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Epigenetic Transgenerational Inheritance of Obesity Susceptibility

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Abstract

The prevalence of obesity and associated diseases has reached pandemic levels. Obesity is often associated with overnutrition and a sedentary lifestyle, but clearly other factors also increase the susceptibility of metabolic disease states. Ancestral and direct exposures to environmental toxicants and altered nutrition have been shown to increase susceptibility for obesity and metabolic dysregulation. Environmental insults can reprogram the epigenome of the germline (sperm and eggs), which transmits the susceptibility for disease to future generations through epigenetic transgenerational inheritance. In this review, we discuss current evidence and molecular mechanisms for epigenetic transgenerational inheritance of obesity susceptibility. Understanding ancestral environmental insults and epigenetic transgenerational impacts on future generations will be critical to fully understand the etiology of obesity and to develop preventative therapy options.

Origins of the Obesity Epidemic

Obesity is rapidly increasing in prevalence worldwide, and has become a public health crisis of pandemic proportions. In 2016, 650 million adults over the age of 18 were considered to be obese, with an overall prevalence of 13%[i]. Within the USA, almost 40% of adults and 18.5% of children are obese [1]. The worldwide prevalence of obesity in adults tripled between 1975 and 2016, and the next generation of children are strongly affected. Global obesity rates in children increased from under 1% in 1975 to 6% in girls, and 8% in boys for 2016[i]. Obesity is defined as a body mass index (BMI) of ≥30 kg/m^2, and is associated with several pathologies, including type 2 diabetes, cardiovascular disease, osteoarthritis, nonalcoholic fatty liver disease, kidney disease, and certain cancers [2]. Sixty five percent of the world lives in a country where obesity has a higher mortality rate than malnutrition[ii]. Elevated BMI contributed to 4 million deaths in 2015, and has an estimated global annual cost of US$2 trillion[iii] [3]. Each five-unit increase in BMI above 25 kg/m^2 also increases the overall mortality risk by 29% [4]. Given the public health consequences of obesity, it is imperative to investigate the etiology and pathogenesis of the disease.

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Resources

[i] www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight

[ii] http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf

[iii] www.mckinsey.com/industries/healthcaresystems-and-services/our-insights/how-the-world-could-better-fightobesity

There have been several proposed etiologies for the obesity pandemic. Traditionally, obesity has been attributed to overnutrition and a sedentary lifestyle; however, over the past decade, it has become clear that additional factors are involved [5]. Between 1998 and 2006, BMI increased by 2.3 kg/m^2 on average in the USA when controlling for dietary intake and exercise levels [6]. In addition, Brown *et al.* [6] found that leisure time spent on exercise in fact increased between 1988 and 2006, indicating that other causes should be investigated [6]. Other potential direct causes of obesity proposed include changes in the gut microbiome [7], effects of air conditioning on thermogenesis [8], chronic sleep deprivation [9], and certain pharmaceutical drugs inducing weight gain [5]. Numerous genome-wide association studies (GWAS) have also been performed to identify a genetically based increased susceptibility to obesity; however, obesity-related genetic variants are limited in predictive power and only account for ~3% of BMI variance [10,11]. In addition, while BMI is commonly used to assess obesity in population-wide studies, the accuracy of BMI in assessing metabolic and cardiovascular health has come into question [12], as discussed further in Box 1.

Epigenetics and the Developmental Origins of Health and Disease

Several recent studies have implicated that a variety of exposures in early life and *in utero* can change metabolism. Barker and colleagues made observations of this phenomenon with several epidemiological studies showing that infants born small for gestational age have an increased susceptibility to cardiovascular disease and metabolic dysfunction [13,14]. The ‘thrifty phenotype hypothesis’ was then proposed from observations of increased adiposity and decreased fat mobilization following poor fetal nutrition [15]. Environmental insults during early development can influence cellular plasticity, thereby increasing the risk of chronic diseases later in life, including obesity and its comorbidities [14,16–18]. The epigenetic mechanisms behind this developmental reprogramming are discussed in Box 2.

One of the most relevant examples of this phenomenon comes from a cohort of patients with severe acute malnutrition between 1963 and 1993 at the University of the West Indies in Kingston, Jamaica, a population used to study the potential origins of malnutrition phenotypes. Severe malnutrition in childhood can lead to differing body composition comprising edematous (i.e., swollen with excessive accumulation of fluid) (kwashiorkor or marasmic kwashiorkor phenotype) or nonedematous (marasmus phenotype). In the Jamaican population, children who developed kwashiorkor had increased birthweight compared with children with marasmus, which implies that early developmental factors may contribute to the different phenotypes from the same nutritional stressor [19]. The individuals in the study who experienced either marasmus or kwashiorkor were followed into adulthood to determine any metabolic changes. Adult survivors of marasmus had increased postchallenge glucose levels, reduced glucose sensitivity, and worsened β cell function compared with adult survivors of kwashiorkor [20]. A study of adult survivors of malnutrition identified a reduction in BMI and bone mineral density in marasmus survivors compared with kwashiorkor survivors. When admitted as infants, marasmus survivors were identified as having a reduced gestational age at birth, birth weight, weight and height for age, neutrophil count, and lymphocyte count compared with kwashiorkor survivors. **DNA methylation** (see Glossary) analysis was performed on muscle biopsies from survivors of severe acute

malnutrition and identified differential DNA methylation patterns associated with metabolic pathways, which may influence the phenotypic origins of kwashiorkor and marasmus [21]. In summary, when faced with an extreme stressor such as severe acute malnutrition, individuals who had a low birth weight had a more benign phenotype of marasmus compared with individuals with a higher birth weight who developed the edematous kwashiorkor. However, while individuals who had a low birth weight and the marasmus phenotype fared better in famine conditions, they had adverse metabolic outcomes as adults outside of famine conditions compared with kwashiorkor survivors. Therefore, environmental insults during early development can influence the adaptive response to metabolic challenges and increase the risk of metabolic disease in later life.

Epigenetic Developmental Reprogramming

Epigenetic developmental plasticity allows an organism to respond to the surrounding environment during cell differentiation, which changes the phenotype and gene expression without modifying the genetic code [22]. **Epigenetics** involves molecular factors and processes around the DNA that regulate genomic activity independent of the DNA sequence, and are mitotically stable [23,24]. Epigenetic changes involve both DNA and chromatin molecular modifications that change the expression of genes and genome activity [25,26]. Epigenetic modifications include DNA methylation of CpG dinucleotide residues, histone modification, most noncoding (nc)RNAs, RNA methylation, and chromatin structure [23] (Figure 1). DNA methylation of CpG dinucleotides is one of the most well-characterized epigenetic marks, and is generally stable and enduring in somatic cells [24]. However, during critical windows of development, the epigenome goes through cycles of methylation changes to accommodate for specific gene expression patterns needed for embryogenesis and fetal development [27]. For example, reduced methylation state is required to obtain a pluripotent stem cell state during development. Changes in environmental conditions during these critical windows of development, such as nutritional imbalances and environmental toxicants, can disrupt these processes, and permanently alter the DNA methylation patterns of the fetal and subsequent somatic cell epigenomes [27,28]. The history of epigenetics and epigenetic inheritance is presented in Box 3.

