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Obesogens: an emerging threat to public health.

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Abstract

Endocrine disrupting chemicals (EDCs) are defined as exogenous chemicals, or mixture of chemicals, that can interfere with any aspect of hormone action. The field of endocrine disruption is historically rooted in wildlife biology and reproductive endocrinology where EDCs are demonstrated contributors to infertility, premature puberty, endometriosis, and other disorders. Recently, EDCs have been implicated in metabolic syndrome and obesity. Adipose tissue is a true endocrine organ and, therefore, an organ which is highly susceptible to disturbance by EDCs. A subset of EDCs, called “obesogens” promote adiposity by altering programming of fat cell development, increasing energy storage in fat tissue, and interfering with neuroendocrine control of appetite and satiety. Obesity adds more than $200 billion to U.S. healthcare costs and the number of obese individuals continues to increase. Hence, there is an urgent, unmet need to understand the mechanisms underlying how exposures to certain EDCs may predispose our population to be obese. In this review, we discuss the history of obesogen discovery from its origins in reproductive biology to its latest role in the transgenerational inheritance of obesity in mice. We discuss the development of adipose tissue in an embryo, maintenance of adipocyte number in adults, how EDC disruption programs stem cells to preferentially make more adipocytes, the mechanisms by which chemicals can permanently alter the germline epigenome, and whether there are barriers to EDCs in the gametes.

Endocrine disrupting chemicals

The field of endocrine disruption is historically rooted in reproductive endocrinology and wildlife biology. Endocrine disrupting chemicals (EDCs) are defined as exogenous chemicals (including pharmaceuticals), or mixture of chemicals, that can interfere with any aspect of
hormone action \(^1\). One poster child EDC, diethylstilbestrol (DES), was prescribed by obstetricians throughout the mid-20\(^{th}\) century with the aim of helping women avoid pregnancy complications \(^2\). Regrettably, children born from DES-treated mothers were at higher risk for clear cell adenocarcinoma, infertility, miscarriage, ectopic pregnancy and breast cancer \(^3-6\). The words “endocrine disruptor” did not enter our scientific literature until 1993 \(^7\). This was long after the first DES-baby was diagnosed \(^8\), and even longer since an accidental polychlorinated biphenyl exposure in cooking oil had contributed to cognitive decline in offspring in Japan \(^9\).

Some of the most widely studied EDCs are chemicals, such as DDT, that alter estrogen and androgen homeostasis in wildlife and contribute to reproductive endpoints such as sex-reversal and/or sterility in marine animals \(^10\). In the United States, media coverage surrounding EDCs intensified when declining male sperm counts were attributed to environmental chemicals \(^11-12\).

Today, EDCs are well-known to be associated with early puberty, infertility, and reproductive dysfunctions later in life in humans and animals \(^13-16\).

**Adipose tissue as an endocrine organ**

At about the same time that reproductive biologists and toxicologists became aware of EDCs, adipose tissue was only beginning to become accepted as an endocrine/paracrine organ (reviewed in \(^17, 18\)), let alone an organ that could be subject to disruption. The identification of fat as an endocrine organ was largely instigated by the discovery of leptin \(^19\) and the master regulator of fat cell development, the nuclear hormone receptor peroxisome proliferator activated receptor gamma (PPAR\(\gamma\)) \(^20\). Adipose tissue is highly connected to steroid hormones (estrogens, androgens, and glucocorticoids) and maintains a close relationship with the immune system via adipokines (reviewed in \(^17, 18\)).
As a result, the EDC field expanded to include adipose as a *bona fide* endocrine organ and, therefore, susceptible to chemical disturbance. The endocrine property of adipose tissue further implied that disruption could contribute to systemic diseases beyond obesity such as diabetes, infertility, and cancer. EDCs have now found a place distinct from reproductive biology and entered the field of metabolic syndrome and obesity. This subset of EDCs, called “obesogens”, or metabolic disruptors \(^\text{21}\) promote adiposity by altering programming of fat cell development (adipogenesis), increasing energy storage in fat tissue, and interfering with neuroendocrine control of appetite and satiety in experimental animals and, presumably, humans. The obesogen field is still in its infancy, but has numerous ramifications for prenatal and postnatal care, and the control/prevention of obesity and metabolic syndrome.

