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The new kids on the block: emerging obesogens

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Abstract

The current obesity epidemic is calling for action in the determination of contributing factors. Although social and life-style factors have been traditionally associated with metabolic disruption, a subset of endocrine-disrupting chemicals (EDCs), called obesogens are garnering increasing attention for their ability to promote adipose tissue differentiation and accumulation. For some chemicals, such as tributyltin, there is conclusive evidence regarding their ability to promote adipogenesis and their mechanism of action. In recent years, the list of chemicals that exert obesogenic potential is increasing. In this chapter, we review current knowledge of the most recent developments in the field of emerging obesogens with a specific focus on food additives, surfactants, sunscreens for many of which the mechanism of action remains unclear. We also review new evidence relative to the obesogenic potential of environmentally relevant chemical mixtures and point to potential therapeutic approaches to minimize the detrimental effects of obesogens. We conclude by discussing the available tools to investigate new obesogenic chemicals, strategies to maximize reproducibility in adipogenic studies, and future directions that will help propel the field forward.

Keywords

adipose tissue; adipogenesis; endocrine disrupting chemicals; emerging obesogens; food additives

1. Introduction

Obesity, defined as body mass index (BMI) > 30 kg/m^2, continues to be among the most prevalent chronic co-morbidities in the US. In the past 2 decades, obesity prevalence has increased from 30.5% in 1999–2000 to 42.4% in 2017–2018 (Hales et al. 2020). Prevalence of severe obesity (BMI > 40 kg/m^2) has also continued to increase from 4.7 to 9.2%, with women being disproportionately more affected by severe obesity than men (11.5 vs. 6.9%, respectively; (Hales et al. 2020). In 2016, obesity prevalence in U.S. children was 18.5%, representing 13 million children age 2 to 19, which is concerning since childhood obesity is

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strongly associated with obesity later in life (Hales et al. 2017). Because monogenic obesity, which is caused by a single gene mutation is relatively rare and obesity is more often considered to be syndromic (associated with other phenotypes, such as neurodevelopmental abnormalities) or polygenic (caused by a combination of genes and the environment) (Thaker 2017), the increasing obesity trends can only be explained by the latter. Notably, obesity prevalence disproportionately affects certain groups, with non-Hispanic African Americans (49.6%) being more affected by obesity compared to age-matched non-Hispanic Whites (44.8%) or Asians (17.4%) (Hales et al. 2020). Other socio-economic factors such as lower education and income have also been associated with a higher obesity prevalence (Ogden et al. 2017). The disparity in prevalence among socioeconomic groups points to non-caloric/non-genetic factors contributing to the increased obesity prevalence. Nutritional and lifestyle habits are thus considered major drivers of obesity. However, growing evidence also supports a contributory role of environmental factors at certain developmental stages to the obesity epidemic. Pioneered in the late 1980s by Barker *et al.* (Barker et al. 1989), studies in the field of developmental origins of health and disease (DOHaD) have demonstrated that pre-conceptional and peri-gestational factors, such as maternal nutrition (Davenport and Cabrero 2009; Strohmaier et al. 2020), maternal glycemic status (Tam et al. 2017), maternal stress (Leppert et al. 2018; Matvienko-Sikar et al. 2020), and/or paternal adiposity (Sharp and Lawlor 2019) can modulate the obesity risk of the progeny. Other environmental factors, such as chemical exposures can also modulate the risk to develop obesity during prenatal, as well as postnatal life (Egusquiza and Blumberg 2020; Heindel et al. 2017; Veiga-Lopez et al. 2018).

2. Adipose tissue: an overview

Obesity is generally described as excessive accumulation of white adipose tissue (WAT) in the body (Rosen and Spiegelman 2006) and it is a risk factor for comorbidities such as cardiovascular disease, type 2 diabetes, and cancer (Meigs et al. 2006). WAT depots differ not only in location, but also in function and developmental stage at which they appear for the first time (Chau et al. 2014). Retroperitoneal, perigonadal and mesenteric WATs are visceral depots, and their dysregulation or misfunction has been associated with adverse health outcomes (Chau et al. 2014), while interscapular and inguinal WAT depots are located subcutaneously, are associated with body thermoregulation, and have lower risks for development of other comorbidities.

