Chapter 19
The Role of Environmental Obesogens in the Obesity Epidemic

Amanda Janesick and Bruce Blumberg

19.1 Introduction

Nearly 260 years ago, prenatal exposure to a particular toxin, ethanol, was observed to have deleterious effects to the developing fetus [1]. During Prohibition in the United States, behavioral problems exhibited by children of "immoderate" alcohol-consuming families were attributed to poor social upbringing [2]. By the 1970s, methodical investigation of infants born to alcoholic mothers firml established a prenatal component contributing to the common display of behavioral and mental problems [3, 4]. Acceptance of fetal alcohol syndrome (FAS) was slow because alcoholics were considered to be morally corrupt, social deviants who bred the same type of people [2]. Today, however, FAS is considered a rather obvious illustration of developmental programming because the teratogenic effects of ethanol are often robust and quickly discernable. Unfortunately, the effects of environmental chemicals that contribute to obesity and metabolic disease are not quite as visible, especially early in life. Major consequences of obesity such as diabetes and heart disease occur much later in life, and even early childhood obesity is more conveniently ascribed to a general lack of dietary restraint and/or lack of exercise. Indeed, the same stigma that plagued the acceptance of FAS is likely to affect the recognition and public awareness of the "obesity before birth" argument.

The conventional wisdom is that people can budget their diets as they do their finances calories eaten should not exceed calories expended, otherwise, the body will drift toward a positive energy balance [5]. Suppose, though, that metabolic set points are perturbed from the beginning of life. For example, babies born to mothers who smoke exhibit a classic trend: low birth weight, with increased risk of obesity and metabolic syndrome later in life [6]. This pattern is characterized in the Developmental Origins of Health and Disease (DOHaD) hypothesis, which was developed after epidemiologists noted that poor maternal nutrition was correlated

A. Janesick (✉)
Department of Developmental and Cell Biology, University of California, Irvine, CA 92697-2300, USA
e-mail: ajanesic@uci.edu

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with reduced perinatal growth followed by a deliberate progression of adult disease in offspring [7–9]. In simple terms, the fetus "anticipates" that it will be born into a nutrient-deprived environment and makes suitable adjustments to its metabolic program. If the environment turns out to be food rich, then the adult will not be able to cope [10]. Low birth weight can be the outcome of multiple environmental stressors (not all nutrient based) and is indicative of a trajectory toward cardiovascular disease, diabetes, and obesity [11–14]. During certain critical developmental time windows (e.g., periconception), with relatively low exposure to a stressor, the fetus might appear completely normal [15], yet subtle developmental cues still exist and will manifest themselves later in life. Most studies supporting the DOHaD hypothesis are primarily nutritional in nature, thus the potential of environmental toxins to modulate developmental pathways that control energy and metabolism is mostly unexplored. Whether the stressor is coming from the outside, or produced endogenously by the parent, virtually all identifie compounds that induce obesity are endocrine disrupting chemicals (EDCs). Many are either direct ligands for hormone receptors or affect components in metabolic signaling pathways under hormonal control.

19.2 An Archetype for Prenatal Chemical Exposure Leading to Obesity (Obesogens): Tributyltin

"Obesogens" are chemical compounds that can promote obesity by increasing the number of fat cells (and fat storage into existing fat cells), by changing the amount of calories burned at rest, by altering energy balance to favor storage of calories, and by altering the mechanisms through which the body regulates appetite and satiety.

Organotins are a family of compounds that contain at least one Sn—C bond. Invented in the 1850s, these "organic bodies of tin" were less a discovery of the compounds themselves than an educational instrument that encouraged the development of organometallic chemistry and the concept of valence number [16]. Currently, organotins are prevalent in industry, used in fungicides, wood preservatives, and heat stabilizers in polyolefi plastics [17]. Organotins were once widely used as antifouling paints on boats in the 1960–1970s, and have since been regulated, but not completely phased out [18]. Because organotins are lipophilic, they readily bioaccumulate in bacteria, algae, and aquatic invertebrates [19]. Concern over the biological consequences of organotin exposure was firs reported when the female gastropod mollusk, exposed to tributyltin (TBT), developed a sperm duct, seminal vesicle, and penis [20, 21]. Neurotoxic [22] and mitochondrial toxic [23, 24] effects were also observed. Reproductive effects were f rst observed in higher vertebrates when it was noted that exposure to TBT masculinized genetically female Japanese flounde [25]. Subsequently, it was found that *Xenopus laevis* tadpoles exposed to low levels of tributyltin exhibited ectopic fat cell production; a novel and unexpected findin [26]. TBT was thus identifie as the f rst "obesogen."