**Economic Impact of Obesogens**

Seventeen percent of American children aged 2 to 19 are now obese (≥ 95th percentile on CDC growth charts) and 32% are overweight (≥ 85th percentile) \(^\text{22}\). More alarming is the rise in obesity rates among children under 2 years of age \(^\text{23}\). Since it is improbable that children in this age group are consuming more food or exercising less than previous generations, it seems likely that an altered *in utero* or postnatal environment affects fat deposition during development. Obesity adds more than $200 billion to U.S. healthcare costs and the number of obese individuals continues to increase \(^\text{22}\). In the European Union (EU), EDCs contribute €157 billion per year (a conservative measurement) to the cost of human disease \(^\text{24}\), with DDE, phthalates, and bisphenol A exposures specifically contributing over €18 billion per year to adult and childhood obesity and diabetes \(^\text{25}\). Given weaker regulations of EDCs in the U.S. versus the EU \(^\text{26}\), the economic cost is likely to be proportionally greater in the U.S. Hence, there is an urgent,
unmet need to understand the mechanisms underlying how EDC exposures can predispose our population to be obese.

A Brief History of Obesogens

In the 1960s, organotins like tributyltin (TBT) were found to be effective in preventing biofouling on ship hulls by marine invertebrates and rapidly replaced copper as a biocide on ships, underwater instruments and oil pipelines. Hardly a decade after organotins were introduced, the first reports of imposex in snails surfaced and subsequent studies identified TBT as the causative agent. Given that snails lack most vertebrate orthologs of sex steroid receptors (e.g., androgen and progesterone receptors), this result did not interest vertebrate developmental biologists until 2003 when TBT was shown to masculinize female fish. The mechanism proposed was that TBT inhibited aromatase action, thereby preventing the biosynthesis of estradiol from testosterone.

Hypothesizing that TBT might function at a transcriptional level via sex steroid receptors, we tested whether TBT could activate steroidal estrogen and androgen receptors in cell culture. The results were negative. Instead, TBT bound to, and activated the nuclear hormone receptors PPARγ and the retinoid X receptor (RXR) from human, mouse and frog with nanomolar affinity, and frogs treated with TBT developed testes containing fat that replaced testicular tissue. This was significant because PPARγ and RXR function as a heterodimer to promote adipose differentiation and lipid storage. In mice, prenatal exposure to TBT led to offspring with an increased propensity to make fat cells at the expense of bone and showed increased adiposity at 10 weeks of age. TBT-exposure during puberty led to weight gain, insulin resistance, increased leptin, and fatty liver in male mice. Structural studies confirmed that TBT possesses
nanomolar binding affinity for RXR, whereas, a related organotin, triphenyltin, bound both PPARγ and RXRα avidly\cite{43-44}. Organotins still remain the only obesogens for which a molecular mechanism has been delineated.

**Evidence supporting the existence of obesogens**

Numerous other chemicals that may be obesogens have been identified (see Table 43.1 in Janesick et al, 2014\cite{45}). The types of evidence supporting the obesogenicity of individual chemicals varies. Some studies are correlative; for example, chlorinated persistent organic pollutants are associated with increased body mass index and/or type II diabetes in humans in cross-sectional epidemiological studies\cite{46}. Other chemicals induce adipogenesis in cells or activate PPARγ, but have not been tested, *in vivo*\cite{47-48}. Some EDCs have only been studied in adults while others predispose a developing fetus to subsequent obesity\cite{49}. We reserve the designation as a *bona fide* obesogen for chemicals that can induce increased fat mass, *in vivo*.

The mechanistic detail underlying obesogen action can also range from limited to thorough. For example, the crystal structure of TBT binding PPARγ and RXR has been solved whereas other obesogens such as bisphenol A may affect multiple numerous endocrine pathways\cite{49}. Whether a chemical can elicit permanent epigenetic changes in an organism and whether exposure occurs during a critical window of development (when germ cells are being programmed) can determine if the effects of an obesogen will be transient or permanent and transmitted throughout multiple generations (discussed below)\cite{50-51}. Therefore, EDCs can have a direct effect on a particular target tissue via a known mechanism of action and can also cause widespread, sometimes subtle effects on multiple organ systems that ultimately promote obesity in the exposed individual and in subsequent generations.
Developmental Origin of Adipose Tissue and Susceptibility to EDC Disruption

Adipogenesis begins in the 14th week of human gestation and continues during the early postnatal period. Adipose tissue turnover in humans persists through childhood and adolescence, then levels off at about 10% renewal per year in adulthood. This phenomenon is mostly independent of BMI, as weight gain/loss in adults is predominantly due to changes in adipocyte size. Adult mice that are challenged with a high fat diet accumulate fat by hypertrophy (increasing fat cell size) in most adipose depots, with the exception of gonadal (visceral) fat which possesses higher capacity to expand by hyperplasia. We and others concluded from these studies that increased adipogenesis during early development permanently establishes an elevated fat cell number in adulthood. Exposure of adult animals to TBT increases fat mass, but it is not known whether this effect is reversible or heritable. Subsequent increases in body fat are primarily derived from a hypertrophic mechanism in the absence of obesogen exposure.