The fact that the location of the WAT plays a role on the severity of obesity implies that each obesity case should be evaluated carefully. Not all obese individuals develop further metabolic complications such as high glucose levels or altered lipid panel, which are often linked to obesity-associated diseases (McLaughlin et al. 2007). The concept of metabolically healthy obesity was first coined in the 1950s and it is based in clinical observations of a cohort of obese patients with reduced predisposition to type 2 diabetes and atherosclerosis compared to metabolically unhealthy obese patients (Vague 1956). Although there is not a clear definition for metabolically healthy obesity, there is some consensus on the fact that it has been associated with the lack of ectopic fat storage, such as in the visceral cavity, liver or muscle tissue, and lack of insulin resistance, hypertension, and/or cardiovascular risk (Bluher 2020). However, obese metabolically healthy individuals are still at a higher risk

of developing adverse comorbidities than lean individuals (Bluher 2020). Before discussing how environmental factors may contribute to the development of unhealthy adipose tissue and obesity, it is necessary to understand adipose tissue development, types, and functions.

2a. White adipose tissue development

WAT contains an heterogeneous cell population that includes mature adipocytes, which only constitutes ~50% of the white adipose tissue mass, and stromal cells (*e.g.*, endothelial, blood cells, and adipocyte precursors) (Rodeheffer et al. 2008). One of the functions of WAT is to act as a nutrient sensor. Thus, in periods of high nutrient availability, adipocytes synthesize and store triglycerides that can be mobilized as free fatty acids in periods of high energy demand (Rosen and Spiegelman 2006). In addition to maintaining nutrient homeostasis, the adipose tissue also plays an important role as an endocrine organ, as it secretes adipokines (e.g., adiponectin and leptin) and enzymes that participate in steroid hormone metabolism (e.g., aromatase) (Kershaw and Flier 2004).

WAT precursors rise during prenatal development in two different forms: precursors that will contribute to the development of adipose tissue, and precursors involved in its homeostasis during adulthood (Jiang et al. 2014). One key characteristic of healthy WAT is its plasticity to adapt depending on environmental cues, such as nutrient availability, energy expenditure or temperature (Pellegrinelli et al. 2016). WAT can expand following two different pathways: hypertrophy (i.e., enlargement of cell size due to increased lipid storage) or hyperplasia (i.e., increased in the number of adipocytes due to proliferation of precursor cells). Hypertrophic adipose tissue is positively associated with chronic inflammation, fibrosis and insulin resistance (Vishvanath and Gupta 2019). In contrast, hyperplasia has been associated with a more metabolically healthy state (Vishvanath and Gupta 2019). Both processes are often accompanied by the remodeling of the extracellular matrix, which provides the tissue with mechanical flexibility and support to grow healthy when necessary, based on environmental stimuli. Unhealthy adipose tissue develops if the excessive growth of the tissue is accompanied by fibrosis, oxidative stress due to impaired angiogenesis, and recruitment of inflammatory cells such as macrophages and lymphocytes (Crewe et al. 2017).

2b. Other types of adipose tissue

Another type of adipose tissue found in mammals is the brown adipose tissue (BAT). In contrast to the detrimental effects of excessive accumulation of WAT, increased BAT accumulation introduces metabolic benefits has traditionally been described as a potential therapeutic target to treat obesity (Harms and Seale 2013). BAT is very vascularized and it is involved in body control of temperature, or thermoregulation, by burning out body fat (Harms and Seale 2013). In newborns, BAT constitutes ~5% of total fat content, reaching the highest percentages during puberty and going down to minimal presence in adulthood (Drubach et al. 2011). The amount of BAT in adults largely depends on each individual's lifestyle, as it has been described that exercise and exposure to low temperatures, increases BAT development (Drubach et al. 2011).

If the different structure, function, and location of WAT and BAT was not complex enough, white adipocytes can acquire brown adipocyte behaviors upon exposure to certain environmental stimuli, such as low temperature (Vitali et al. 2012), and become *beige* adipocytes (Harms and Seale 2013).

The difference between adipose tissue type (WAT, BAT, and *beige*), its location in the body (subcutaneous *vs*. visceral), and whether it is healthy or dysfunctional can dramatically impact the health of the individual and contributes to whether the obese subject is metabolically healthy or unhealthy. Environmental factors have been shown to influence the accumulation and function of both WAT and BAT, and the capacity of WAT to become *beige* cells (Symonds et al. 2021; Xiao et al. 2020). As such, environmental factors play a critical role not only in the increasing prevalence of obesity worldwide, but also in its association with other disease such as type 2 diabetes and cardiovascular disease.