In mice, a single, prenatal exposure to TBT during gestation, results in premature accumulation of fat in adipose tissues [26] (Fig. 19.1). In accord with nutritional experiments supporting the DOHaD hypothesis, the mice were born slightly underweight, but had already stored fat at the expense of total body mass [26]. By 6 months, the mice were significantl heavier, despite normal diet and exercise (unpublished). Histological sections of newborn liver, testis, mammary gland, and inguinal adipose tissue (which normally do not store lipids before feeding commences) all showed pronounced lipid accumulation in the pups born to TBT-treated mothers [26]. Hence, the tendency to store excess fat was already programmed before the mouse was born, simply due to a single exposure of TBT, at a dose equivalent to what humans might acquire, inadvertently, from their environment. TBT is one of few examples of an exogenous chemical that generates an obese phenotype in both sexes, solely due to in utero exposure. The mechanisms underlying why early life exposure to chemicals, like TBT, might increase a person's propensity toward obesity is the focus of this review.

19.3 Obesogens Acting on Estrogen/Androgen Metabolism

Estrogens in the adult are protective against android (abdominal) obesity and metabolic disease. The ovariectomized rat (a model for menopause in women and estrogen deficien y in men) has abdominal obesity, which is reversed upon treatment with estrogen [27, 28]. In 3T3-L1 preadipocytes constitutively expressing the estrogen receptor, triglyceride levels, and lipoprotein lipase expression are decreased [29]. Conversely, knockout of the estrogen receptor leads to increased white adipose depot size, central weight gain, and impaired glucose metabolism [30, 31]. The same phenotypes seen with estrogen insufficien y can also be simulated by the inhibition of aromatase, an enzyme that converts testosterone to estradiol. Aromatase knockout mice are obese and this phenotype is attributed to an estrogen deficit as opposed to testosterone excess [32]. Metabolic disease, fatty liver, and abdominal obesity have also been demonstrated in a human case study where CYP19A1, the gene encoding aromatase, is rendered non-functional [33]. Interestingly, tributyltin (TBT) is an inhibitor of P450-mediated aromatase expression and/or action, in both f sh and humans [34, 35]. The masculinization of female mollusks by TBT is thought to result from inhibition of aromatase; although there is also evidence that TBT acts through nuclear receptors to inhibit aromatase expression. Whether and to what degree TBT perturbs estrogen availability in mammals to affect adiposity is currently unknown.

Prenatal exposure to excess estrogen does not protect the fetus against obesity, despite the fact that estrogen promotes leanness in adults. The most prevalent model for studying prenatal estrogen exposure is the diethylstilbestrol (DES) mouse. DES is a synthetic estrogen that actually binds to the estrogen receptor two to three times stronger than does the natural ligand, 17β-estradiol [36]. When mice are treated prenatally with low-dose DES, they give birth to pups that are initially smaller, but

