20 Impact of Environmental Obesogens

Focus on Mechanisms Linking to Childhood Obesity

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THE OBESITY EPIDEMIC: BEYOND DIET AND EXERCISE

Although the prevailing paradigm of obesity remains one of energy intake versus energy expenditure, the abrupt rise in global childhood obesity rates has led researchers to explore alternative contributors. While genetics undoubtedly bestow some obesity risk, the handful of genetic loci associated with obesity in human studies account for <2% of variance in body weight [1], as reviewed in Chapter 13. This observation is not surprising given the abrupt time line of the obesity epidemic. Beyond excess caloric intake and sedentarism, well-studied environmental risk factors for obesity include stress, smoking, sleep patterns, and the microbiome, as reviewed in other chapters in this book. In this chapter, we will discuss mounting evidence implicating developmental exposure to xenobiotic compounds as a hitherto underinvestigated contributor to the global obesity epidemic [2]. This chapter complements Chapter 19, with a focus on understanding the potential mechanisms that might link obesogens to obesity development.

ENVIRONMENTAL CHEMICALS AND OBESITY

A study setting out to examine the potential effects of the environment on obesity observed over 20,000 animals, representing 12 distinct populations and 8 different species, living in proximity to

industrialized societies [3]. These animals included pets (cats and dogs), laboratory animals (mice, rats, and primates) that were fed controlled diets, and feral rats. Notably, nearly every one of these populations showed positive trends in both weight gain and odds of obesity over the past several decades. The chance of these populations all concomitantly exhibiting the same trend in obesity was calculated as approximately 1 in 10 million [3]. These data strongly suggest that an environmental insult, independent of diet and exercise, is responsible for the parallel trend in obesity in humans and animals.

The use of synthetic chemicals in commerce has grown exponentially since the 1940s, numbering in the tens of thousands today [4]. Of particular concern is a subset of nearly 3000 chemicals, termed *endocrine disrupting chemicals* (EDCs), that interfere with any aspect of hormone action [5,6]. The "endocrine" label can be misleading as EDCs can perturb the action of any chemical messenger, endocrine or otherwise (autocrine, paracrine, neurotransmitter). EDCs can alter hormone synthesis and transport, or they can interfere with the cell signaling and receptor systems that regulate hormone response in target tissues. Since hormones play critical roles in development and metabolism, investigators suspect that EDCs might interfere with hormonal systems to promote weight gain and ultimately obesity [7].

THE OBESOGEN HYPOTHESIS

In 2006, our group proposed the obesogen hypothesis, which asserts that there are EDCs in the environment that confer obesity risk on exposed individuals, principally those exposed during critical windows of development [8]. These "obesogens" promote adiposity through a variety of mechanisms that include

- Disturbing normal fat development, thereby increasing the number of fat cells
- Encouraging the storage of energy within fat cells, increasing fat cell size
- Altering metabolic set points programed during development
- Interfering with neurologic and hormonal control of hedonic reward and appetite

A number of obesogens have been identified in humans and animals and the list continues to lengthen. Obesogens identified in animal studies include estrogenic chemicals (such as diethylstilbestrol [DES] [9], genistein [10], Bisphenol A [BPA] [11], and nonylphenol [12]); organotins, such as tributyltin (TBT) [13]; organochlorine pesticides (including polychlorinated biphenyls [PCBs] [14], dichlorodiphenyltrichloroethane [DDT] [15], triflumizole [16], and tolylfluanid [17]); organophosphates (such as chlorpyrifos [18] and diazinon [19]); brominated [20] and nonbrominated flame-retardants [21] and a number of other chemicals including nicotine [22], benzo[a]pyrene [23], phthalates [24]; and perfluorooctanoic acid (PFOA) [25]. In humans, urinary phthalates are associated with waist circumference and insulin resistance in adults [26] and in children and adolescents [27,28]. Likewise, multiple studies of US [29–31] and Chinese [32] children associate urine BPA levels with obesity prevalence. Urinary phenol pesticides are correlated with obesity in adolescents [33]. Serum levels of several persistent organic pollutants (POPs), including dichlorodiphenyldichloroethylene (DDE, a metabolite of DDT), PCBs, hexachlorobenzene (HCB), and β-hexachlorocyclohexane (β-HCH), are associated with BMI in adults [34]. Prenatal exposure to PCBs [35], DDE [36], and HCB [37] are associated with obesity later in life. Chapter 19 provides a detailed overview of the findings from human cohort studies that have examined the effects of these obesogens on obesity development.