Epigenetic Transgenerational Inheritance Mechanisms

Changes in methylation patterns in the germline due to environmental insults can induce a transgenerational phenotype. **Epigenetic transgenerational inheritance** is the germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that leads to phenotypic variation [24,29]. There are two main exposure mechanisms behind epigenetic transgenerational inheritance. Environmental exposures can induce an altered cascade of epigenetic change, such as DNA methylation, in the fetus of gestating females during the developmental period of gonadal development and primordial germ cell migration (Figure 2). Aberrant DNA methylation of the germline can be heritable and is referred to as germline **epimutations** [30]. The exposure of an F0 generation gestating female to an environmental insult also exposes the developing F1 generation embryo (Figure 3). In addition, alterations of the epigenome in the developing germ cells within the F1 generation fetus can influence the F2 generation. If the altered

DNA methylation patterns are heritable to the subsequent F3 generation, the transmission of these epimutations is considered epigenetic transgenerational inheritance [29,31]. Preconception exposure-mediated epigenetic transgenerational inheritance can be induced by exposing the F0 generation male or female to an environmental insult that can affect the epigenome of the germline. The germline, which eventually becomes the F1 generation, has been directly exposed to the environmental exposure, and is not considered to be transgenerational. Therefore, the F2 generation is considered to be the first nonexposed transgenerational offspring in this preconception exposure instance [29] (Figure 3).

The altered germline epigenetics has the potential ability to change the transcriptome and epigenetics of the totipotent cells in the early embryo. This can subsequently alter the epigenetics of all somatic cell types derived from these stem cells. During cellular and tissue differentiation, a cascade of gene expression changes occurs simultaneously with a cascade of epigenetic changes (Figure 2). The early stages of development are more susceptible to environmental insults that alter this cascade of epigenetic change. Therefore, the environmentally altered differential epigenetic state can influence genome activity and the cell type-specific differentiated transcriptome to subsequently increase susceptibility for diseases, such as obesity (Figure 2). The integrated genetic and **epigenetic processes** that occur through these developmental periods establish the physiology and susceptibility to disease in later life stages [23]. Therefore, the environmentally induced epigenetic transgenerational inheritance of disease susceptibility, such as lifestyle and diet, then promotes the susceptibility for diseases, such as obesity.

Evidence for Epigenetic Transgenerational Inheritance

Although non-Mendelian forms of inheritance have been observed over the past century, such as Kammerer’s midwife toad [32] and Waddington’s heat-induced fly wing structure alteration [33], the molecular mechanisms involved were unknown and observations were not considered distinct from genetic inheritance. This was not demonstrated until recently, when more molecular information was available regarding epigenetics (Box 3). One of the first observations of environmentally induced epigenetic transgenerational inheritance was the exposure of gestating rats to the agricultural fungicide vinclozolin, which was found to promote transgenerational male testis disease and germline DNA methylation changes [34]. The number of examples of epigenetic transgenerational inheritance has increased dramatically over the past few decades, and the phenomenon has now been demonstrated in both plants and animals (Figure 4). In plants, partial reprogramming of epigenetic marks in both male and female gametes occurs immediately after fertilization in plants [35]. Both temperature and drought have been shown to promote epigenetic transgenerational phenotypic changes in both flowering and growth characteristics [36,37]. A well-known plant example of epigenetic transgenerational inheritance involves a change in symmetry in *Linaria vulgaris* flowers [38]. Increased DNA methylation in the promoter region of the *Lcyc* locus changed the floral symmetry phenotype from bilateral to radial, and was transgenerationally transmitted for >100 generations [39].

In non-mammalian animals, there are also a variety of species that have demonstrated the capacity for epigenetic inheritance. The model insect *Drosophila melanogaster* has

demonstrated epigenetic transgenerational inheritance in many studies [40–42]. Other members of the phylum Arthropoda, such as *Artemia* [43] and *Daphnia magna* [44], have also been shown to exhibit epigenetic transgenerational inheritance. The nematode, *Caenorhabditis elegans*, has shown transgenerational inheritance of both histone modification and DNA methylation [45,46]. Several species of fish have also demonstrated epigenetic inheritance, such as the zebrafish [47–49] and the pipefish *Syngnathus typhle* [50]. Birds, such as quail [51] and the Muscovy duck [52], have also exhibited environmentally induced heritable changes. While most mammalian research is performed on rodents [24], there have been other examples of epigenetic transgenerational inheritance occurring in domestic pigs [53] and the common marmoset [54]. Several epidemiological studies, such as the Dutch and Swedish Famine Cohorts, have also identified transgenerational inheritance in humans [55,56]. Therefore, epigenetic transgenerational inheritance appears to be a highly conserved adaptive response among various species from plants to mammals (Figure 4).

Numerous environmental insults (Table 1) have been identified to induce epigenetic transgenerational inheritance, including heat exposure [40,43,57,58], salt stress [57], drought [59], stress and trauma [60–63], a high-fat diet (HFD) [64–66], nutritional deprivation [55,56], diabetes and/or prediabetes [67,68], folate [69], smoking [70,71], and alcohol [72,73] (Figure 4). Several studies have revealed that environmental toxicants, including the fungicide vinclozolin [34,74–76]; the herbicides atrazine and glyphosate [77,78]; plasticizers, such as bisphenol A [79,80] and phthalates [80]; the pesticides diethyltoluamide (DEET) with permethrin [81] and methoxychlor [82]; the hydrocarbons jet fuel (JP8) [83] and benzo[a]pyrene [84]; the antifouling agent tributyltin [85]; mercury [47]; and dioxins [86–88], promote increased rates of disease and sperm epimutations. Ancestral exposure to environmental insults have been shown to induce a variety of diseases and phenotypic abnormalities. Transgenerationally increased rates of disease include testis abnormalities [80,89–94], prostate disease [86,89], ovarian disease [80–83,86,92], uterine disease [87,95], kidney disease [82,86,89,96,97], immune system abnormalities [89], and tumor development [89]. More details regarding the history of epigenetics and epigenetic transgenerational inheritance are provided in Box 3.