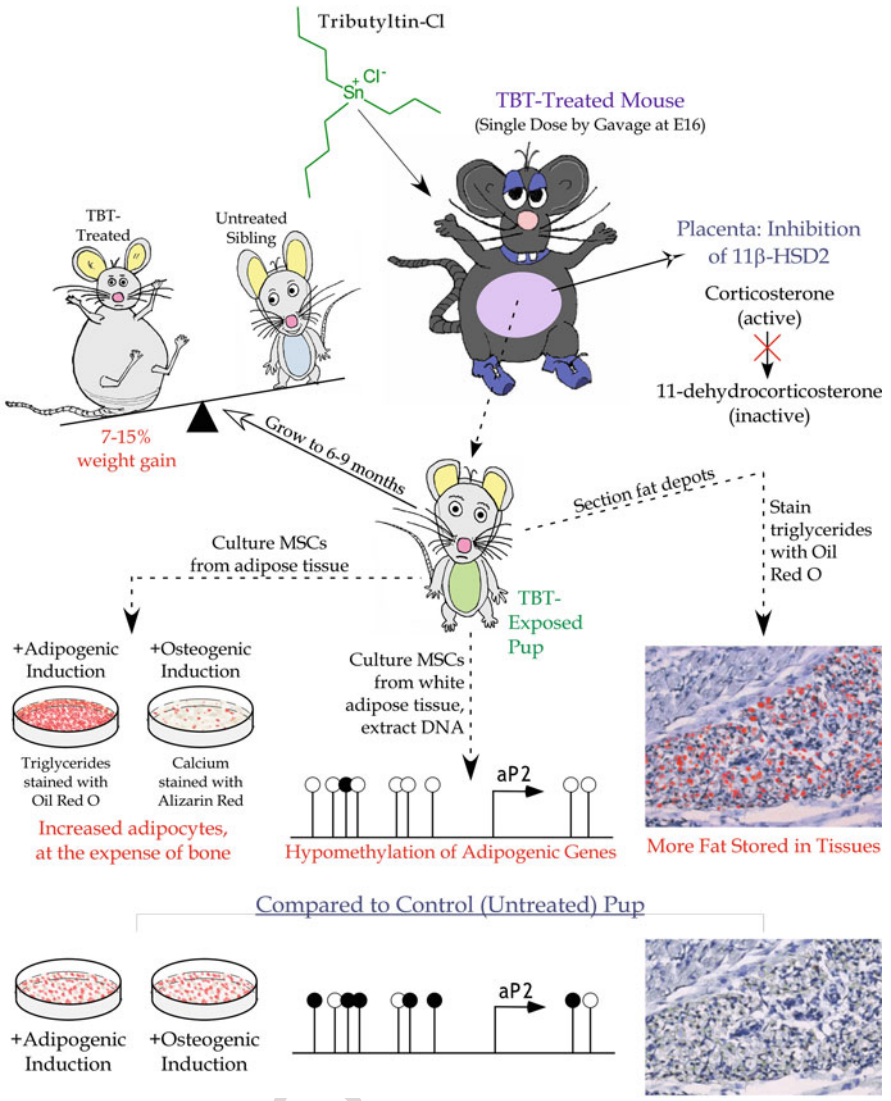

Fig. 19.1 Prenatal exposure to tributyltin (TBT) predisposes an organism to be obese. A single exposure to TBT at embryonic day 16 causes a host of changes in developing animals. TBT exposure alters the expression of 11β-HSD2 in the placenta, which could increase glucocorticoid levels. Prenatal TBT exposure produces pups that have already stored fat in tissues at birth, compared with control animals that do not. Multipotent stromal cells from exposed animals are predisposed to develop into adipocytes at the expense of bone cells. Promoters of some PPARγ target genes are undermethylated in MSCs from exposed animals compared to controls. This increased number of adipocytes may be important in the 7–15% weight gain seen in exposed animals from 6 to 9 months of age

become heavier later in life [37]. The environmental estrogen bisphenol-A (BPA), activates the estrogen receptor and is also an inverse antagonist (i.e., activator) of the estrogen-related receptor [38]. BPA treatment of pregnant dams results in smaller offspring that exhibit "catch-up" growth and are significantl heavier by 6 weeks of age [39]. Dichlorodiphenyl-dichloroethylene (DDE), the major metabolite of the pesticide DDT, is both an estrogen receptor activator and an anti-androgen [40, 41]. Mothers who lived along the Lake Michigan shoreline, exposed to high levels of DDT, were more likely to have a child that exhibited elevated BMI in adulthood [42]. One area of active study concerning the obesogenic effects of estrogen is the regulation of leptin and its receptor by estrogens [43]. High levels of maternal leptin are correlated with high fetal leptin [44] which is a precursor to weight gain later in life. The obesogenic effects of estrogenic chemicals are explored more fully in Chapter 17.

19.4 Obesogens Acting on Glucocorticoid Metabolism

Disruption of glucocorticoid homeostasis also contributes to the pathogenesis of obesity and metabolic syndrome. Obesity is linked to a general increase of positive feedback within the hypothalamic–pituitary–adrenocortical (HPA) axis, leading to an oversecretion of cortisol from the adrenal gland [45–49]. This does not necessarily translate to higher circulating cortisol levels in the blood; and for that reason, obesity is not associated with systemic hypercortisolism, as in the case of Cushing's syndrome. Rather, the hypercortisolism observed in obesity is intracellular and peripheral in nature, often characterized by the impaired ability to clear or inactivate cortisol in adipose tissue, particularly visceral adipose tissue [50]. Visceral adiposity is particularly dangerous since it is associated with all the major health consequences and mortality risk of obesity (reviewed in [51]). In addition, the visceral adipose tissue itself is populated with more glucocorticoid receptors (GRs) than subcutaneous tissue [52, 53]. Since glucocorticoids increase adipocyte proliferation and their differentiation from stromal cells, the presence of excess glucocorticoids or GRs will undoubtedly stimulate adipogenesis locally [54–56].