Mesenchymal stem cells harvested from adipose tissue or bone marrow can be induced to differentiate into fat, bone, cartilage, and other lineages in culture; although, it is uncertain whether they have the same plasticity, in vivo. Commitment to each of these lineages is largely mutually exclusive and irreversible. Transformation of a mesenchymal stem cell into an adipocyte requires initial commitment to the adipose lineage, followed by terminal differentiation into a mature adipocyte (reviewed in). The initial commitment is mediated by various transcription factors which function to sensitize cells to BMP signaling, repress osteogenic Wnt signaling and promote PPARγ expression. Terminal differentiation is marked by an induction of metabolic genes and adipokines associated with mature adipocytes.
This step is primarily controlled by PPAR$\gamma$ and CCAAT-enhancer-binding proteins (C/EBP) -$\alpha$, -$\beta$, and -$\delta$\textsuperscript{68-69}. Treatment of committed pre-adipocytes with an “adipogenic cocktail” (glucocorticoids, cAMP agonists, and insulin) increases expression of PPAR$\gamma$ and C/EBP proteins which establish a sustained positive feedback loop\textsuperscript{68, 70-71}.

Together with adipogenic cocktail, activation of PPAR$\gamma$ via exogenous ligands such as rosiglitazone or TBT strongly promotes adipocyte differentiation and maintenance; together with the expression of genes involved in lipid droplet formation, glucose uptake, fatty acid synthesis, and adipokine secretion\textsuperscript{50, 72}. Obesogens such as TBT, or the fungicide triflumizole, can commit mesenchymal stem cells to the adipocyte fate while diverting them away from the osteogenic lineage. A single dose of TBT given to mice prenatally caused the mesenchymal stem cell population in offspring to veer towards the adipose lineage at the expense of bone\textsuperscript{41}. When mice were exposed to triflumizole in the water throughout pregnancy, mesenchymal stem cells preferentially differentiated into adipocytes in a PPAR$\gamma$-dependent process\textsuperscript{73}. Prenatally TBT-treated animals had more and larger fat cells and substantial fat accumulation in the liver\textsuperscript{50}. Based on the experimental design in this study, skewing the mesenchymal stem cell lineage towards adipocytes was judged likely to be an epigenetic phenomenon rather than a genetic mutation. The action of obesogens early in development can be written into the epigenetic code consisting of DNA methylation or histone modifications in the mesenchymal stem cell population\textsuperscript{41, 50, 74}. Such alterations will ultimately poise adipogenic genes to become more transcriptionally active and osteogenic genes to be transcriptionally silent during cellular differentiation.

Potential consequences of obesogens on metabolic setpoint in humans
Obese humans have more fat cells\textsuperscript{53}, and likely developed them early in life, by mechanisms outlined above. We theorize that adipogenic stimuli (such as obesogen exposure) received perinatally, or during adolescence, permanently increase fat cell number, thereby creating an altered metabolic set-point. If true, the implications are profound: The higher number of fat cells from the beginning of life cannot be reduced by diet, exercise, or even surgery\textsuperscript{53}. Visceral fat depot sizes can be expanded in adults via proliferation\textsuperscript{54-56}, but permanently decreasing cell number by weight loss has not been documented. Rigorous and faithful adherence to a restrictive diet and a vigorous exercise regimen can successfully shrink, or even empty existing fat cells. However, 83-87\% of those who achieve significant weight loss regain the weight within a few years\textsuperscript{75-76}, supporting the existence of altered metabolic setpoints. There is no evidence that empty fat cells automatically undergo apoptosis; such a scenario is evolutionarily unlikely because healthy fat cells would be required in order for the organism to survive periods of fasting. Moreover, it is likely that shrunken fat cells would "crave to be filled" because expression of the satiety hormone, leptin, closely parallels fat mass and small fat cells secrete the least leptin\textsuperscript{77}.