3. Obesogens, environmental factors that interfere with adipose tissue metabolism

Since the industrial revolution in the 1950s, an increasing number of chemicals have been introduced into the manufacturing chain of consumer products, with over 40,000 of them currently in commerce (EPA 2020). Their production volume also continues to increase with production of plastics worldwide from 1950 to 2018 increasing up to 6,300 metric tons / year (Geyer et al. 2017). Notably, some of these chemicals can interfere with normal endocrine function and are known as endocrine disrupting chemicals (EDCs) (Gore et al. 2015). Baillie-Hamilton proposed in 2002 evidence that certain chemicals can alter adipose tissue deposition contributing to obesity, which would later be known as obesogens (Baillie-Hamilton 2002; Grun et al. 2006). *Bona fide* obesogens are those that induce white adipose tissue storage *in vivo*, while potential obesogens are those that have only been shown to promote adipogenesis *in vitro* (Egusquiza and Blumberg 2020). Currently, ~20 chemicals are recognized as *bona fide* obesogens and include organotins, polychlorinated biphenyls, bisphenols, phthalates, parabens, and non-steroidal estrogens, among others (Andrews et al. 2020). These chemicals are components of common consumer products, such as plastic bottles, toys, vinyl flooring, food preservatives, disinfectants, and medical equipment, but also of building materials (paints, resins, solvents) and agrochemicals (Ren et al. 2020; Veiga-Lopez et al. 2018). Hence, exposure to these chemicals is ubiquitous and thus, human exposure virtually unavoidable. Since exposures to these chemicals occur throughout an individual's lifetime (Hendryx and Luo 2018; Shim et al. 2017; Woodruff et al. 2011; Zota et al. 2014), recognizing the major events that shape an individual's adipose tissue development is key in understanding the major windows of susceptibility to obesogenic chemical exposures.

For some obesogenic chemicals, there is conclusive evidence regarding their ability to modulate adipogenesis, as they activate the peroxisome proliferator-activated receptor gamma (PPARγ) or its heterodimeric partner, the retinoid X receptor alpha (RXRα) (Egusquiza and Blumberg 2020; Heindel et al. 2015; Veiga-Lopez et al. 2018). Other chemicals increase adipogenic differentiation and adipose tissue accumulation by

modulating other receptors such liver X receptor (LXR), thyroid receptor-beta (TRβ), glucocorticoid receptor (GR), estrogen receptor (ER), androgen receptor (AR), and others (Chappell et al. 2018; Kassotis et al. 2019; Niemelä et al. 2008).

PPARγ is considered the "master" regulator of adipogenesis as it is involved in the initial steps of adipocyte differentiation (Tontonoz and Spiegelman 2008), while RXRα has been shown to play a critical role in the commitment of stem cell precursors into the adipogenic pathway (Shoucri et al. 2017). One example of *bona fide* obesogen that activates both PPARγ and RXRα is tributyltin (TBT) (Grun et al. 2006; Yanik et al. 2011), which increases adipogenic commitment and differentiation *in vitro* (Kirchner et al. 2010; Li et al. 2011). *In vivo*, TBT can induce adipose tissue accumulation upon postnatal exposure in mammals and fish (Penza et al. 2011; Tingaud-Sequeira et al. 2011) and lipid accumulation in non-adipose tissues, such as liver (Zuo et al. 2011), brain (Lyssimachou et al. 2015), and ovary (de Araujo et al. 2018). More importantly, prenatal TBT exposures can reprogram adipose tissue development and these traits are transmitted transgenerationally through epigenetic mechanisms (Chamorro-Garcia et al. 2017; Chamorro-Garcia et al. 2013; Diaz-Castillo et al. 2019; Kirchner et al. 2010). Other organotins, such as triphenyltin and dibutyltin have also shown to exert obesogenic effects (Chamorro-Garcia et al. 2018; Lutfi et al. 2017; Milton et al. 2017).

Although relatively underexplored, certain obesogens induce development of dysfunctional adipocytes *in vitro* by altering glucose uptake, reduction of antidiabetic adipokine adiponectin, and reduction in the cell ability to inhibit expression of proinflammatory and profibrotic markers (Ariemma et al. 2016; Regnier et al. 2015; Shoucri et al. 2018). One example of obesogen that promotes development of dysfunctional adipocytes is TBT which has been attributed to its capability of activating RXRα (Shoucri et al. 2018). The mechanistic evidence is less definitive for other chemicals, including polychlorinated biphenyls, bisphenols, phthalates, parabens, and non-steroidal estrogens, which has been reviewed elsewhere (Egusquiza and Blumberg 2020; Ren et al. 2020; Veiga-Lopez et al. 2018). To note, other tissues, such as the liver are also targets of obesogenic chemicals and have been linked to chronic metabolic diseases such as non-alcoholic fatty liver disease (Foulds et al. 2017). In this review, we have focused on chemicals that have been recently reported to display obesogenic potential with a central focus on the adipose tissue, but for which mechanism of action remain unclear (See Table 1 and Figure 1).