In addition to these environmental exposures, there is a wide range of obesogenic pharmaceuticals for which weight gain is an established side effect. These include first- and second-generation antipsychotics [38], selective serotonin-reuptake inhibitors (SSRIs) [39], systemic glucocorticoids [40], and the antidiabetic thiazolidinediones (rosi-, pio-, and troglitazone) [41]. If clinicians have already accepted weight gain as an established side effect of these medications, then it is not unreasonable

to infer that exposure to physiologically relevant doses of nonpharmaceutical xenobiotic compounds (such as EDCs) could have the same effect.

DEVELOPMENTAL OBESOGEN EXPOSURE

Applying the developmental origins of health and disease (DOHaD) model to the obesogen hypothesis requires monitoring of early-life EDC exposure during sensitive developmental windows together with subsequent observation of obesity and metabolic disease throughout life. Given the relatively recent establishment of the DOHaD and EDC fields, such studies are limited in number and by low sample size and lack of long-term follow-up. The strongest epidemiological evidence of an environmental obesogen programing obesity risk *in utero* is maternal smoking. It is well established that the children of smoking mothers are born small-for-gestational-age; however, these children experience catch-up growth in the first year of life and eventually outpace their peers. Tens of epidemiological studies have all shown an increased overweight/obesity risk in children whose mothers smoked during pregnancy [42].

A number of studies have evaluated perinatal exposure to POPs, which have long half-lives and persist in the environment despite significant regulation and outright bans on many (reviewed in Chapter 19). Taken together, these studies indicate that POPs may affect intrauterine and postnatal growth to increase risk of obesity later in childhood [43–46]. The continued follow up of ongoing cohorts will be informative in addition to new, well-designed prospective studies of precise exposure windows that track obesity and metabolic health into adulthood. It must also be accepted that human studies will always be limited by overall numbers together with confounding and interacting variables. Therefore, these should be supplemented with cell culture and animal studies that can provide a controlled environment to carry out exposures and study them mechanistically.

DEVELOPMENT OF FAT

One facet of the obesogen hypothesis that has received ample attention is the notion that EDCs can promote the excessive development of fat tissue. Adipocytes appear during the second trimester of pregnancy and proliferate through childhood and adolescence before leveling off at approximately 10% renewal per year in adulthood [47]. This phenomenon is independent of BMI, as weight gain/loss in adults is predominantly attributed to changes in cell size rather than cell number [47]. Visceral fat, which is linked to insulin resistance, may be the exception to this rule. In humans, visceral depot size is determined by cell number [48], and adult mice fed a high-fat diet generate new fat cells in visceral depots [49]. Therefore, adipogenic stimuli (such as an obesogen) during gestation and early life establish the number of fat cells in an individual, while fat mass in adults is regulated both by cell number and cell size in a depot-specific manner.

Adipocytes originate from the mesodermal lineage via the mesenchymal stem cell (MSC or multipotent stromal cell), a multipotent cell capable of forming bone, muscle, cartilage, tendon, fat, and other tissues. MSCs and their lineage-restricted derivatives can be found in the perivascular niche of any vascularized organ, including adipose tissue [50]. Transformation of an MSC into a mature adipocyte requires initial commitment to the adipose lineage, followed by terminal differentiation into a functioning fat cell [51]. Adipose lineage commitment requires the concerted action of multiple signaling cascades regulated by adipogenic transcription factors that induce the expression of the peroxisome proliferator-activated receptor gamma (PPARγ), the "master regulator" of adipogenesis [52]. PPARγ, a member of the nuclear receptor family, is a ligand-activated, DNA-binding transcription factor that dimerizes with the retinoid X receptor (RXR) to bind and regulate genomic targets that promote adipose differentiation [53]. Both MSCs and mouse 3T3-L1 cells (a committed preadipocyte cell line) have become valuable *in vitro* tools for screening candidate obesogens and characterizing their mechanisms of action.