One popular hypothesis that links obesity to the HPA axis is the dysregulation of 11β-hydroxysteroid dehydrogenase type-1 (11βHSD1) (see Chapter 13). 11βHSD1 is expressed wherever the glucocorticoid receptor is found (i.e., ubiquitously) and primarily functions to convert the inactive cortisone (or 11-dehydrocorticosterone in rodents) into the active cortisol (or corticosterone in rodents) (reviewed in [57]). In humans, obesity and metabolic syndrome are associated with elevated 11βHSD1 [50, 58, 59]. The phenomenon is also observed in obese Zucker rats, where 11βHSD1 is increased specificall in omental adipose tissue [60]. Concomitantly, the Zucker rats showed decreased 11βHSD1 expression in the liver, which might lead to a compensatory effect whereby the HPA is stimulated to release more corticosterone [50]. The alternate dehydrogenase (11βHSD2) functions in the opposite direction; it converts active cortisol (or corticosterone)

to inactive cortisone (or 11-dehydrocorticosterone) and is mostly expressed in the kidney, colon, salivary glands, and placenta, where mineralocorticoid receptors are present [61–63]. 11βHSD2 is expressed at low levels in adipose tissue and its reduction might be correlated with obesity [64, 65]. However, deficien y of 11βHSD2 at the kidney results primary in increased mineralocorticoid receptor activation, hypokalemia, and hypertension [66].

Excess glucocorticoid exposure during pregnancy is commonly associated with lower birth weights, but increased risk of cardiovascular disease, diabetes, and hypertension in the adult offspring (reviewed in [67]). Endogenous stimulation of glucocorticoid levels in the mother can potentially wreak havoc on fetal metabolism. In studies of maternal distress, where the mother's physiological state is negative or depressed, the mother produces more corticotropin-releasing hormone, which in turn leads to increased cortisol secretion, and reduced birth weight in the offspring (reviewed in [68]). Exogenous stimulation of glucocorticoid levels in the mother is also common. For example, ethanol exposure in guinea pigs led to elevated levels of cortisol in the mother (measured in the saliva) which was transmitted to fetal blood and amniotic flui [69]. When pregnant rats were given the synthetic glucocorticoid dexamethasone, the birth weight of the offspring was reduced, followed by an increased risk for hypertension in adults [70]. Monkeys treated with dexamethasone during pregnancy had offspring that were normal at birth, but exhibited significan weight gain at 2 months of age [71]. These monkey infants grew to develop obesity and demonstrated all signs of metabolic syndrome (increased blood pressure, high total cholesterol, decreased HDL, and insulin resistance).

Recently, a study showed that increased cortisol production rates in adults were associated with visceral adiposity. Importantly, however, weight loss did not return cortisol production to normal [72]. Obesity was not the source of the elevated cortisol production rates; rather, a perturbed HPA axis was a predisposing mechanism for obesity. This mechanism might account in part for why people cannot lose weight effectively. In this view, any environmental toxin that perturbs the HPA axis in early life could also contribute to obesity and subsequent resistance to weight loss. There are many possible mechanisms by which xenobiotic chemicals can influenc glucocorticoid metabolism to disrupt energy balance, appetite, and the stress response (reviewed in [73]). Given that 11βHSD1 catalyzes the conversion of inactive to active glucocorticoids, one possible way to disturb the HPA axis would be to render 11βHSD1 hyperactive. Adult rats given a daily dose of alcohol throughout the f rst 22 days of gestation gave birth to offspring that demonstrated a significan increase in 11βHSD1 activity, with a subsequent increase in serum cortisol, in both liver and adipose tissue [74]. Hence, it is no surprise that alcohol exposure in utero leads to an activated HPA axis [75] and is associated with glucose intolerance in the adult [76, 77].