Epigenetic Transgenerational Inheritance of Susceptibility for Obesity and Metabolic Dysfunction

Transgenerationally increased susceptibility to obesity and its comorbidities has been observed following ancestral exposure to several environmental insults (Table 2). Most studies have investigated the potential for transgenerational inheritance of obesity and metabolic dysfunction through exposure to a HFD and caloric restriction. Ancestral exposure to a maternal HFD was shown to result in paternal transmission of increased body size in F3 generation female mice [64]. The F2 generation male offspring of mice, ancestrally exposed to neonatal lactational over-nutrition, developed glucose intolerance and fasting hyperglycemia [98]. Paternal HFD in the F0 generation of mice was associated with increased adiposity and with alterations in sperm miRNA, with a reduction in total global germ cell methylation in F2 generation offspring [99]. In another study, F2 generation

offspring ancestrally exposed to a paternal HFD had reduced birth weight and resistance to weight gain, with adult females developing glucose intolerance. Additionally, the ancestral paternal HFD was also associated with differential expression of the let-7c miRNA in sperm, and subsequent expression in the adipose tissue of the offspring [100]. Paternal HFD also demonstrated an increase in adiposity and serum leptin in F2 generation males [101]. Ancestral exposure to paternal HFD and streptozotocin induced prediabetes, and also predisposed the F2 generation to impaired insulin sensitivity and glucose intolerance through germline-mediated epigenetic transgenerational inheritance [68]. The daughters of women who experienced the Dutch Hunger Winter of World War II *in utero* had 1.8 times more chronic diseases as adults compared with nonexposed women [102]. The F2 generation grand-offspring of fathers who experienced the Dutch Hunger Winter of World War II had significantly increased BMI compared with exposed mothers or the unexposed population [55]. A preconception paternal exposure transgenerational impact appears in the F2 generation (Figure 3). In Överkalix, an isolated town in Northern Sweden, paternal ancestor nutrition appeared to significantly influence the incidence of cardiovascular disease and diabetes in their grand-offspring [103]. These results indicate that ancestral exposure to HFD and malnutrition (caloric restriction) can promote germline epimutations that can induce a transgenerational obesity and metabolic dysregulation phenotype in future generations.

A recent study by Risal *et al.* [104] observed a fivefold increase in polycystic ovary syndrome (PCOS) in daughters whose mothers also had PCOS. Daughters of women with PCOS had increased free androgen levels as well as metabolic dysfunction-related symptoms, such as increased BMI, waist circumference, and diastolic blood pressure. To determine whether prenatal exposure to androgens can promote PCOS-like symptoms in the transgenerational F3 generation [104], F0 generation mice were exposed to either dihydrotestosterone, a high fat and high sucrose diet, dihydrotestosterone plus a high fat and high sugar diet, or vehicle control. F3 generation androgenized lineage females had increased weight and fat mass, and reduced energy expenditure. PCOS-like reproductive and metabolic dysfunctions were observed in the F3 generation of the androgen lineage, including increased weight, fat mass, adipocyte size, liver triglyceride concentrations, and altered adipogenesis [104]. In the high fat, high sugar diet lineage, metabolic dysfunction was observed in the F1 generation, but was less substantial in the F2 and F3 generations. In the androgen plus high fat and high sugar diet lineage, the embryonic development of the F2 generation was detrimentally affected to the extent that only one female survived and no F3 offspring were obtained [104]. These results indicate that androgen exposure, as well as a high fat, high sugar diet, can promote metabolic dysfunction in the F3 generation. A combination of both exposures appears to be mostly fatal by the F2 generation [104]. Interestingly, single cell RNA-seq identified transcriptomic alterations in the oocytes of F1 through F3 generations of both the obesity and androgen lineages, which may be a potential mechanism for the inheritance of these phenotypes [104]. Further investigation is needed to determine whether the altered transcriptome is associated with epigenetic alterations in the germline required for epigenetic inheritance [23].

Environmental toxicants have also been indicated in the epigenetic transgenerational inheritance of obesity susceptibility. In 2006, Grün and Blumberg developed the term

'obesogen' to refer to environmental toxicants that can induce obesity [105]. Several environmental toxicants have since been shown to induce a transgenerational obese phenotype in the F3 generation of rats following ancestral prenatal exposure. A recent study showed that rats ancestrally exposed to a cadmium and mercury mixture demonstrated increased abdominal adiposity and impaired glucose tolerance through the F4 generation [106]. Ancestral exposure to a mixture of plastics derived endocrine disruptors [bisphenol-A (BPA), bis(2-ethylhexyl)phthalate (DEHP), and dibutyl phthalate (DBP)] increased the susceptibility to obesity in the F3 generation of male and female rats [80]. Additionally, several differential DNA methylated regions (DMR) in the sperm of the F3 generation plastics lineage males were associated with genes previously shown to be relevant to obesity [80]. A transgenerational obese phenotype was observed in the F3 generation of males and females ancestrally exposed to jet fuel hydrocarbons as well as to the pesticide methoxychlor [82,83]. These studies identified germline epimutation signatures that contribute to the obesity phenotype. However, further investigations into the molecular mechanisms behind the transgenerational inheritance of obesity susceptibility are needed.

Ancestral exposure to the pesticide dichlorodiphenyltrichloroethane (DDT) was shown to dramatically increase the susceptibility to obesity in F3 generation male and female rats [97,107]. DDT was historically one of the most commonly used pesticides against insect vectors of disease, with widespread use starting during the 1940s and 1950s [108]. In 1973, the compound was banned in the USA following health and environmental concerns; however, the World Health Organization (WHO) has continued to recommend indoor use to combat malaria[iv]. In 2013, Skinner *et al.* identified a 50% incidence of obesity in rats ancestrally exposed to DDT, and several F3 generation DDT sperm DMR were associated with known obesity genes [107]. A follow-up study utilizing an expanded assessment of obesity identified similar patterns, as well as a unique obesity-specific sperm epimutation signature [97]. F3 generation DDT lineage rats were bred to the F4 generation with wild-type rats in a maternal outcross (MOC) and paternal outcross (POC). The obesity phenotype present in the F3 DDT lineage generation females did not appear in either F4 generation POC or MOC outcrossed females however, the F4 generation males had significantly increased obesity in both outcross lineages [109]. The results indicate that the male obesity phenotype may be inherited through either the male or female germline, whereas the female obesity phenotype may require inheritance of both paternal and maternal alleles. Interestingly, the great-grandchildren (F3 generation) of many of the F0 generation human females exposed to DDT during pregnancy are adults today. Therefore, ancestral exposures to environmental toxicants such as DDT should be considered a potential component of the current obesity epidemic (Figure 5).

Another obesogen capable of inducing transgenerational obesity is tributyltin, an organotin antifouling agent. Tributyltin acts as an agonist of peroxisome proliferator-activated receptor γ (PPARγ) and retinoid X receptor (RXR), which are important activators of adipocyte differentiation from mesenchymal stem cells [110–112]. Interestingly, *in utero* exposure of tributyltin increased gonadal fat deposition, but did not increase overall body weight in the

[iv] http://apps.who.int/iris/bitstream/10665/69945/1/WHO_HTM_GMP_2011_eng.pdf

F1 generation, because there was a change in the lean: fat mass ratio [113]. This effect was also transmitted to the F3 generation of mice ancestrally exposed to tributyltin, as evidenced by increased adipose tissue deposition, adipocyte size, and adipocyte number without a change in body weight. Ancestral tributyltin exposure transgenerationally reprogrammed F3 generation mesenchymal stem cells to increase adipogenesis and attenuate the osteogenesis pathway [85]. A 'thrifty phenotype' appeared in F4 generation males ancestrally exposed to tributyltin, characterized by increased potential for weight gain when fed a HFD, and decreased capacity to lose weight during fasting compared with controls [114]. These metabolic alterations were coupled with changes in chromatin structure and DNA methylation associated with increased expression of the leptin gene in gonadal white adipose tissue [115]. These observations provide insights into the molecular etiology of epigenetic transgenerational inheritance of obesity and, thus, further studies into the molecular mechanisms of other obesogens are necessary.