TRIBUTYLTIN: A MODEL OBESOGEN

We and others first showed that the TBT binds and activates both PPARγ and its heterodimeric partner, RXR, to promote adipogenesis and alter lipid homeostasis [13,54]. Human and mouse MSCs, as well as mouse 3T3–L1 preadipocytes exposed to environmentally relevant levels (nanomolar) of TBT, or the pharmaceutical PPARγ agonist rosiglitazone, were shunted toward the adipocyte lineage via a PPARγ-dependent pathway [55,56]. Mice exposed to TBT, *in utero,* showed lipid accumulation in adipose depots, livers, and testes, and have MSCs biased toward the adipose lineage and away from the bone lineage [13,56,57]. Treatment of adult mice or rats with TBT resulted in obesity and fatty liver [58,59], as well as disrupted thyroid function [60].

Beyond concerns over organotin exposure, there is an expanding group of obesogens to which humans are exposed that also activate PPARγ. These include phthalates [61]; triflumizole [16]; flavanones [62,63]; bixin [64]; dioctyl sodium sulfosuccinate (DOSS), a component of the oil dispersant COREXIT [65]; and several flame-retardants including BDE-47 [66], tetrabromo- and tetrachloro-BPA (TBBPA, TCBPA) [67], and triphenyl phosphate (TPP), a component of the flame-retardant Firemaster® 550 (FM550) [68].

Phthalates are widely used as plasticizers and solubilizing agents and are commonly found in personal-care products, medications, and medical equipment. Phthalates and their metabolites can be detected in the urine of nearly all humans [69], including infants [70]. These chemicals promote adipose differentiation of 3T3-L1 cells [71] and they stimulate adipogenesis and suppress osteogenesis in mouse MSCs [72]. Several *in vivo* studies show that prenatal phthalate exposure promotes obesity in adult mice [24,73]. In addition to activation of PPARγ, phthalates may program obesity risk through their effects on PPARα or PPARδ, thyroid metabolism, or gestational growth [74]. Urinary phthalates are associated with obesity and insulin resistance in children, adolescents, and adults [26–28,75]. Studies of prenatal phthalate exposure that examine obesity as a primary outcome are sparse, though one study of African American and Dominican mothers in New York showed a negative correlation between third trimester urine phthalates and BMI of the offspring at 5 and 7 years [76].

AU: In sentence beginning ‘ For a half century’ can you please provide full definition for PBDE?

For a half century, brominated chemicals, such as PBDEs and hexabromocyclododecane (HBCD), have been used as flame-retardants in a variety of products [77]. Due to safety concerns, several PBDEs were phased out of U.S. production in 2005, though these chemicals linger in products and migrate into house dust, a major source of human exposure [77]. BDE-47 induces adipogenesis in 3T3-L1 cells [78], in part due to a weak activation of PPARγ [66]. A recent screen of flame-retardants and their metabolites revealed that 3-hydroxy-BDE-47 activates PPARγ with the same potency as the pharmaceutical rosiglitazone [79]. TBBPA and TCBPA have not been phased out and are still widely used. Recently, these halogenated bisphenols were identified as PPARγ agonists that stimulate differentiation of 3T3-L1 preadipocytes [67,79]. The phase out of PBDEs increased demand for alternative flame-retardants such as the organophosphate-based FM550. Perinatal FM550 exposure results in varied phenotypes in rat offspring including obesity, advanced puberty, cardiac hypertrophy, and anxiety [21]. In a subsequent study, FM550 was shown to be a PPARγ activator along with TPP, a triaryl phosphate that comprises 10%–20% of FM550 [68]. FM550 and TPP were further shown to increase adipogenesis and inhibit osteogenic differentiation of mouse MSCs [80].

