   | USA NHANES data 1999–2006 children and adolescents ages 3–19 years | Lead exposure | BMI | Inverse association between blood lead levels and BMI |                                                                                                                                                                                                               | [63] |
| Tian (2009)         | 19404590   | China n = 106 infants measured again at ~4.5 years | Prenatal cadmium exposure | Birth weight and height, weight and height at ~4.5 years, WPPSI-R | Higher levels of cord blood cadmium associated with lower birth weight and length and at ~4.5 years, lower height and WPPSI-R-IQ full scores |                                                                                                                                                                                                               | [11] |
| Leasure (2008)      | 18335103   | In vivo (mice) | Early-life lead exposure    | Body weight, motor activity, dopamine levels | Late onset obesity in 1-year-old male mice and motor abnormalities in male mice |                                                                                                                                                                                                               | [69] |

HDL: High-density lipoprotein; LDC: Low dose cadmium; LDL: Low-density lipoprotein; SGA: Small for gestational age; TC: Total cholesterol; TG: Triglycerides; TMBW: Term mean birth weight; TLBW: Term low birth weight; VLDL: Very low-density lipoprotein.
Table 2. Relationships between cadmium or lead exposure and obesity and its correlates (cont.).

| Author (year) | PMID  | Study location | Sample size/characteristics | Exposure | Measured outcomes | Results                                                                 | Ref. |
|---------------|-------|----------------|-----------------------------|----------|------------------|--------------------------------------------------------------------------|------|
| Berkowitz (2006) | 16376613 | USA | n = 169,878 birth certificate data for five communities in proximity to the Bunker Hill Superfund site | Prenatal lead exposure (due to lead smelter fire). Air emissions of high concentrations of lead | Preterm birth, SGA, TLBW and TMBW among term infants | Maternal lead exposure associated with increased risk of TLBW and SGA, and reduced TMBW | [9] |
| Sanin (2001) | 11331680 | Mexico | n = 329 mother–infant pairs | Early-life lead exposure | Weight at age 1 month and weight gain from birth to 1 month | Maternal lead burden inversely associated with infant weight at one month of age and weight gain between birth and one month of age | [60] |
| Gonzalez-Cossio (1997) | 9346987 | Mexico | n = 272 mother–infant pairs | Early-life lead exposure | Birth weight | Maternal bone-lead burden inversely associated with birth weight | [10] |
| Kim (1995) | 8529592 | USA | n = 236 at age ~7 years (1975–1978) and follow-up 13 years later n = 58 at age ~20 years (1989–1990) | Lead exposure | Weight and height | Dentin lead levels were positively associated with BMI in 1975–1978 and increase in BMI between 1975–1978 and 1989–1990 | [64] |

HDL: High-density lipoprotein; LDC: Low dose cadmium; LDL: Low-density lipoprotein; SGA: Small for gestational age; TC: Total cholesterol; TG: Triglycerides; TLBW: Term low birth weight; TMBW: Term mean birth weight; VLDL: Very low-density lipoprotein.
| Author (year) | PMID | Study location | Sample size/characteristics | Exposure | Measured outcomes | Tissue source and assay type | Results | Ref. |
|-------------|------|----------------|----------------------------|----------|------------------|-----------------------------|---------|-----|
| Nye (2016)  | NA   | USA            | n = 321 mother–infant pairs | Prenatal lead exposure | Birth weight, changes in WHZ between birth to 1 year, 1–2 years, and 2–3 years of age, and DNA methylation | Peripheral blood leukocytes (umbilical cord); pyrosequencing of H19, MEG3, PEG3, and PLAG1 DMRs | Prenatal lead exposure inversely associated with birth weight, positively associated with WHZ change by 2–3 years, and hypermethylation at the MEG3 DMR regulatory region | [74] |
| Sen (2015)  | 26417717 | USA            | n = 35 mother–infant pairs | Prenatal lead exposure | DNA methylation | Dried blood spots: MNBS, CNBS, CCBS; 450K | 564 loci with altered DNA methylation in the CNBS of children whose mothers had high neonatal blood lead levels | [75] |
| Vidal (2015) | 26173596 | USA            | n = 319 mother–infant pairs | Prenatal cadmium exposure | Birth weight and DNA methylation | Peripheral blood leukocytes (umbilical cord); pyrosequencing of IGF2/H19, MEG3, MEST, NNAT, PEG3, SGCE/PEG10, and PLAG1 | Higher maternal cadmium levels associated with lower birth weight and lower DNA methylation at the PEG3 DMR in female infants | [12] |
| Li (2016)   | 26115033 | USA            | n = 64 females and n = 41 males ages 25–30 years (Blood lead concentration data available for these individuals at ages birth to 78 months) | Early-life lead exposure | DNA methylation of 22 imprinted genes | Peripheral blood leukocytes; MassARRAY EpiTYPER | Early-life lead exposure associated with sex-dependent DNA methylation differences in the imprinted gene DMRs of PEG3, IGF2/H19, and PLAG1/HYMA1 | [76] |
| Sen (2015)  | 26077427 | USA            | n = 25 males and n = 18 females from ages 3 months to 5 years | Early-life lead exposure | DNA methylation | Dried blood spots; 450K | Early-life lead exposure leads to 5-mC clustering into three sub-types: sex-specific and conserved. In the conserved subtype, increased DNA methylation around the transcription start site of LEP was identified. HIF3A is among the genes differentially methylated and associated with lead exposure in females | [77] |