To build on previous transgenerational obesity research, King *et al.* used a purified cell population of mature white adipocytes for epigenetic analysis rather than adipose tissue [116]. Epigenetic modifications regulate gene expression in a cell-specific manner, and individual cell types have their own differential epigenetic pattern [117–119]. Adipose tissue has many varying cell types, and the cell composition is highly plastic [120]. Mature adipocytes comprise one-third of the cell population, while other cells include preadipocytes, endothelial precursor cells, mesenchymal stem cells, and immune cells at varying population levels [121]. Therefore, investigating an individual cell type, such as mature white adipocytes or preadipocytes, may provide a more metabolically relevant epigenome. Optimally, analysis of epimutations in a purified cell population such as white adipocytes, brown adipocytes, preadipocytes, and/or mesenchymal stem cells, as well as the germline, are needed to provide insight into potential epigenetic changes associated with obesity etiology. Elucidation of the impacts of ancestral exposures on metabolically relevant cells, such as adipocytes, will help elucidate the molecular etiology of obesity.

In the 2019 study by King *et al.*, adipocytes were isolated from the gonadal fat pad of lean, normal, and obese rats ancestrally exposed to the herbicide atrazine (lean phenotype) [122], the pesticide DDT (obese phenotype) [123], or vehicle control [116]. DNA methylation analysis identified unique adipocyte DMR patterns specific to control lineage obesity and DDT lineage obesity, indicating the potential for a unique molecular etiology to environmental toxicant-mediated obesity. Additionally, there were unique adipocyte DMR patterns identified between the F3 generation DDT lineage males and females, which suggests a potential sex-specific effect. Interestingly, a comparison of epigenetic alterations at a reduced stringency identified an extensive set of common potential genes and pathways affected by changes in DNA methylation between the lean and obese phenotypes. The overlapping DMR identified were associated with genes previously correlated with obesity, type 2 diabetes, and metabolic syndrome. The most commonly identified genes included *Caln1*, *Ikzf1*, *Iqsec3*, *Kcnma1*, *Ksr2*, *Mycbp2*, *Myo16*, *Negr1*, *Nr1h5*, *Rbms3*, and *Tmem236*. These genes, commonly modulated by DNA methylation in adipocytes, should be further investigated with transcriptomic analysis to determine whether there are gene expression changes associated with their methylation. This study also only examined DNA methylation, and subsequent analysis of other epigenetic marks, such as ncRNA and histone

modifications, would be valuable. Additionally, further studies are needed to determine whether similar epigenetic mechanisms are present in the visceral adipocytes of lean and obese humans, which may identify potential therapeutic targets for metabolic pathologies [116]. The regulatory impacts of these genes should be further investigated as novel modulators of adipocyte metabolism and function. Epigenetic transgenerational inheritance is a novel mechanism to consider in the etiology of obesity, and further research and eventual human studies may provide insight into potential therapeutics for metabolic diseases.

Concluding Remarks and Future Directions

Obesity has become a worldwide public health crisis, with a rapid increase in prevalence over the past 30 years. Although poor diet and an increasingly sedentary lifestyle have been traditionally indicated as the cause for this increase in obesity, it is clear that other factors also increase susceptibility to obesity. Various environmental insults have been shown to modify the germline epigenome and induce a transgenerational phenotype in future generations in the absence of continued exposure. Epigenetic transgenerational inheritance is a highly conserved mechanism for adaptive response to the environment, and has been identified in a variety of both plant and animal species. Several ancestral environmental exposures can transgenerationally increase the susceptibility to obesity and adult onset diseases. A number of different studies have investigated heritable epigenomic changes in the male germline [80,82,97,99,100,107] and, recently, a study attempted to identify a potential obesity-specific epigenetic signature in sperm [97]. This research should be developed further to determine the plausibility of a preconception biomarker of increased susceptibility to obesity in offspring. Identification of biomarkers in the sperm may aid in early development interventions to prevent adverse metabolic outcomes.

Unfortunately, little is known about the transgenerational effects in the female germline. Although molecular analysis of oocytes has proven challenging due to the inability to collect adequate numbers of cells [123], there have been attempts to identify female-germline specific disease phenotype inheritance through parent-of-origin allelic transmission [82,107,109]. Maternal and paternal outcrosses of DDT and vinclozolin lineage rats identified transgenerational disease phenotypes that may require both male and female germline-mediated changes [109]. Recent advancements in single cell or low cell count sequencing technologies are under development and have started to be applied to recent transgenerational studies [104,123]. Applying single cell sequencing technology to transgenerational studies will not only give a more accurate understanding of the molecular etiology of the inheritance of obesity, but also has the potential to identify novel mechanisms of epigenetic inheritance.

Many studies are attempting to identify the germline-mediated mechanisms behind the transgenerational inheritance of obesity; however, few focus on identifying the molecular changes at the tissue or somatic cell level. Previous studies have shown that epigenetic transgenerational inheritance of obesity following ancestral exposure can modify adipocyte differentiation and the epigenome of adipose tissue and adipocytes [85,114,116]. These studies have identified epigenetic modifications of certain genes and pathways that have the

potential to be used as pharmaceutical targets. Further studies are needed to investigate whether similar epigenetic changes are present in humans (see Outstanding Questions).

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Glossary

DNA methylation
addition of a methyl group to a cytosine in a 5′-cytosine-phosphate-guanine-3′ (CpG) dinucleotide residue sequence to form 5-methylcytosine

Epigenetic processes
DNA methylation, histone modification, ncRNAs, RNA methylation, and chromatin structure

Epigenetic transgenerational inheritance
germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that leads to phenotypic variation

Epigenetics
molecular factors and processes around the DNA that regulate genomic activity independent of DNA sequence, and are mitotically stable

Epimutations
mitotically stable epigenetic alterations, such as an environmentally induced DNA methylation, at a specific CpG site

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Highlights

The prevalence of obesity has increased dramatically over the past 30 years, and cannot be explained by genetics, diet, and exercise alone.

A variety of early life and *in utero* exposures to environmental insults can change metabolic outcomes through developmental epigenetic reprogramming.

Epigenetic transgenerational inheritance of obesity has been observed following ancestral exposure to a high-fat diet, malnutrition, and several environmental toxicants.

Unique obesity-specific sperm epimutation signatures have been identified in the transgenerational F3 generation of animals ancestrally exposed to environmental toxicants.

Numerous genes modified by DNA methylation in a variety of phenotypes and ancestral exposures have been found to be potential novel modulators of adipocyte (fat cell) metabolism and function.