PFOA is a persistent fluorochemical with hundreds of industrial applications that is found in the serum of most humans living in the United States [81]. PFOA purportedly activates PPARγ [82], though this assertion is controversial [83], and PFOA does not induce adipogenesis in 3T3-L1 preadipocytes [78]. However, *in utero* exposure to low-dose PFOA results in increased body weight and elevated serum insulin and leptin in postpubertal female mice [25]. These animal data were mirrored in a prospective study of 665 Danish pregnant mothers whose gestational PFOA exposure was associated with the BMI of female, but not male, offspring at 20 years of age [84]. Another Danish cohort showed no such associations [85].

Taken together, these results indicate a continued need to screen for industrial chemicals that can activate PPARγ, since there is sufficient evidence in cells, animals, and humans to believe these compounds will act as obesogens, *in vivo*.

ESTROGENIC OBESOGENS

Estrogens are protective against obesity and cardiovascular disease in adults, as is well demonstrated by the onset of abdominal obesity and dyslipidemia following the loss of estrogen at menopause. Emerging research, however, implicates early-life exposure to low-dose estrogens to be obesogenic. Prenatal exposure to the estrogenic EDCs, DES, genistein, and BPA results in obese adult animals [86], and urine BPA is associated with obesity prevalence in children [29,30,32]. DDT and its metabolite DDE are estrogenic and antiandrogenic, respectively, and have been implicated as obesogens in humans and animals [15,36].

DES is a synthetic estrogen that was widely prescribed to pregnant women in the mid-twentieth- century to prevent miscarriage. Though mothers were unaffected, among the millions of children born to DES-treated mothers, there was a well-documented increase in several rare pathologies of the reproductive tract. Data from the National Cancer Institute's DES Follow-Up Study showed a modest increase in obesity risk among females prenatally exposed to DES, and this risk was higher in those exposed to lower doses [87]. Mice exposed to low doses of DES prenatally become obese later in life, while high-dose exposure resulted in decreased birth weight followed by catch-up growth and subsequent obesity [9]. Similar results were observed in mice exposed postnatally, during the first 5 days of life [86,88]. Importantly, these results were recapitulated with other estrogens (2- and 4-hydroxyestradiol), suggesting an estrogen-dependent mechanism [9].

Of all the data on estrogenic EDCs, data implicating BPA as a potent obesogen are most concerning. BPA, used in polycarbonate plastics and epoxy resins, is produced in millions of tons annually and can be detected in most humans [11,69]. BPA is a potent activator of the estrogen receptor (ER) in the nucleus and also at the cell membrane where it induces rapid cell signaling events [89]. BPA promotes differentiation of 3T3-L1 preadipocytes [90] and human preadipocytes via an ER-dependent mechanism [91]. Low-dose prenatal BPA exposure in animals results in increased body weight in adult life [92]. Perinatal BPA exposure results in increased visceral fat depot size in females at weaning, as well as adipocyte hypertrophy and increased expression of adipogenic and lipogenic genes [93]. Both Trasande et al. and Bhandari et al. have shown a correlation between urinary BPA and obesity prevalence in US children from the National Health and Nutrition Examination Survey (NHANES) [27,29], results echoed in a Chinese cohort [32]. Despite these extensive data (and data implicating BPA in numerous other pathologies), regulatory agencies do not believe levels of BPA exposure are sufficient to result in adverse outcomes, and production of the high-volume chemical continues.

OTHER OBESOGENS AND THEIR MECHANISMS OF ACTION

Much attention has been paid to the ability of obesogens to act as hormone mimics that can bind nuclear receptors. Numerous EDCs have been shown to bind PPARγ and ER, though other nuclear receptors are known to be obesogen targets. BPA, whose obesogenic effects are largely attributed to its ability to bind and activate ER, is also an activator of the steroid and xenobiotic receptor (SXR) [94], the glucocorticoid receptor (GR) [95], and an antagonist of the androgen receptor (AR) [96]. Likewise, phthalates activate all three PPAR receptors (α, δ, γ) [71,97] as well as SXR [9]. The obesogenic effects of PCB 77 were shown to be dependent on the activation of the aryl hydrocarbon receptor (AhR) both *in vitro* and *in vivo* [14]. Tolylfluanid, a fungicide commonly used in Europe, promotes adipose differentiation of 3T3-L1 cells through activation of the GR [99], and mice fed a diet supplemented with tolylfluanid gain more weight and fat mass than

controls [17]. Hence, obesogens can act through several members of the nuclear receptor family, at times simultaneously, to promote obesity.