4. Emerging obesogens: the new kids on the block

Here, we discuss current knowledge on emerging obesogens defined as chemicals or mixture of chemicals for which there is preliminary evidence for their role to disturb adipose tissue development, but whose mechanisms of action remain unclear.

4a. Food additives

Food additives are compounds that help preserve food or change its qualities. Over 3,000 food additives are databased in the US Food and Drug Administration (FDA) and are classified into numerous categories including stabilizers, preservatives, coloring agents, flavor enhancers, antioxidants, and emulsifiers among others. While some food

additives are natural products, such as vinegar or salt, others are not, but are considered by the US FDA as safe compounds. Here, we have focused on food additives, such as surfactants, emulsifiers, antioxidants, and flavor enhancers, that have been reported to enhance adipogenic differentiation and result in intracellular lipid accumulation.

Originally evaluated as a component of the oil dispersant COREXIT used in the clean-up of the Deepwater Horizon spill (USEPA 2010), dioctyl sodium sulfosuccinate (DOSS) is used as stabilizer, thickener, wetting agent, solubilizing agent, emulsifier, and flavoring agent in numerous food products, such as cream cheese, salad dressings, and milk (e-CFR 2020). Also known as docusate, DOSS is also approved for use as a stool softener. DOSS is rapidly absorbed and metabolized in the body making, which difficult its detection in human fluids (WHO 1975). DOSS has been reported to exert a dose-response increase in lipid accumulation in 3T3-L1 preadipocytes, with 118.6 μM of DOSS resulting in the same adipogenic differentiation as 1 μM of rosiglitazone (Temkin et al. 2016). DOSS's adipogenic induction occurs through PPARγ (Bowers et al. 2016), but not RXRα activation (Bowers et al. 2016). Because of the rapid rate at which DOSS is metabolized in humans and other animals tested, it would be necessary to determine whether the results observed *in vitro* are indeed caused by DOSS or any of its metabolites. Sorbitan monooleate, also known as Span80, is another emulsifier part of COREXIT that can also enhance adipogenic differentiation of 3T3-L1 preadipocytes in a dose-dependent manner through transactivation of PPARγ and RXRα (Bowers et al. 2016). Polyoxyethylene (20) sorbitan monooleate, another emulsifier commonly known as Tween 80 or Polysorbate 80, can also enhance 3T3-L1 preadipocyte differentiation through RXRα activation (Bowers et al. 2016). Both, Span 80 and Tween 80 are derived from oleic acid, which has the ability to enhance adipogenic differentiation especially in the presence of insulin and dexamethasone through PPARγ (Madsen et al. 2005). Other surfactants, such as polyoxyethylene stearates (8 and 40), and polyethene sorbitans (monolaurate, monoleate, monopalmitate, monostearate, and tristearate), also containing saturated and monounsaturated fatty acids backbones, which have the ability to stimulate adipogenic differentiation (Madsen et al. 2005), have not been evaluated in the context of adipogenesis. Notably, mixture of these surfactants, such as DOSS and Span80, leads to significant increases adipocyte differentiation above that of each individual exposures (Bowers et al. 2016). Among all surfactants studied to date, only DOSS has been tested *in vivo*. A gestational DOSS exposure with an oral dose representing the use of stool softener (5μg/g BW) resulted in male, but not female mice offspring with increased body and visceral adipose tissue mass and accompanying glucose intolerance (Temkin et al. 2019). Additional studies should investigate whether surfactant mixtures with fatty acids backbones can lead to adipose tissue accumulation *in vivo*.