Box 1.

Challenges in the Assessment of Obesity and Disease Risk

According to the WHO[i], obesity is defined as excess fat accumulation that may impact health. Therefore, it is imperative that current methods of determining obesity accurately assess risk factors for cardiovascular and metabolic comorbidities. BMI is calculated by weight in kilograms divided by height in meters squared (Equation I) [2]:

$$\text{BMI} = \text{weight}/\text{height}^2 \quad [\text{I}]$$

Although a BMI of ≥30 kg/m^2 is often used as a threshold to define obesity [2], there are potential weaknesses in exclusively utilizing height and weight as anthropometric measurements. For example, BMI does not compensate for body composition differences associated with age, sex, or ethnicity [124–126]. Specifically, the phenotype of individuals with the same BMI is highly heterogeneous, with vast differences in the anatomical distribution of fat as well as cardiometabolic health [127].

Recently, several attempts have been made to formulate novel anthropometric measures that more accurately predict cardiovascular and metabolic risk. The body adiposity index (BAI) attempts to measure the percentage of body fat by using a modified ratio of hip circumference to height (Equation II) [128]:

$$BAI = \text{hip}/\text{height}^{1.5} \quad [\text{II}]$$

The Clínica Universidad de Navarra-Body Adiposity Estimator (CUN-BAE) similarly attempts to estimate total body fat percentage by using a modified BMI equation that accounts for both age and sex as risk factors [129]. Both equations are built on the principle that excess adiposity, as opposed to excess body weight, correlates better to cardiometabolic risk; however, these measurements do not account for fat depot-specific risk. White adipose tissue is categorized as subcutaneous or visceral adipose tissue [130]. Subcutaneous adipose tissue lays directly under the skin, while visceral adipose tissue involves fat pads that lay deep in the abdomen and next to visceral organs [130]. Visceral adiposity has been linked to increased risk of cardiovascular and metabolic diseases compared with subcutaneous adiposity [131]. Therefore, it is important to consider visceral or abdominal adiposity as a measurement of cardiometabolic risk in a clinical setting.

Several cohorts have used waist circumference (WC) or waist-to-height ratio (WHtR) as alternative anthropometric measures for abdominal adiposity because they correlate significantly better with cardiovascular risk factors compared with BMI [12,132]. To further refine these measurements, a body shape index (ABSI) was developed to adjust WC for height and weight (Equation III) [133]:

$$\text{ABSI} = \text{WC}/\text{BMI}^{2/3}\,\text{height}^{1/2} \quad [\text{III}]$$

ABSI predicted mortality risk in different age, sex, and weight using a population data set from the USA, although the measurement had weaknesses in accuracy depending on

ethnic group [133]. A recent review of 66 studies using 25 different novel anthropometric parameters including a variety of ethnic and socioeconomic demographics found that these new measurements were generally positively correlated with risk factors and disease outcomes [134]. The authors also cautioned that specific cut-off values may not be applicable to all populations and further research is needed to determine the accuracy of these measurements [134]. BMI should still be utilized in obesity studies due to ease of measurement and historical use to determine trends in obesity data. However, future cohorts should consider including various measurements being used in these novel anthropometric formulas, such as thigh, waist, and hip circumference, to account for the cardiometabolic risk associated with variance in fat deposition. Perhaps an epigenetic biomarker for obesity susceptibility and/or obesity will be developed in the future.

Box 2.

Epigenetics and the Developmental Origins of Health and Disease

A variety of exposures in early life and *in utero* can change metabolic outcomes in adulthood. In 1934, Kermack *et al.* observed a significant drop in mortality rate within the UK and Sweden between 1751 and 1930, which the authors attributed to an improved early-life environment [135]. In 1960, Widdowson and McCance observed that rats born to small litters ($n = 3$) with greater maternal nutrition access grew more rapidly, reached sexual maturity earlier, and maintained a larger body size into adulthood compared with rats from larger litters ($n = 15–20$) [136]. These results provided evidence that there are critical windows of development susceptible to changes in nutrition that have long-lasting effects in adulthood. In 1962, J.V. Neel developed what is now known as the ‘thrifty genotype’ hypothesis from the observations that babies born from women with diabetes often have macrosomia and develop diabetes in adulthood. Neel postulated that this genotype is more efficient at energy intake and efficiency, evolutionarily gaining an energy reserve advantage during famine conditions, which may be detrimental under nutritional surplus [137].

Later studies concluded that the timing of the critical window (i.e., the specific trimester or early childhood) in addition to changes in environment in later life may change the phenotype. In 1976, Ravelli *et al.* investigated prenatal and postnatal nutrition levels during the 1944–1945 Dutch famine and the potential for increased susceptibility to obesity. If individuals experienced famine during the last trimester of pregnancy or within the first few months of life, the rates of obesity significantly decreased. However, if individuals experienced famine during the first half of pregnancy, they had a significantly increased rate of obesity [138]. In 1977, Forsdahl hypothesized that individuals born in poverty who experienced affluence in later life had a reduced tolerance to a HFD, increasing the susceptibility to arteriosclerosis compared with individuals who never experienced poverty [139]. In 1985, Wadsworth *et al.* identified a similar correlation between cardiovascular disease and socioeconomic status [140]. Both men and women who came from families with the lowest socioeconomic class had significantly higher mean systolic blood pressures compared with those from the highest socioeconomic status [140]. Interestingly, men who grew up in the lowest socioeconomic status but rose in socioeconomic status had even higher mean systolic blood pressure compared with those that stayed within the same socioeconomic class in adulthood [140]. Barker and colleagues made observations related to this phenomenon, with several epidemiological studies showing that infants born small for gestational age had an increased susceptibility to cardiovascular disease and metabolic dysfunction [13,14]. Derived from Neel’s ‘thrifty gene hypothesis’ [137], the ‘thrifty phenotype hypothesis’ was proposed from observations of increased adiposity and decreased fat mobilization following poor fetal nutrition [15].

Thus, the molecular mechanisms involved in developmental origins of health and disease phenomena will be affected by environmental influences on the epigenome, with early-

life developmental origins of epigenetic alterations generating later-life impacts on health and disease.

Box 3.

History and Mechanisms of Epigenetics and Epigenetic Inheritance

During the 1940s, Conrad Waddington coined the term ‘epigenetics’ in reference to environment–gene interactions that could not be explained by classic Mendelian genetics [141]. Waddington found that heat shock could induce a change in *Drosophila* wing structure phenotype that was heritable for 16 generations [40]. Three decades later, one of the first epigenetic marks identified was DNA methylation, the addition of a methyl group to a CpG DNA residue [142–144]. Other epigenetic processes were identified in the following decades. During the 1980s and 1990s, histone modifications were found to be associated with changes in gene expression [145]. Eventually, ncRNA, chromatin structure, and RNA modifications were identified as epigenetic factors [23]. As both the field and technology advances, it is likely that other epigenetic molecular marks and factors will be identified.