Not all obesogens are nuclear receptor ligands, and obesogens that do activate nuclear receptors may also act through alternative pathways. For example, TBT, BPA, and phthalates, all of which activate nuclear receptors, also inhibit the enzyme 11β-hydroxysteroid dehydrogenase, a critical regulator of active/inactive intracellular glucocorticoid levels [100]. TBT is further known to inhibit aromatase [101] and isocitrate dehydrogenase [102]. Prenatal exposure to nicotine results in obesity and metabolic complications [22,103], presumably through its action on nicotinic acetylcholine receptors (nAChRs), plasma membrane–associated ion channels present in the brain, hypothalamus, adrenal medulla, and other organs [104]. The pharmaceutical obesogen lithium, which has diverse mechanisms of action, promotes weight gain through increased appetite, hypothyroidism, and even a combination of thirst and improved mood that leads to the consumption of high-calorie beverages [105]. Therefore, obesogens can act through varied nuclear receptor–independent mechanisms to promote weight gain.

EPIGENETICS AND THE ENVIRONMENT

A central tenet of the DOHaD hypothesis is the notion of "developmental plasticity," whereby the developing fetus adapts to environmental stimuli, permanently altering phenotypic expression [106]. While these adaptations may benefit the fetus in the short term, they may confer disease risk later in life within a different environmental context [107]. The definitive example of this concept is the "thrifty phenotype" seen in the offspring of malnourished mothers. These children are programed to survive in a food-scarce environment, but when faced with caloric excess in adult life these adaptations increase the risk for cardiometabolic diseases [108]. Crucially, the genotype of these individuals remains unchanged, though environmental inputs during development have permanently altered their phenotype. That is, there are changes in gene expression during development without any alteration of DNA sequence. Of the mechanisms thought to be responsible for such a phenomenon, epigenetics is the most widely accepted, and its role in childhood obesity is discussed in detail in Chapter 14.

Epigenetics is the study of heritable changes in phenotype that are not the result of altered DNA sequence, but rather environmentally influenced modifications of the genome. These modifications include methylation and/or hydroxymethylation of DNA at cytosine residues of 5' to guanine (CpG sites), chemical modifications of the histone proteins that package DNA into chromatin, and expression of noncoding RNAs. Epigenetic marks can alter chromatin accessibility by encouraging or disrupting transcription factor/cofactor binding to regulatory elements and recruiting silencing complexes to the genome. In mammalian development there are two major epigenetic reprograming events during which there is a genome-wide erasure of DNA methylation marks and subsequent remethylation [109]. The first reprograming occurs in the preimplantation embryo and the second in the developing primordial germ cells. This process plays a critical role in regulating the potency of developing cell populations from pluripotency through lineage commitment and eventual terminal differentiation [110].

There is ample evidence that environmental inputs during development can alter the epigenetic landscape to alter gene expression, development, and phenotype [111,112]. A classic model of this phenomenon is the viable yellow agouti (A^{vy}) mouse described in Chapter 14. Studies in wild-type animals have explored the effects of maternal and paternal nutrition on the epigenetic landscape and phenotype of the offspring [113]. The progeny of rat dams fed a low-protein diet had livers with promoter hypomethylation and increased expression of PPARα (Ppara) and the GR (NR3C1) [114], later attributed to a reduction in DNA methyltransferase 1 (DNMT1) expression [115]. Maternal protein restriction in mice resulted in fetal livers with promoter hypermethylation and underexpression of the liver X receptor alpha gene (LXR-alpha) in addition to several of its target genes [116]. Maternal high-fat diet altered the feeding behavior of the

offspring via altered methylation and expression of genes in the dopamine and opioid pathways within areas of the brain associated with reward [117]. Interestingly, a paternal high-fat diet resulted in glucose intolerance and pancreatic β-cell dysfunction in female offspring, as well as hypomethylation and upregulation of a member of the JAK-STAT signaling pathway in pancreatic islets [118]. This study suggests that the phenotype seen in female offspring is due to high fat diet–induced epigenetic modifications of the paternal germ line. Recent work furthered this notion using a fly model, where as little as 2 days of paternal dietary intervention prior to mating resulted in obese progeny [119]. The authors went on to show this phenotype was passed through the male germ line via modifications of histone marks passed on from sperm to developing embryos [119].