Introduced in the food industry in the 1950's, 3-tertbutyl-4-hydroxyanisole (3-BHA) has been used as an antioxidant to prevent fat rancidification of foods. 3-BHA is also used in cholesterol lowering medications, such as lovastatin, and simvastatin and it is considered an EDC for its estrogenic and anti-estrogenic activities (Kang et al. 2005; Pop et al. 2013). Present not only in foodstuffs, but also in the environment (Liu et al. 2015; Wang et al. 2016; Wang and Kannan 2018), human exposure burden to synthetic phenolic antioxidants (SPA) is widespread; with BHA displaying lower percentage exposure and concentration compared to other SPAs (median: 0.014 ng/ml) (Liu and Mabury 2018). 3-BHA, but not 2-

tert-butyl-4-hydroxyanisole (2-BHA) has been reported to enhance adipogenesis in 3T3-L1 preadipocytes (Sun et al. 2019). This effect however was driven by activation of upstream PPARγ modulators, cAMP-response element binding protein (CREB), upregulation of CAAT/enhancer-binding proteins β (C/EBPβ), but not direct PPARγ binding (Sun et al. 2019). Chronic (18 weeks) exposure to 3-BHA in male C57BL/6J mice did not affect body weight or insulin sensitivity, but increased percent inguinal subcutaneous adipose tissue, adipocyte size, and total cholesterol at 1 mg/kg BW, a dose well above human exposure ranges (Sun et al. 2020b). Whether these effects are reproducible at lower exposure ranges or can induce programming effects during gestational exposures has yet to be evaluated.

4b. Alkylphenols

Alkyphenols are organic compounds that belong to a class of synthetic-nonionic surfactants widely employed in the manufacture of detergents, adhesives, cosmetics, emulsifiers and household cleaners (Acir and Guenther 2018). Their global consumption of over 600 kilotonnes per year (Zgola-Grzeskowiak et al. 2015) makes them ubiquitous in the environment and human tissues. Human urine levels of alkylphenols have been estimated in ~12 ng/ml (You et al. 2011) Alkylphenols are considered xenoestrogens (Soto et al. 1991) and their effects on the nervous and immune systems have been widely studied (Acir and Guenther 2018). Because alkylphenols accumulate in human adipose tissue (Lopez-Espinosa et al. 2009; Muller et al. 1998) non-ethoxylated alkylphenols, such as 4-nonyphenol and octylphenol have been studied in the context of adipogenesis. For over a decade, we have known that both, 4-nonyphenol and octylphenol can lead to pro-adipogenic effects *in vitro* (Lee et al. 2008; Masuno et al. 2003; Miyawaki et al. 2008) and *in vivo* (Hao et al. 2012; Kim et al. 2015; Zhang et al. 2014). However, the effects of ethoxylated-alkylphenol on adipogenesis have only been explored recently. A study evaluating several alcohol- and ethoxylated-alkylphenols with chain lengths between 11 and 16 base carbons, reported that ethoxylated alkylphenols promote triglyceride accumulation with longer ethoxylate chains length resulting in a greater effect (Kassotis et al. 2018a). This effect was shown as low as 0.1 μM (0.1 ng/ml) and to be independent of PPARγ activation but partially driven by the TRβ antagonism in 3T3-L1 cells (Kassotis et al. 2018a). More recently, the alkylphenol 4-hexylphenol was also reported to enhance adipogenesis in 3T3-L1 cells and lipid accumulation in HepG2 cells (Sun et al. 2020a). However, *in vivo* exposure studies have not been reported to date.

4c. Sunscreens

Other chemicals that have recently been reported to enhance adipogenesis include those used as organic ingredients of sunscreen, such as benzophenones (Kim and Choi 2014). Bezonphenone 3 (BP-3) is present in human urine at 22.9 μg/l, while avobenzone is present in human plasma at 3.3–7.1 ng/ml for up to 21 days after the original exposure (Matta et al. 2020; Zamoiski et al. 2015). Considered EDCs for their ability to bind to the pregnane X receptor (Mikamo et al. 2003), benzophenones can block ultraviolet (UV) light and are thus also used to protect products, such as perfumes and soaps, from UV damage. Stemming from a microarray study, avobenzone, a dibenzoylmethane derivative used in sunscreens to absorb UV-A rays, was discovered to increase PPARγ transcription (Ahn et al. 2019). Using human bone marrow mesenchymal stem cells (hBM-MSCs), avobenzone was observed to

promote adipogenesis (at 10 μM), but independently of the PPARγ pathway (Ahn et al. 2019). Using the same *in vitro* approach, benzonphenone (BP)-3 and BP-8 (at 30 μM) promoted adipogenic differentiation through PPARγ binding, with BP-3 being a full and BP-8 a partial PPARγ agonist (Shin et al. 2020). *In vivo* studies have yet to be conducted to validate these results at human exposure doses.