Several historic observations have suggested the presence of non-Mendelian inheritance processes, including observations by Mendel with peas and by Krammerer during the early 1900s with the midwife toad [74]. These observations were not generally accepted during the early 1900s due to the rediscovery of genetics. The first person to help establish the field of epigenetics was Waddington [40,141] who coined the term and again observed non-Mendelian inheritance phenomenon with *Drosophila*. Subsequent observations with paramutation in plants supported this process, but was suggested to be a genetic phenomenon [146]. The first indirect observations of the link of epigenetics and inheritance came from imprinted genes. Imprinted genes involve monoallelic gene expression, are transmitted in a parent-of-origin (maternal or paternal) manner in the germline, and involve modifications of DNA methylation, histone modification, and ncRNA expression [76,147,148]. The control and inheritance of genomic activity involves interdependent mechanisms of several epigenetic and genetic processes.

One of the first studies associating changes in epigenetic marks and transgenerational inheritance correlated ancestral exposure to vinclozolin with DNA methylation changes in the germline in 2005 [34]. Epigenetic transgenerational inheritance requires the germline transmission of these imprinted-like epigenetic modifications by altering the epigenome of developing embryonic stem cells in the next generation [76]. Within 15 years, the field expanded rapidly and current research has demonstrated that the phenomenon of epigenetic transgenerational inheritance can occur in a variety of species and ancestral exposures [76].

The term ‘epigenetic inheritance’ refers to any epigenetic effect on subsequent generations, namely a combination of direct multigenerational exposures and transgenerational exposures [29]. This is distinct from epigenetic transgenerational inheritance, which requires the transmission of germline information between generations in the absence of any continued direct exposure [76]. A distinction between multigenerational exposure or intergenerational epigenetic inheritance and epigenetic transgenerational inheritance is required due to the distinct mechanisms and biological

impacts of the two processes. Therefore, to distinguish the two, the term ‘epigenetic transgenerational inheritance’ is used as originally proposed [34].

Outstanding Questions

Few environmental insults have been assessed for the effects of ancestral exposure. What other environmental insults have the capacity to induce the transgenerational inheritance of obesity?

How does the epigenetic profile related to obesity in sperm influence the physiology of adipose tissue? How do other epigenetic marks, such as histone retention and ncRNA, in the sperm come into play in obesity phenotypes?

How is the development of adipose tissue affected in the epigenetic transgenerational inheritance of obesity? Are there effects on brown (thermogenic) adipose tissue?

Are there epigenetic changes in adipocyte precursor cell types, such as preadipocytes and mesenchymal stem cells?

Given that obesity and metabolic disorders are multifaceted and likely have multiple causes, are other somatic tissues involved in these transgenerational obesity phenotypes? Many of the animals in these studies are fed *ad lib*. Could there be transgenerational neurological changes, such as hyperphasia and changes in satiation, related to these phenotypes? Could transgenerational inheritance of endocrine changes, such as hypothyroidism, be involved?

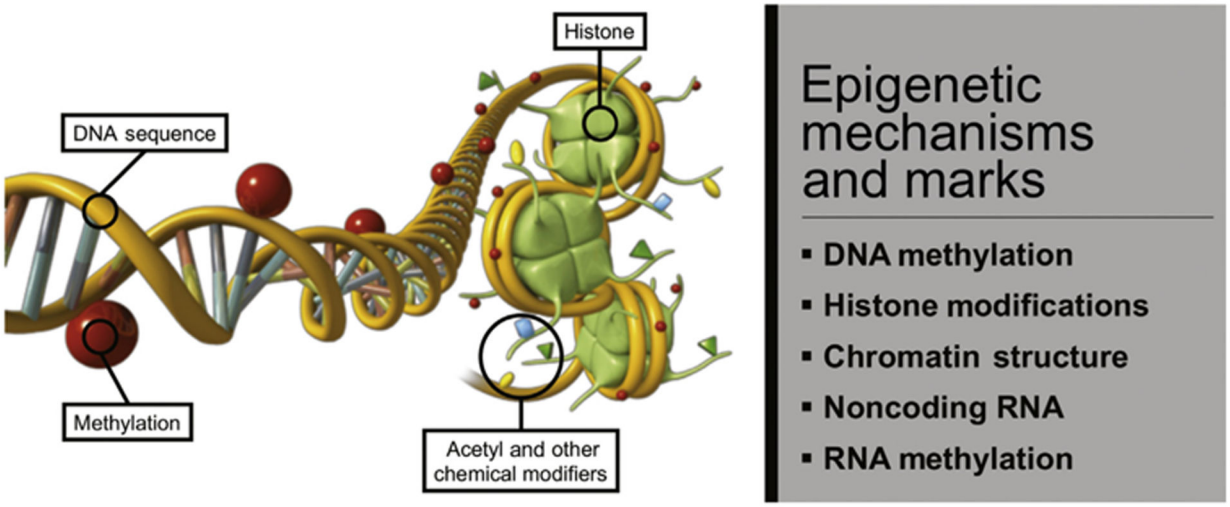

Figure 1. Epigenetic Processes and Marks.
Modified from [149].

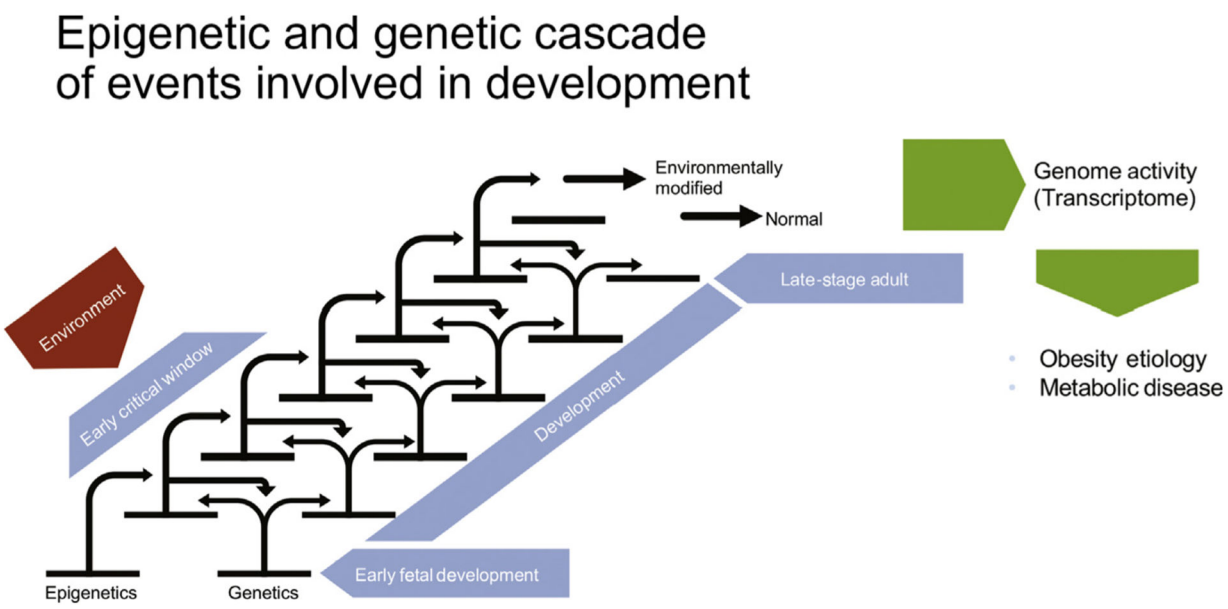

Figure 2. Epigenetic and Genetic Cascade of Events Involved in Development.