Transgenerational Inheritance of Obesity
A startling recent finding in the EDC field is the identification of transgenerational effects which do not follow Mendelian inheritance. This research took root in the reproductive endocrinology field when Michael Skinner and colleagues found that the fungicide, vinclozolin, given only to the pregnant rat reduced fertility in subsequent generations, including F3 (great-grandchildren) and F4 (great-great-grandchildren), that were not exposed to the chemical\textsuperscript{78}. In a
landmark study of humans from Sweden, it was demonstrated that food availability during the prepubescent period (8-12 years old) affected longevity and mortality from cardiovascular disease of that individual’s grandchildren. A single winter of overeating could lead to a 6 year decrease in longevity of a prepubescent boy’s grandsons, but not granddaughters. Other examples of heritable effects of environmental chemicals on obesity in rats have been demonstrated, albeit at relatively high doses. These compounds include plastic components such as BPA, diethylhexyl and dibutyl phthalates, a mixed hydrocarbon mixture (jet fuel JP-8) and the once widely used pesticide, DDT.

Pregnant F0 mice treated with low doses of TBT in their drinking water produced offspring that had larger fat depots, increased expression of adipogenic markers and fatty livers, despite a normal chow diet providing only 13.2% of calories from fat. Mesenchymal stem cells from these animals showed decreased expression of bone markers and a gene expression pattern indicating a bias toward the adipogenic lineage. Strikingly, these impacts of TBT treatment in pregnant F0 animals persisted through at least the F3 generation. Therefore, prenatal TBT exposure caused heritable alterations in the directly exposed F1 fetuses (and/or F2 germ cells), predisposing the MSC compartment toward the adipocyte lineage even in the F3 generation which was not directly exposed to the chemical.

Transgenerational effects, such as those elicited by TBT, are likely to be epigenetic in origin. That is, they involve changes in gene expression without accompanying changes in the DNA sequence. The major factors underlying epigenetic inheritance include expression of non-coding RNAs and alterations in chromatin structure and transcriptional activity resulting from changes in DNA and histone methylation (which are heritable). Other histone modifications (acetylation, phosphorylation, ubiquitination) affect gene expression, but these are not thought to
be heritable. Perhaps the most commonly cited changes in the epigenome resulting from EDC exposure is DNA methylation. DNA methylation also happens to be the modification easiest to measure genome-wide, and is associated with the most evidence for heritability. The usual function of DNA methylation is to regulate the transcriptional repression of certain genes in order to promote and stabilize a particular cell lineage. For example, if the promoters of osteogenic genes are methylated in the mesenchymal stem cell population but those of adipogenic genes are demethylated, the adipogenic lineage would be favored due to increased expression of adipogenic genes.

EDC alteration of DNA methylation in somatic cells is well-documented, however, it is more challenging to prove that EDCs are eliciting permanent changes in germ cell methylation as would be required for transgenerational inheritance. The currently favored mechanism underlying transgenerational inheritance of altered DNA methylation is that EDCs improperly cause regions of DNA to evade erasure of methylation marks during the two main demethylation events that occur during development. Various enzymes (e.g., Uhrf1, Dnmt1) are responsible for global demethylation, and EDCs could disrupt, or locally prevent the expression of these genes. Skinner and colleagues have identified germline epimutations caused by the EDC, vinclozolin, that persist through multiple generations. By comparing DNA methylation in male primordial germ cells (when DNA methylation is erased) to that in prospermatogonia (when DNA methylation is reestablished), they clearly demonstrated that some methylation marks are not erased in the EDC-treated cells.

**EDC exposure in the gametes**
Another important question is whether EDC exposure is acting directly on the gametes, or if more systemic effects are at play. There are two barriers that protect the germline from environmental chemicals and obesogens: the blood-fo llicular-barrier (females) and Sertoli cell barrier (males). Numerous chemicals of various sizes and charge can pass the blood follicular barrier\textsuperscript{91}. Lipid-soluble compounds penetrate the Sertoli cell barrier quite effectively, while larger hydrophilic compounds do not readily diffuse and are not actively transported\textsuperscript{92}. The extent to which environmental chemicals cross these barriers is largely unknown. The PPAR\textgreek{y} agonist, rosiglitazone, likely crosses both the Sertoli cell\textsuperscript{93} and blood-fo llicular\textsuperscript{94} barriers. Rosiglitazone decreases fatty acid oxidation in mouse cumulus oocyte complexes that are matured in vitro. While the resultant eggs fertilized efficiently, fewer embryos developed to the morula stage, and even fewer into hatching blastocysts\textsuperscript{94}. This suggests that xenobiotic chemicals can exert effects not only at the level of the adipocyte in the parental generation, but can also dysregulate lipid homeostasis at the level of the ovary.