4d. Chemical mixtures

The complexity of studying chemical mixtures (Martin et al. 2020) have resulted in only a handful of studies that have attempted to evaluate the effect of a combination of chemicals on adipogenic differentiation. Using the 3T3-L1 cell model, Kassotis et al., 2018 (Kassotis et al. 2018b) tested a mixture of 23 commonly used unconventional oil and gas chemicals (UOG), including acrylamide, alkylphenols, benzenes, bronopol, diethanolamine, ethanols, ethylene glycol, propylene glycol, styrene, toluene, and xylens. This mixture resulted in an increase in tryglyceride accumulation and preadipocyte proliferation at 10 μM and 1 μM, respectively (Kassotis et al. 2018b). This effect was observed in both, laboratory mixtures as well as samples from UOG-impacted samples (Kassotis et al. 2018b). Notably, some UOG-impacted waters promoted PPARγ, but not the laboratory UOG mixture, demonstrating that UOG-induced triglyceride activation is PPARγ independent. These findings further demonstrate that chemicals that can independently promote adipogenesis, such as acrylamide and alkylphenols (Kassotis et al. 2018a; Lee and Pyo 2019) can act as obesogens in environmentally collected samples containing a complex chemical mixture. However, developmental exposure to a similar UOG mixture altered body weight and energy expenditure, but not body composition in C57BL/6 mice (Balise et al. 2019a; Balise et al. 2019b), which highlights the need to validate *in vitro* findings using animal models.

Other developmental exposures (prenatal and postnatal until day 140 of life) to complex mixtures, such as those found in bisphenol A- and nonylphenol-containing wastewaters have reported an increased adipose deposition and weight gain during adulthood in male mice (Biasiotto et al. 2016). Other chemical mixtures that have been tested for adipogenesis include those present in oil sands process-affected water (OSPW). This complex mixture that includes thousands of chemicals (Headley et al. 2013; Pereira et al. 2013; Pereira and Martin 2015) was found to have PPARγ activity (Peng et al. 2016). However, the pro-adipogenic effect of OSPW was restricted to the polar fractions at environmentally relevant concentrations (Peng et al. 2016). While these studies provide with a proof of principle regarding real-life exposures, the high complexity of the mixtures does not allow to make in-depth conclusions regarding the specific chemical(s) that drive the adipogenic effect. Less complex mixtures have also been evaluated. Using an EDC mixture design based on serum concentrations reported in pregnant women of a Swedish mother-child cohort (EDC mixture #1 in Table 1, the developmental effects of a mixture (4 phthalates, triclosan, and 2 perfluorinated chemicals; dose ranges: 3 – 28 nM per chemical) on adipogenesis and lipid storage have been evaluated in zebrafish (Mentor et al. 2020). With an exposure from 3 h until 17 days post-fertilization, the EDC mixture increased adipocyte number and visceral adipose tissue, but mild effects on adipocyte-related genes (Mentor et al. 2020). While these findings further support previous studies linking phthalates to adipogenic outcomes, not all studies are in support of the obesogenic nature of phthalates (Wassenaar

et al. 2017). Another EDC mixture consisting of di(2-ethylhexyl) phthalate, bisphenol A, 2,3,7,8-tetrachlorodibenzo-pdioxine (TCDD), and polychlorinated biphenyl 153 (PCB153) below the NOAEL was used to expose adult male mice for 15 weeks (EDC mixture #2 in Table 1). This EDC mixture did not increase adipose tissue under standard diet conditions but resulted in lower plasma free fatty acids and triglycerides and mild expression changes in subcutaneous adipose tissue related to adipogenic differentiation (Naville et al. 2019). Whether these EDC mixtures exert sex-specific differences in adipose tissue depots of mammalian species or different developmental windows result in obesogenic outcomes, such as those seen in individual chemical exposures (Egusquiza and Blumberg 2020; Veiga-Lopez et al. 2018), warrants further research.