Development and cellular differentiation are dependent on the impacts of a cascade of genetic and epigenetic changes. Early life environmental exposures have an increased impact on the transcriptome and physiology of an organism compared with exposures later in development. In organisms that have finished development, most cells have already been fully differentiated. However, early developmental exposures can affect stem cells and cell differentiation to increase the susceptibility to altered transcriptomes and impact disease etiology and phenotypic variation. Modified from [24].

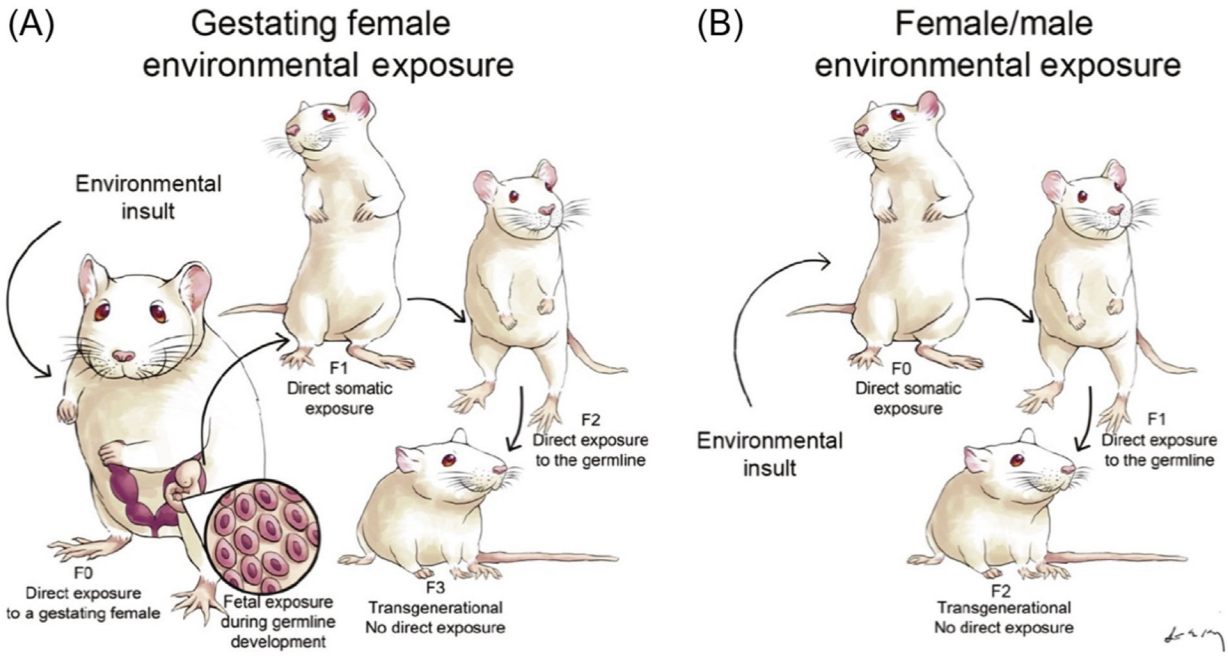

Figure 3. Exposure Mechanisms of Environmentally Induced Epigenetic Transgenerational Inheritance.

(A) The exposure of an F0 generation gestating female to an environmental insult also exposes the developing F1 generation embryo. In addition, alterations of the epigenome in the developing germ cells can also influence the F2 generation if the altered methylation patterns are heritable by the subsequent F3 generations. The transmission of these epimutations is considered epigenetic transgenerational inheritance. (B) Preconception exposure-mediated epigenetic transgenerational inheritance can be induced by exposing the F0 generation to an environmental insult that can affect the epigenome of the germline. The germline, which eventually becomes the F1 generation, has been directly exposed to the environmental toxicant and is not considered to be transgenerational. Therefore, the F2 generation is considered to be the first transgenerational offspring.

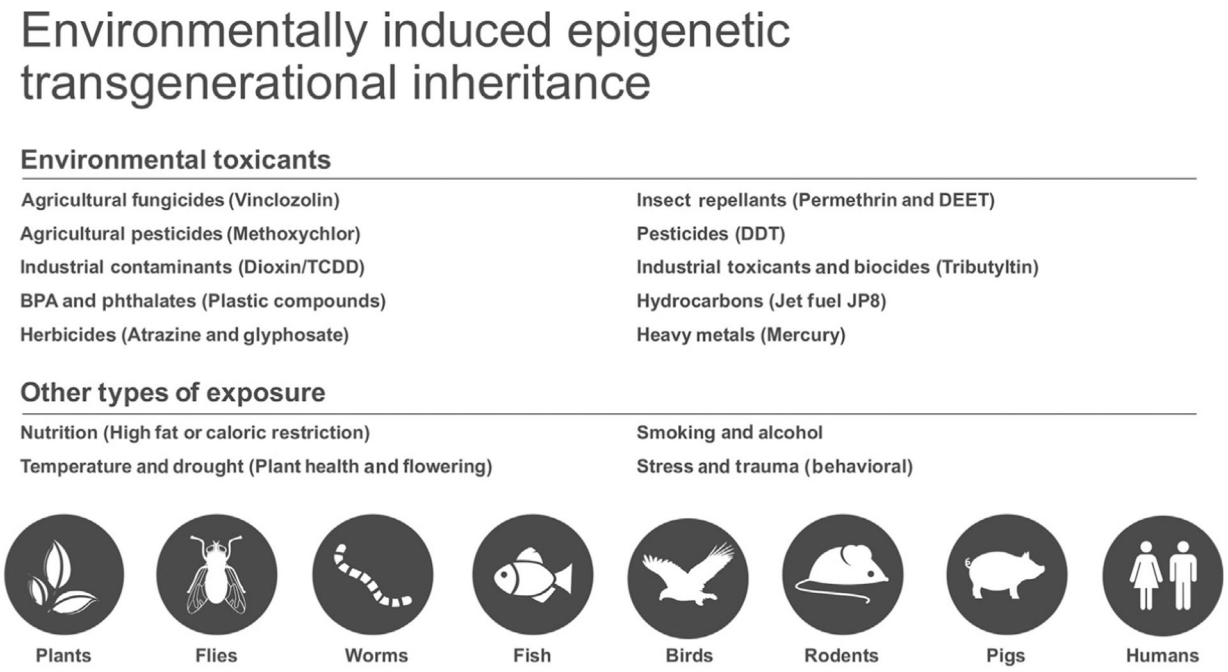

Figure 4. Environmentally Induced Epigenetic Transgenerational Inheritance.