CNBS: Child neonatal blood spots; CCBS: Child’s current blood spot; Dnmts: DNA methyl-transferases; hESCs: Human embryonic stem cells; MIRA: Methylated CpG island recovery assay; MNBS: Maternal neonatal blood spots; NA: Not available; WHZ: Weight-for-height Z score.
Table 3. Relationships between cadmium or lead exposure and epigenetic alterations (cont.).

| Author (year) | PMID      | Study location | Sample size characteristics | Exposure | Measured outcomes | Tissue source and assay type | Results                                                                 | Ref. |
|---------------|-----------|----------------|----------------------------|----------|------------------|----------------------------|-------------------------------------------------------------------------|------|
| Sen (2015)    | 26046694  | Mexico         | n = 24 female and n = 24 male infants and *in vitro* (hESCs) | Prenatal lead exposure | DNA methylation | Umbilical cord blood; 450K and MeDIP-450K (modified 450K) | Lead exposure associated 5-mC and 5-hmC clusters identified. These can be divided into sex-independent and sex-dependent categories with possible roles as early biomarkers of lead exposure | [78] |
| Senut (2014)  | 24519525  | In vitro (hESCs) | Lead exposure | Neuronal differentiation and DNA methylation | hESCs; 450K | Lead exposure affects neuronal differentiation of hESCs altering number and morphology of generated neurons. Lead exposure also alters DNA methylation of genes involved in neuro-developmental pathways | [112] |
| Sanders (2014) | 24169490  | USA n = 17 mother–infant pairs | Prenatal cadmium exposure | DNA methylation | Maternal venous blood and umbilical cord blood; MIRA | Cadmium exposure associated patterns of DNA methylation in maternal and newborn DNA | [80] |
| Faulk (2013)  | 24059796  | In vivo (viable yellow agouti [A^vy] mice) | Early-life lead exposure | Body weight and DNA methylation | Tail DNA; coat color classification and pyrosequencing of imprinted *Igf2* and *Igf2r*, and metastable epiallele loci *Cabp* and *Avy* | Dose and sex-specific effects were identified. Increase in wean body weight in males developmentally exposed to lead. Male specific effects at A^vy locus. Altered coat color in A^vy/a offspring | [71] |
| Kippler (2013) | 23644563  | Bangladesh     | n = 127 mother–infant pairs and n = 56 children age 4.5 years | Prenatal cadmium exposure | Birth weight and DNA methylation | Umbilical cord blood and blood mononuclear cells from the 4.5-year-old children; 450K | Maternal cadmium exposure associated with sex-specific changes to DNA methylation. CpG sites associated with cadmium exposure identified in both newborns and 4.5 year old children and cadmium-associated changes in methylation related to lower birth weight | [81] |

CNBS: Child neonatal blood spots; CCBS: Child’s current blood spot; Dnmts: DNA methyl-transferases; hESCs: Human embryonic stem cells; MIRA: Methylated CpG island recovery assay; MNBS: Maternal neonatal blood spots; NA: Not available; WHZ: Weight-for-height Z score.
search for articles in the reference sections of relevant papers pertaining to epigenetics, birth weight or adiposity generating 18 additional articles, for a total of 23 articles included in Tables 2 & 3.

Studies of targeted methylation analysis of DNA from human embryonic kidney cells exposed in culture to lead, and tissues exposed in vivo to lead report epigenetic perturbations at the regulatory regions of imprinted genes and altered expression of DNA methyltransferases [79,82,83]. Targeted DNA methylation analysis identified differential methylation at the imprinted loci of PEG3, PEG1/MEST, IGF2/H19 and DLK1/MEG3 that is attributable to prenatal cadmium or lead exposure, and associated with dysregulated growth outcomes in both mice and humans [12,71,73,74].

In humans, agnostic approaches using global methylation screening of Alu and LINE-1 elements demonstrated hypomethylation of LINE-1 related to increased patellar lead levels [84,85]. Sex-specific effects resulting from cadmium [81] and lead [77,78] exposure as well as multigenerational effects from lead exposure [75] have also been reported.