Humans exposed to famine early, but not late, in gestation have a slight hypomethylation of the maternally imprinted insulin-like growth factor 2 gene (IGF2), as compared with their unexposed, same-gender siblings at 60 years of age [120]. Strikingly, the degree of methylation of a single CpG residue associated with the RXA alpha (RXRA) gene in an umbilical cord at birth predicts adiposity of the offspring at 9 years of age [121]. An ensuing study showed that hypermethylation of 4 CpGs in a differentially methylated region (DMR) upstream of RXRA was inversely associated with bone mineral density of offspring at 4 years [122]. Finally, recent data show that obesity is associated with altered small noncoding RNA expression and DNA methylation of sperm, though further studies are needed to assess whether and how these alterations of the germ line are manifested in offspring [123].

EPIGENETICS AND EDCS

Substantial research shows that developmental exposure to EDCs alters the epigenome. Maternal exposure of agouti (A^{vy}) mice to BPA [124] or the phytoestrogen genistein [125] results in hypo- or hyperretrotransposon methylation, respectively, and a corresponding obese or lean phenotype in the offspring. Wild-type mice exposed to DES [126] or BPA [127] *in utero* have increased uterine expression and altered methylation of the Homeobox A10 gene (HOXA10), which plays critical roles in uterine development and the maintenance of mature endometrium. Rats treated postnatally with PCBs have diminished global liver DNA methylation and decreased hepatic expression of DNA methyltransferases (DNMTs) [128]. Prenatal PCB exposure in rats induces liver expression of histone-modifying enzymes that subsequently reduce the transcriptional activating histone marks H3K4me3 and H4K16ac [129]. Perinatal BPA exposure results in hepatocellular damage in adult male rats and decreased hepatic expression of the β-oxidative gene carnitine palmitoyltransferase 1a (CPT1a) at birth, attributed to altered DNA methylation, transcription factor binding, and histone modification of CPT1a [130]. Hence, perinatal exposure to EDCs can permanently alter the development and function of varied tissues at least in part through stable alterations of the epigenome.

Adipogenesis is also regulated by epigenetic mechanisms that respond to environmental influences [131,132]. 3T3-L1 preadipocytes treated with a panel of EDCs, including TBT and BPA, experienced global changes in DNA methylation during adipose differentiation [78]. Bone marrow MSCs from mice treated with dexamethasone favor an adipose fate over bone due to reduced promoter methylation of the proadipogenic gene CCAAT/enhancer binding protein alpha (CEBPA) [133]. Mice prenatally exposed to TBT have MSCs biased toward the adipose lineage and a hypomethylated promoter region of the PPARγ target gene, fatty acid binding protein 4 (FABP4) [56]. Postnatal genistein exposure resulted in increased fat mass in female rats and diminished adipose expression and hypermethylation of wingless-type MMTV integration site 10B (WNT-10B), a regulator of adipose lineage commitment [134]. Finally, prenatal exposure to polycyclic aromatic hydrocarbon (PAH) increased weight and fat mass of the offspring and adipose expression of PPARγ, which correlated with promoter methylation of a single CpG site [135]. The ability of EDCs to epigenetically reprogram the MSC compartment to favor the fat lineage is an emerging and exciting area of research.