Of course, the fetus is normally shielded against excess glucocorticoids because placental 11βHSD2 is highly expressed throughout pregnancy to reduce fetal cortisol exposure [78]. Prenatal inhibition of 11βHSD2 by carbenoxolone administration throughout pregnancy leads to reduced birth weight, anxious behavior, and increased secretion of corticotropin-releasing hormone in rats [79]. Cadmium,

dithiocarbamates, and organotins all have the potential to reduce 11βHSD2 activity in the placenta [80–82]. Hence, one plausible mechanism for obesity in offspring from mothers exposed to tributyltin (TBT) is that placental 11βHSD2 activity is reduced. In fact, TBT is readily transferred from the mother and accumulates in the fetal placenta, liver, and brain [83]. Hence, the leaking of glucocorticoids to the fetus through the activation of 11βHSD1, or placental inhibition of 11βHSD2, is a potential mode of action for environmental chemicals. This is an attractive idea because the dehydrogenases function in the direct activation or inactivation of cortisol. However, due to the complexity of the HPA axis, there are many more avenues of endocrine disruption on glucocorticoid metabolism yet to be uncovered. For example, corticosteroid-binding globulin (CBG) activity is negatively correlated with BMI, waist-to-hip ratio, and insulin resistance in healthy adults [84]. Adipose tissue that is deficien in CBG cannot evacuate cortisol to the blood. In humans with CBG deficien y, preadipocytes readily proliferate and differentiate into adipocytes [85]. Hence, an EDC that lessened CBG activity might also lead to obesity in the adult.

19.5 Obesogens Acting on Peroxisome Proliferator-Activated Receptors

Unlike steroid receptors, which have long been associated with growth, metabolism, and reproductive development, the peroxisome proliferator-activated receptors (PPARs) were originally recognized because certain chemicals activated these receptors to increase peroxisome proliferation in rats [86, 87]. Recent research has focused on PPAR involvement in lipid metabolism and adipogenesis. PPARα is primarily expressed in the liver and is stimulated upon starvation [88], where free fatty acids, once liberated from adipose tissue and transported into the blood, are oxidized in the liver, resulting in ketone bodies that provide energy to muscular tissue and the brain (reviewed in [89]). Fatty acids are natural ligands for PPARα, although drugs such as fenofibrat and gemfibrozi (used to lower LDL levels) also increase PPARα activity [90]. PPARγ is primarily expressed in adipocytes [91] and upon activation, increases adipogenesis [92]. Thiazolidinediones (TZDs), which combat type 2 diabetes, are potent activators of PPARγ [93]. PPARα activation is thought to protect against obesity, whereas stimulation of PPARγ is obesogenic. Therefore, PPARγ has become a focus of many recent obesity-related studies. Nevertheless, chronic stimulation of PPARα leads to glucose intolerance, as well as deterioration of the heart muscle due to lipotoxicity [94–96]. Thus, while PPARα converts fat into energy, it can limit the uptake of glucose into tissues, leading to diabetes.

Like all PPARs, PPARγ forms a heterodimer with the retinoid-X receptor (RXR), a binding partner for multiple nuclear receptors. The ligand-binding pocket of PPARγ is large [97] and can accommodate various chemical structures [98]. PPARγ promiscuity is well demonstrated by the fact that tributyltin (TBT) has nanomolar affinit for PPARγ, yet its structure is highly dissimilar to fatty acids and TZDs

[26, 99]. Indeed, the mechanistic basis for TBT-promoted adipogenesis is most strongly supported by evidence that TBT is an agonist for PPARγ and RXR [100]. Competitive binding assays show that TBT has comparable affinit for RXR, as do synthetic RXR agonists [26]. TBT was actually a better activator of PPARγ even compared to TZDs such as troglitazone [26, 100]. The crystal structure of TBT along with the RXRα ligand-binding domain plus a coactivator fragment shows that TBT binds covalently to RXR [101], which means that it will not readily dissociate once attached. While there is no structural evidence for TBT association with PPARγ, the PPARγ antagonist, T0070907 [102], reduced TBT-stimulated adipogenesis, indicating that the obesogenic activity of TBT is likely mediated through activation of PPARγ [103].