The potential for epigenetic transgenerational inheritance is induced by various environmental insults, including environmental toxicants. This phenomenon has been observed in a variety of organisms, including plants and animals. Adapted from [19]. Abbreviations: BPA, bisphenol-A; DEET, diethyltoluamide; DDT, dichlorodiphenyltrichloroethane; TCDD, dioxin.

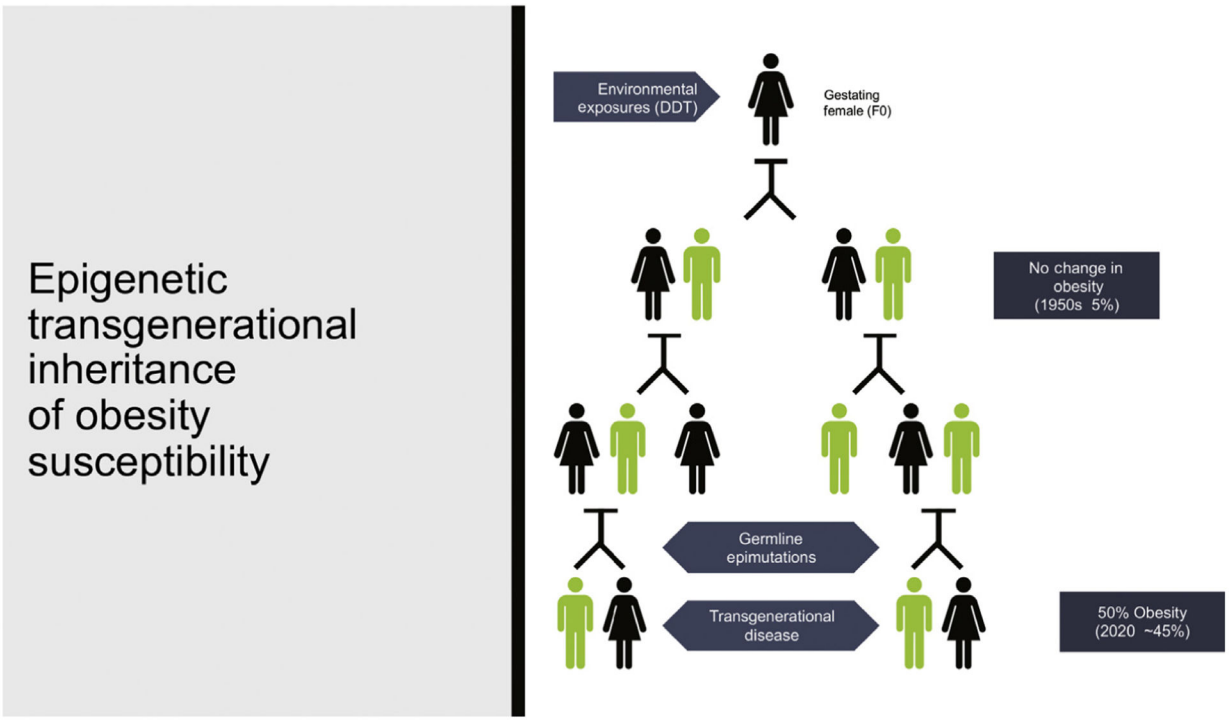

Figure 5. Environmentally [e.g., Dichlorodiphenyltrichloroethane (DDT)]-Induced Epigenetic Transgenerational Inheritance of Obesity.

Obesity susceptibility epigenetically inherited from ancestral exposures. During the 1950s, the entire North American population was exposed to high levels of the pesticide DDT, when the obesity rate was <5% of the population. Three generations later, the obesity frequency in North America is now ~45% of the population.

Table 1.

Environmental Insults That Can Induce Epigenetic Transgenerational Inheritance

Exposure	Effects	Refs
Vinclozolin	Testis disease, prostate disease, kidney disease, age of puberty, male infertility, immune system abnormalities, tumor development	[29,34,76,89]
Methoxychlor	Kidney disease, ovary disease, obesity, male infertility	[34,82]
Permethrin/DEET	Pubertal abnormalities, testis disease, ovary disease	[81]
Dioxin	Prostate disease, ovary disease, kidney disease, uterine disease, testis disease, increased risk of preterm birth	[86,87,95]
BPA/phthalates	Pubertal abnormalities, testis disease, obesity, ovarian disease	[80]
BPA	Heart disorders, reduced fertility, changes in social behavior	[79,150,151]
Hydrocarbon mixture (jet fuel)	Ovary disease, obesity	[83]
DDT	Obesity, testis disease, ovary disease, kidney disease	[107]
Benzo[a]pyrene	Behavioral changes, infertility, increased BMI	[48,84]
Tributyltin	Obesity	[85,105,114]
Glyphosate	Obesity, testis, kidney, ovary, and prostate disease	[78]
Mercury	Behavioral changes	[47]
Caloric restriction	Cardiovascular mortality, increased chronic disease, increased BMI	[55,102,103]
High-fat diet	Increased adiposity, mammary cancer, hyperglycemia	[64–66]
Folate		[69]
Stress	Depressive-like behaviors, increased risk taking, and glucose dysregulation; reduced anxiety and serum cortisol; reduced growth and delayed behavioral development	[57,60–63]
Drought	Changes in DNA methylation	[36,59]
Heat/salt stress	Accelerated flowering, increased tolerance	[57]
Prediabetes/diabetes	Impaired insulin sensitivity	[67,68]
Smoking	Abnormal pulmonary function, increased fat mass	[70,71]
Alcohol	Neurological defects	[72,73]
Heat stress	Increased tolerance to heat stress in plants; wing structure change in *Drosophila melanogaster*	[43,58]

Table 2.

Environmental Insults That Can Transgenerationally Increase the Susceptibility to Obesity and Its Comorbidities

Exposure	Effects	Refs
Maternal HFD	Increased body size in females	[64]
Paternal HFD	Increased adiposity and sperm miRNA changes; reduced birth weight, resistance to weight gain with glucose intolerance; increased adiposity and serum leptin	[99–101]
Paternal HFD and prediabetes	Impaired insulin sensitivity and glucose intolerance	[68]
Paternal overnutrition	Glucose intolerance and fasting hyperglycemia in males	[98]
Maternal famine	Increased rate of chronic disease	[102]
Paternal famine	Increased BMI; increased incidence of cardiovascular disease and diabetes	[55,103]
BPA/phthalates	Increased adiposity and sperm methylation changes associated with obesity genes	[80]
DDT	Increased adiposity and sperm methylation changes associated with obesity genes	[107]
Methoxychlor	Increased adiposity	[82]
Tributyltin	Increased fat:lean tissue ratio, increased weight gain on a HFD, decreased weight loss when fasting, changes in chromatin structure and DNA methylation, increased expression of leptin	[85,114]
Glyphosate	Increased adiposity and adipose size, obesity and sperm DNA methylation	[78]