For future work to be comprehensive, tools such as whole-genome bisulfite sequencing are needed to identify DNA methylation patterns and genes that are dysregulated by exposure to cadmium or lead in cell types relevant to the genesis of obesity. It will also be important to identify the overlap in epigenetic profiles associated with cadmium or lead exposure and those associated with obesity.

**Potential mechanisms by which cadmium & lead may alter obesity risk**

Cadmium and lead have well-established roles as neurotoxins impacting neurodevelopment [86–88]. The relationship between obesity and brain function is also established [89,90]. The role of neurodegeneration on obesity mediated by neurotoxic heavy metals was reviewed [91]. One mechanism by which heavy metal exposure might lead to obesity may involve the effects of metal neurotoxicity on brain function and signaling related to appetite and satiety. Since brain development is affected by both lead and cadmium, a disruption in energy balance could result from dysregulated appetite and satiety response, with consequent increased caloric intake. For example, both cadmium and lead exposures have been shown to reduce the levels of BDNF [8,92,93], an obesity related gene that regulates energy balance [94]. Meanwhile, lower methylation of BDNF promoter in the salivary DNA of obese adolescents has also been reported [36], while increased adiposity is related to decreased levels of circulating BDNF [95]. Likewise, prenatal lead exposure results in decreased sponta-
Figure 1. Hypothesized relationships linking exposure, epigenetics and obesity. This schema summarizes the hypothesized relationships between an exposure such as to heavy metals and increased risk of obesity and its comorbidities including cardiovascular disease, Type 2 diabetes and dyslipidemia. Epigenetic alterations may provide a means by which metal exposure alters obesity risk, but the known neurodevelopmental effects and remodeling of gut microbiota by metal exposures may also contribute, influencing behavior and metabolism. Bidirectional interactions between neurodevelopmental effects, the microbiome and the epigenome could together alter each of these factors to individually or synergistically contribute to obesity. The suggested complexity of interactions highlights the need for comprehensive ascertainment of exposure and their effects.

Conclusions
Epigenetics can be a powerful tool in understanding the etiology of complex diseases. In the context of obesity, a multifactorial and chronic disease, epigenetic patterns may contribute to delineating the pathways that contribute to comorbidities and severity. Furthermore, connecting exposure and effect is often challenging and epigenetics has the potential to elucidate relationships between the two. In this report, we review and discuss the utility and application of comprehensive DNA methylation analysis as an epigenetic screening tool in childhood obesity, however, methodological shortcomings remain.

Future perspective
This report provides a summary of how patterns of epigenetic response could be used to characterize early exposure to ubiquitous environmental toxicants of concern such as cadmium and lead. This approach could be useful in endotyping obesity, improving exposure assessment, identifying epigenetic profiles to serve as indicators for specific heavy metal exposures and also for clarifying the role of the microbiota–gut–brain axis. Furthermore, studying populations with known exposures to heavy metals [9,10] and a higher incidence of obesity [11] may help expand and clarify these links. This can only be accomplished by increasing the capability of next-generation sequencing to produce whole-genome methylation maps from humans and animal models for multiple exposures known to be risk factors for common chronic diseases including obesity. We anticipate that these methylation maps will have the ability to:
subdivide obesity phenotypes; and provide gene targets for expression studies and therapeutic intervention. We also anticipate that animal models will soon determine the extent of epigenetic alterations due to chronic low-level exposure to heavy metals, and alterations in the gut–brain axis. It is in this context that human studies can identify, with specificity, heavy metal-induced epigenetic changes that occur during early development that contribute to obesity risk. Overall, the identification of epigenetic alterations in response to environmental exposures such as cadmium and lead exposure will elucidate mechanisms that may be involved in the genesis of obesity and cardiometabolic disease, allow for exposure detection, and provide a new means for reducing obesity incidence and its severity.

Executive summary

• Epigenetic alterations as a consequence of early exposure to ubiquitous environmental pollutants, such as cadmium and lead, may contribute to our understanding of the development of obesity, and effects on lifetime risk.
• A mechanism by which early cadmium or lead exposure could initiate obesity is through its neurotoxic role as the brain is a target for both metals. Altered brain function could lead to subsequently dysregulated appetite, impulsivity and lack of satiety, thereby resulting in increased caloric intake and altered energy expenditure.
• The role of the gut microbiome on obesity in the context of cadmium or lead exposure, is another area that warrants further investigation.
• Changes in the epigenome may provide insight into the genesis of heavy metal-induced obesity, and serve as a reliable method for predicting its development.
• The keys to understanding how metal exposure affects obesity are to improve direct exposure assessment, and establish epigenetic profiles that serve as markers for specific exposures.
• This report summarizes studies identifying DNA methylation profiles associated with childhood obesity, and the extent to which they can be used to link early cadmium and lead exposure to obesity, potentially providing novel endotypes of obesity in children.

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