Inappropriate stimulation of PPARs by EDCs is a potential factor underlying the etiology of many forms of obesity. This point is perhaps best made by pharmaceutical obesogens (TZDs) used to treat diabetes. Because TZDs are effective agonists of PPARγ, they promote weight gain in humans by increasing adipose tissue differentiation [104, 105], even as they improve insulin resistance and blood glucose. Since drugs aberrantly modulate PPARγ to stimulate adipogenesis, EDCs like TBT can do the same. However, TBT is not the most ubiquitous EDC to which humans are exposed. Some phthalates (see Chapter 16) are also PPARγ agonists [106] and stimulate the proliferation of adipocytes in the 3T3-L1 cell culture model [107]. Phthalate metabolites are associated with increased waist circumference in men [108], and therefore, are predicted to be obesogenic. Phthalates are ubiquitous organic chemicals that give plastics such as polyvinyl chloride (PVC) with more fl xibility and durability. Since phthalates are not permanently bonded to PVC, they readily leach into food, from medical devices and materials used in construction and manufacturing. Phthalates are probably able to cross the feto-placental barrier, as demonstrated by a simulated human placental perfusion ex vivo model [109]. Identifying the role of phthalates as obesogens is an important topic for future research.

19.6 Epigenetics: The Gene–Environment Connection

Obesogens are predicted to influenc the prenatal programming of obesity through epigenetic modificatio of gene activity; an attractive mechanism frequently invoked to explain complex environment–gene interactions [110]. There are more than 6,000 genes expected to influenc body weight. Of these, about 10 times more genes favor the increase rather than decrease of weight [111]. Epigenetic modifica tion of any of these genes theoretically could result in "developmental plasticity," which allows an organism to make rapid adaptations to changing environments by altering levels of gene expression via DNA methylation, modificatio of histone proteins, or alteration of mitochondrial function [112–114]. During development, the embryo undergoes genome-wide DNA demethylation and remethylation [115–117], which is coupled to corresponding changes in histone acetyl transferase activity

[118]. The first wave of methylation reprogramming occurs prior to the blastocyst stage and establishes which cells will commit to a particular lineage [119]. The second wave occurs during the development of the testes or ovaries, where the primordial germ cells incorporate sex-specific imprinting patterns [120]. During both of these time windows, an exogenous chemical can impose changes (in some cases permanent) in the methylation status of DNA.

Alterations in the methylation status of certain genes have been observed in offspring that experience malnutrition in the womb. Fetal liver derived from rats fed a low-protein diet showed promoter hypermethylation in the liver X-receptor (LXR) [121] and hypomethylation in PPARα [122]. The methylation-deficient status of PPARα was rescued by supplementing the low-protein diet with folic acid, a methyl donor [122]. These modification could plausibly disturb normal lipid homeostasis and impair metabolism, perhaps leading to obesity. Chemical-induced alterations in DNA methylation status have also been observed for diethylstilbestrol (DES) [123] and the dioxin TCDD [124]. In preimplantation embryos, DNA methyltransferase activity was altered depending on whether the embryo was exposed to TCDD, DES, or polychlorinated biphenyl-153 (PCB153) [125]. Liver nuclear extracts derived from TBT-treated rats showed increased histone acetyl transferase activity [126]. Therefore, by affecting the levels of enzymes, such as DNA methyltransferase and histone acetyl transferase, which have broad impacts on gene regulation, these EDCs can conceivably transmogrify the entire epigenetic landscape. Hence, exposure to environmental chemicals has the potential to remodel the behavior of the myriad of genes that are connected to obesity.

should read "wherein"

The maternal programming of adipose tissue development is fundamentally concerned with epigenetic modification that alter stem cell fate. Adipogenesis is a differentiation event in the mesodermal lineage where in multipotent stromal cells (MSCs) or more restricted derivatives give rise to fat cells. MSCs have the potential to mature into various tissues including bone, muscle, cartilage, or adipose [127]. MSCs harvested from epididymal or ovarian fat pads of mice exposed to TBT in utero differentiate into significantl more fat cells, compared to controls [103]. Indeed, adipogenesis occurred at the expense of osteogenesis, suggesting that prenatal exposure to TBT biased the MSC population toward the adipogenic lineage [103]. TBT likely induces epigenetic changes within the MSC compartment that promote demethylation of adipogenic genes. It is currently controversial whether active demethylation of adipogenic genes (with a corresponding up-regulation in expression) is indicative of MSC differentiation into adipocytes. Recent evidence shows that in a population of pure adipose-derived MSCs, adipogenic genes are already hypomethylated in the MSCs, suggesting that MSCs are predisposed to adipogenesis [128]. Hence, an environmental chemical might augment the innate hypomethylated state of adipogenic genes or increase the methylation of other lineages, such that the MSC pool is biased prior to adipocyte differentiation. In support of this model, uninduced MSCs harvested from mice exposed to TBT in utero show decreased methylation in the promoter region of fatty acid-binding protein 4 (FABP4), a marker of adipocytes; suggesting that the MSC population has already been epigenetically modifie to favor adipogenesis [103]. Future experiments will

be required to identify what other genes are required to mediate the effects of prenatal TBT exposure in the MSC compartment, committing more MSCs to the adipogenic lineage.