**How to cope with obesogen exposure**

Obesity adversely affects many reproductive health outcomes, including infertility and menstrual disorders, early puberty, and pregnancy complications\textsuperscript{95-96}. While the aftermath and consequences of obesity are familiar, how to prevent its development is less certain. An article published by AJOG brought attention to EDCs and discussed the worthwhile endeavor of taking histories regarding environmental exposures from mothers before conception and during pregnancy\textsuperscript{97}. Although many obstetricians believe that counseling patients would help avoid environmental contaminants, only 45% routinely discuss mercury, 20% discuss...
pesticides/insecticides, and 5-10% discuss PCBs, BPA, and phthalates during prenatal care\(^9\)–\(^8\). Presumably, obesogens are discussed with even fewer patients.

It should be obvious that it is impossible to conclusively establish a causal link between chemical exposure in humans and any adverse health outcome except in cases of accidental exposure (e.g., Minamata mercury poisoning\(^9\), Yushō disease from PCB poisoning\(^10\)) or unanticipated side effects of prescription drug treatment\(^11\) because one simply does not perform double-blind, placebo-controlled chemical exposures on humans. The medical community readily accepts evidence of a drug’s effectiveness in preclinical animal studies as adequate justification to move drug candidates into human clinical trials. Therefore, we believe that evidence of harm from chemical exposure in animal studies should be sufficiently persuasive to counsel patients to show caution toward EDC exposure, including obesogens. In our opinion, EDCs should be routinely discussed by obstetricians with their patients. One way to minimize EDC exposure is to consume organic fruits, vegetables and grain products insofar as possible. An increasing number of fungicides routinely applied to fruits and vegetables are being identified as obesogens and metabolic disruptors\(^7\),\(^3\)\(^1\)\(^2\) and the levels of agrochemical residues such as glyphosate on corn, wheat and rice continues to rise\(^3\)\(^1\). It may also be reasonable to recommend that women minimize the use of cosmetics and personal care products containing EDCs (such as parabens and phthalates).

While we have focused on xenobiotic EDCs and obesogens that are encountered via exposures to plasities, pesticides, herbicides, industrial products, personal care products, etc, it should be noted that there are numerous chemicals shown to be obesogens in animals (and humans\(^1\)) that are intentionally added to foods\(^1\)\(^5\). These include (but are not limited to) artificial sweeteners\(^1\)\(^6\)–\(^1\)\(^7\), phytoestrogens\(^1\)\(^8\), preservatives\(^1\)\(^9\) and added sugars, in particular
high fructose corn syrup (extensively reviewed in\textsuperscript{110}). Women hoping to minimize even potential exposures to obesogens may consider limiting these chemicals during pregnancy.

Such behavior modifications have already been tested on a limited basis. One study showed that simply replacing foods with organic, non-packaged, fresh foods had a significant effect on lowering BPA and DEHP levels in the urine\textsuperscript{111}. Counter intuitively, in another study where households were provided catered, local, organic foods delivered in wood crates, glass containers, prepared without use of plastic containers/utensils, and eaten in ceramic dishes with metal utensils, the levels of urinary DEHP metabolites increased during this period of intervention\textsuperscript{112}. Remarkably, the authors traced this DEHP contamination to ground cinnamon and cayenne pepper used in the catered food\textsuperscript{112}, suggesting that the spices we consume can be a significant source of EDC exposure. No studies have yet been performed on the effects of specifically removing obesogenic EDCs, but one might reasonably expect at least a modest benefit.

In summary, an extraordinary amount of evidence is mounting to support adverse health effects of endocrine disruptors, obesogens and metabolic disruptors. Obesogens have the potential to alter metabolic setpoints and program obesity early in life. Some of these effects might be epigenetically transmitted to future generations. While direct cause-effect relationships between specific chemical exposures and corresponding harm in humans may never be established to a substantial certainty, there is obvious potential benefit in counseling patients to avoid exposure to EDCs, with no discernible risk. This “precautionary principle” is endorsed by the American Medical Association\textsuperscript{13}, and is an eminently sensible strategy for protecting public health compared with waiting until the legal threshold is reached for triggering action to ban or restrict the use of particular chemicals.
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