5. Approaches and Reproducibility

Chemicals described in this chapter represent only a subset of chemicals that have been recently evaluated for their potential role as obesogens, which suggests the existence of many other obesogens in the environment that have not been described yet. To determine whether a chemical is considered an obesogen, rigorous approaches to improve reproducibility should be followed considering all potential limitations. *In vitro* models allow for a first approximation to identify potential obesogens, that could subsequently be tested in animal models. Although there are significant efforts focused on standardizing the methodologies to determine the role environmental factors in human obesity (Trasande et al. 2016), differences across laboratories affect not only replication of results, but also data interpretation. One key example is the use of 3T3-L1 cells as a model of adipogenesis differentiation. Kassotis *et al.*, made the observation that the source of the cell line, the plastic used to culture the cells (i.e., whether or not it is chemically treated by the manufacturer), and the protocol used for their differentiation, play a critical role in the detection of new potential obesogens *in vitro* (Kassotis et al. 2017). Determining the limiting factors and setting up strategies to implement reproducibility standards will be the ideal path forward to inform *in vivo* experimental approaches to confirm *bona fide* obesogens. Moving the field forward also requires high throughput strategies that allows screening of chemicals for not only receptor binding potential using classic binding assays (Foley et al. 2017; Hartman et al. 2018) or molecular docking-based methods (Jaladanki et al. 2021) but also for adipogenic potential (Graham et al. 2020). Importantly, these high throughput tools need to be coupled with efficient and automated analytical capabilities (Adomshick et al. 2020; Yuan et al. 2019).

Most of the studies reviewed here use the 3T3-L1 preadipocyte cell line as model to study adipogenesis. The limitation of 3T3-L1 cells is that they are committed to differentiate into adipocytes. Thus, they cannot be used to study the potential of chemicals to induce adipogenic commitment or their effects over other potential differentiation pathways precursors might follow prior to commitment. An alternative is the use of mesenchymal stem cells (MSCs). Derived from various adult tissues in mammals (e.g., adipose tissue, bone marrow, umbilical cord), these primary cells are commercially available from different species, and they are able to differentiate into various cell types, including adipocytes, depending on the specific *in vitro* stimuli (Ullah et al. 2015). MSCs are, therefore, and excellent tool to study commitment effects. Although further analyses are needed, Shoucri *et*

al. showed that RXRα activators are potential inducers of adipogenic commitment (Shoucri et al. 2017). The food additives Span 80 and Tween 80 have been shown to induce final adipocyte differentiation in 3T3-L1 cells and to activate RXRα. These two food ingredients are potential candidates to be tested for their adipocyte commitment capacity in MSCs. Although some chemicals such as TBT and bisphenols have been demonstrated to have similar effects across species *in vitro* and *in vivo* (Gingrich et al. 2021; Pu et al. 2019; Veiga-Lopez et al. 2015), a potential limitation is that 3T3-L1 are murine cells, which limits the capability of drawing conclusions about mechanisms of action in humans. MSCs can be isolated from multiple species, including humans, which would broaden the capacity to evaluate interspecies effect of potential obesogens. Moreover, the use of a single cell line, stem cells isolated from a single individual, or representing only one sex, does not capture human inter-individual genetic and environmental exposure differences. Thus, to enhance study robustness and reproducibility, and whenever possible, multiple systems (cell lines, primary cultured cells from different individuals, and/or both sexes) should be included within the same study.

6. Conclusions & Future directions

Alteration of adipose tissue size and homeostasis play an important role not only in obesity, but also in the development of other metabolic co-morbidities such as type 2 diabetes and cardiovascular diseases (Bluher 2020). To note, obesity also induces wide-reaching systemic effects on other systems, such as the reproductive and the immune system (Francisco et al. 2018; Leisegang et al. 2021; Snider and Wood 2019). Thus, determining factors contributing to obesity, critical windows of susceptibility and mechanisms of action is instrumental to generate strategies for future prevention and treatment of a broad range of metabolic and reproductive disorders.

Epidemiological studies have shown the association between exposure to environmental factors and metabolic disruption (Kahn et al. 2020). These studies are extremely valuable to understand the impact of these factors on human health. However, due to the ethical and logistic limitations of the use of human tissues at certain stages in life, performing further analyses to confirm these associations becomes challenging. As mentioned in this chapter, adipose tissue structure is very dynamic throughout life in response to environmental cues (Pellegrinelli et al. 2016). Since the development of the adipose tissue starts prenatally (Jiang et al. 2014), environmental exposures during *in utero* development may contribute to lasting metabolic disruptions compromising health during adulthood (Figure 2). Animal models, from flies and worms, to fish, rodents, and large mammals and non-human primates, allow the scientific community to narrow down mechanisms of action during all stages in life that would not be possible to determine in humans (for review see Kleinert et al. 2018).