About 40 years ago, in an effort to properly measure and defin obesity, researchers postulated that the number of fat cells in the adult is f xed and that people gain or lose weight by fillin or emptying these cells with fat [129, 130]. Recently, this hypothesis was proven correct, but with an important modification The number of fat cells in an adult is essentially constant as had been supposed. However, in contrast to previous dogma, adipocytes are continually undergoing apoptosis [131] and being replenished [132]. Using the same [^{14}C]-labeling method employed to prove that adults have the capacity to produce new brain cells, researchers have shown that the life span of a fat cell is approximately 10 years in both obese and lean subjects [133]. During severe weight loss (e.g., gastric bypass), the number of fat cells remained constant, while cell volume decreased [133]. This suggests that obese individuals possess a pool of MSCs that is intrinsically biased toward replenishing fat cells, i.e., that obese individuals have a steady state level of adipocytes that may be higher than non-obese people. Such a bias could be regulated by epigenetic changes due to exposure to environmental cues experienced during critical developmental windows. Chemicals like TBT influenc the number of MSCs committed toward the adipogenic fate by remodeling the chromatin, favoring the expression of genes that promote adipogenesis. Notwithstanding these recent advances, there is much yet to be learned about how epigenetic contributions modulate the prenatal programming of MSC fate and what role obesogens play in this process.

19.7 Conclusion

Prenatal exposure to obesogens is likely to be an underestimated contributor to the obesity epidemic. Although unhealthy food consumed in large portions together with insufficien physical activity are likely to be among the chief substrates of weight gain, rates of obesity have increased in infants [134], as well as children and adults. This suggests that obesity is being programmed prenatally or in early childhood. There is increasing evidence that supports the proposal that environmental chemicals may contribute to the prenatal programming of obesity (see Chapter 16). Prenatal exposure to tributyltin, a chemical for which the mechanism of action is known, predisposes organisms to obesity, suggesting that the DOHaD model is applicable to effects of chemical exposure, in addition to altered nutrition. There are numerous EDCs more prevalent in the environment than tributyltin that have been linked to metabolic disease and whose increased usage mirrors the rising trend of obesity. The metabolic pathways targeted by most of these chemicals remain to be determined and fir links between chemical exposure and obesity should be based on understanding the underlying mechanisms. Understanding how chemicals enter the body and are transferred to the developing fetus is still not well understood. Epigenetics and the regulation of the stem cell lineages will

provide answers to the mechanistic questions regarding how obesogens disrupt the endocrine system. This will inform the development of therapeutics and perhaps even non-pharmacologic solutions to the obesity problem.

Lastly, an important policy issue raised by our work is that of personal responsibility. While it is undeniable that the balance of our food intake and activity is reflecte in total body weight, it is equally true that obesity is a multifactorial disease with inputs from many different developmental pathways. Works in our laboratory and elsewhere have revealed critical roles for EDCs in body weight. A more complete understanding of how these pathways impact the body's homeostatic mechanisms for energy balance, adipocyte number, appetite, and satiety will allow future research to move forward without being limited by the simplistic model that caloric intake and exercise can be trivially balanced like a checkbook to achieve optimum weight.

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Chapter 19

Q. No.	Query
AQ1	"A. Janesick" has been set as corresponding author. Please check.
AQ2	Please provide complete details for reference "18".
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AQ2: Reference 18 is a web site http://nsglc.olemiss.edu/Advisory/Antifouling.pdf

AQ4: Reference 71 is an abstract from the 11th Annual Meeting of the Neuroendocrinology Section of the German Society of Endocrinology (DGE). It was published in the Experimental and Clinical Endocrinology & Diabetes (Volume 115, Issue 8). There is no page number given, but the abstract number is N14.