ENVIRONMENTAL EXPOSURES CAN HAVE TRANSGENERATIONAL CONSEQUENCES

Of great concern is accumulating evidence linking developmental EDC exposure to disease risk not only in offspring, but also in multiple generations of unexposed descendants. Skinner and colleagues showed that high-dose exposure of pregnant F0 rats to the fungicide vinclozolin caused reproductive abnormalities in male rats through four generations (F1–F4) [136]. While the F1 fetus and F2 primordial germ cells were exposed to vinclozolin *in utero*, the F3 and F4 generations received no direct exposure and hence their phenotype is considered transgenerational. This study went on to show that the F3 and F4 phenotype was due to heritable epigenetic alterations of the male germ line [136]. Similar adverse effects on male reproductive health were demonstrated in F1–F3 male descendants of rodents exposed to BPA [137] and phthalates [138]. Our group first showed that developmental EDC exposure could result in a transgenerational obesity phenotype [57]. The F1, F2, and F3 progeny of F0 mothers exposed to environmentally relevant doses of TBT display increased adipose depot weights, hepatic steatosis, and MSCs reprogramed to favor the adipocyte lineage [57]. There is a small, but growing list of environmental chemicals that induce a heritable, transgenerational obesity phenotype, including a mixture of plastics-derived EDCs (BPA and phthalates) [139], a hydrocarbon mixture (jet fuel, JP-8) [140], and DDT [141].

How exactly these developmental exposures propagate disease phenotypes to unexposed generations remains an open question. Some assert that an altered intrauterine environment is sufficient to propagate a phenotype through multiple generations, independent of epigenetic changes to the germ line [142]. This assertion is contradicted by evidence of transgenerational phenotypes following paternal exposures and studies showing phenotypes beyond the F2 generation [143]. DNA methylation marks are stable through mitosis and meiosis; hence, altered epigenetic reprograming of the germ line is a key mechanism through which EDCs are proposed to cause transgenerational phenotypes [112]. DNA methylation remains the most studied epigenetic factor responsible for EDC-induced transgenerational phenomena. However, DNA hydroxymethylation, histone modifications, and a variety of noncoding RNAs have all been implicated in epigenetic inheritance [144]. That developmental EDC exposure may contribute to the vast and abrupt rise in global obesity through several generations raises the stakes of identifying obesogens, studying their mechanisms of action, and ultimately reducing human exposure.

ECONOMIC BURDEN OF EDCS

A series of studies set out to estimate the economic burden of EDC exposure in the European Union [44,145]. The total cost of EDC exposure in health-care expenditures and lost productivity were conservatively estimated to be $209 billion annually, with the true cost likely being many times higher [145]. The cost of obesity and diabetes due to EDC exposure was in the range of $20–30 billion annually [44]. It should be noted that this analysis only assessed three EDCs (DDE, phthalates, and BPA) that were backed by the strongest animal studies and longitudinal epidemiological studies in humans with measurements of prenatal exposure. EDCs, for which animal data are strong but human studies are sparse, cross-sectional and/or inconclusive (e.g., PFOA and TBT) were not included. Moreover, this study did not take into account the harrowing possibility that EDCs are programing transgenerational disease susceptibility into multiple generations of humans. Hence, the actual societal burden of EDC exposure is likely to be many fold higher than the conservative estimate.

CONCLUSIONS

The tremendous cost of obesity warrants full consideration of all risk factors that may contribute to the disease. While physicians continue to prescribe diet and exercise as a panacea for obesity, the collective

weight of the US population continues to rise, even at the bottom of the BMI distribution [146]. Current clinical management of obesity and its comorbidities remains fixated on disease prevention in adults whose health is already deteriorating. Lifestyle interventions are rarely successful, yet physicians continue to attribute these failures to genetics or even to a lack of will and determination. We have presented strong evidence that environmental exposures (EDCs, in particular) in the womb and during early development can program our obesity risk for the rest of our adult lives and possibly the lives of future generations. With this in mind, it would be appropriate to shift our focus away from adults that are already in poor health and toward young adults that are planning to have children, pregnant mothers, infants, and children. On the side of industry, there are some efforts to design chemicals that lack bioactivity [147]. However, these efforts cannot counter the vast production of EDCs worldwide and governments must take action to regulate these chemicals or incentivize industry to screen for bioactivity prior to their introduction into the manufacturing process.

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