Potential therapeutic approaches to counteract the detrimental effects of obesogens would be the introduction of other dietary supplements that have been shown to promote beneficial metabolic outcomes. For example, there are food additives such as curcumin and capsaicin, that induce the development of BAT and mitochondrial biogenesis and that could therefore be used as therapeutics to counteract obesogen exposure. Curcumin and capsaicin, the natural polyphenol resveratrol (Burns et al. 2002) and polyunsaturated fatty acids (PUFAs)

stimulate brown adipogenesis via the PPARγ/PRDM16 complex (for review see El Hadi et al. 2018). Green tea and menthol have also shown to modulate mitochondrial biogenesis (a characteristic of BAT development) via phosphodiesterase inhibition or the transient receptor potential cation channel melastatin (TRPM8) (El Hadi et al. 2018). Activators of other anti-adipogenic pathways such as the AMP-activated Protein Kinase (AMPK) (Ahmad et al. 2020), could be potential therapeutic targets for the treatment of obesity.

While the work performed using *in vitro* models contribute to a better understanding of mechanisms of action of emerging obesogens, multiple strategies to increase reproducibility, such as the use of multiple *in vitro* model systems, multiple species, and *in vivo* validation is highly warranted. This coupled with high throughput screenings to identify emerging obesogens will help fast-track the field and establish the contribution that obesogens have in the current obesity epidemic.

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Non-standard Abbreviations

2-BHA	2-tertbutyl-4-hydroxyanisole
3-BHA	3-tertbutyl-4-hydroxyanisole
AMPK	AMP-activated protein kinase
AR	Androgen receptor
BAT	Brown adipose tissue
BMI	Body mass index
BW	Body weight
C/EBPb	CAAT/enhancer-binding proteins β
CREB	cAMP-response element binding protein
DOSS	Dioctyl sodium sulfosuccinate
EDCs	Endocrine disrupting chemicals
ER	Estrogen receptor
FDA	Food and Drug Administration
GR	Glucocorticoid receptor
hBM-MSCs	human bone marrow mesenchymal stem cells
LXR	Liver X Receptor

NOAEL	Non-observed adverse effect level
PCB153	Polychlorinated biphenyl 153
PPARγ	Peroxisome proliferator-activator receptor gamma
OSPW	Oil sands process-affected water
PUFAs	Polyunsaturated fatty acids
RXRα	Retinoid X receptor alpha
SPA	Synthetic phenolic antioxidants
TBT	Tributyltin
TCDD	2,3,7,8-tetrachlorodibenzo-pdioxine
TRb	Thyroid receptor-beta
TRPM8	Transient receptor potential cation channel melastatin
UOG	Unconventional oil and gas chemicals
WAT	White adipose tissue

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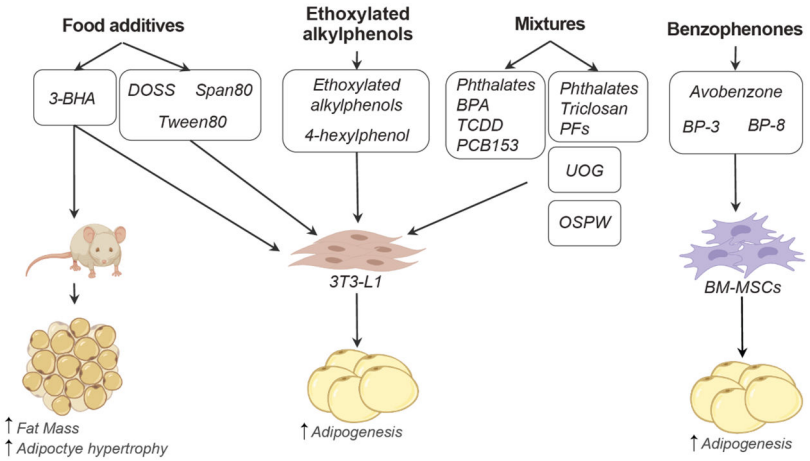

Figure 1. Effect of emerging chemicals on adipogenesis and adipose tissue accumulation.

Emerging obesogenic chemicals are classified as food additives, ethoxylated alkylphenols, benzophenones, or chemical mixtures (*top*). Testing models that have been used so far for these emerging obesogens include cell-based models, such as the preadipocyte cell line 3T3-L1 and bone marrow mesenchymal stem cells (BM-MSCs) and mice. Additional in vivo studies to validate in vitro observations are needed. 3-BHA: 3-tertbutyl-4-hydroxyanisole, BP: benzophenone, BPA: bisphenol A, DOSS: dioctyl sodium sulfosuccinate, TCDD: 2,3,7,8-tetrachlorodibenzo-pdioxine, PCB: polychlorinated biphenyl, PF: perfluorinated chemicals, and UOG: unconventional oil and gas chemicals.

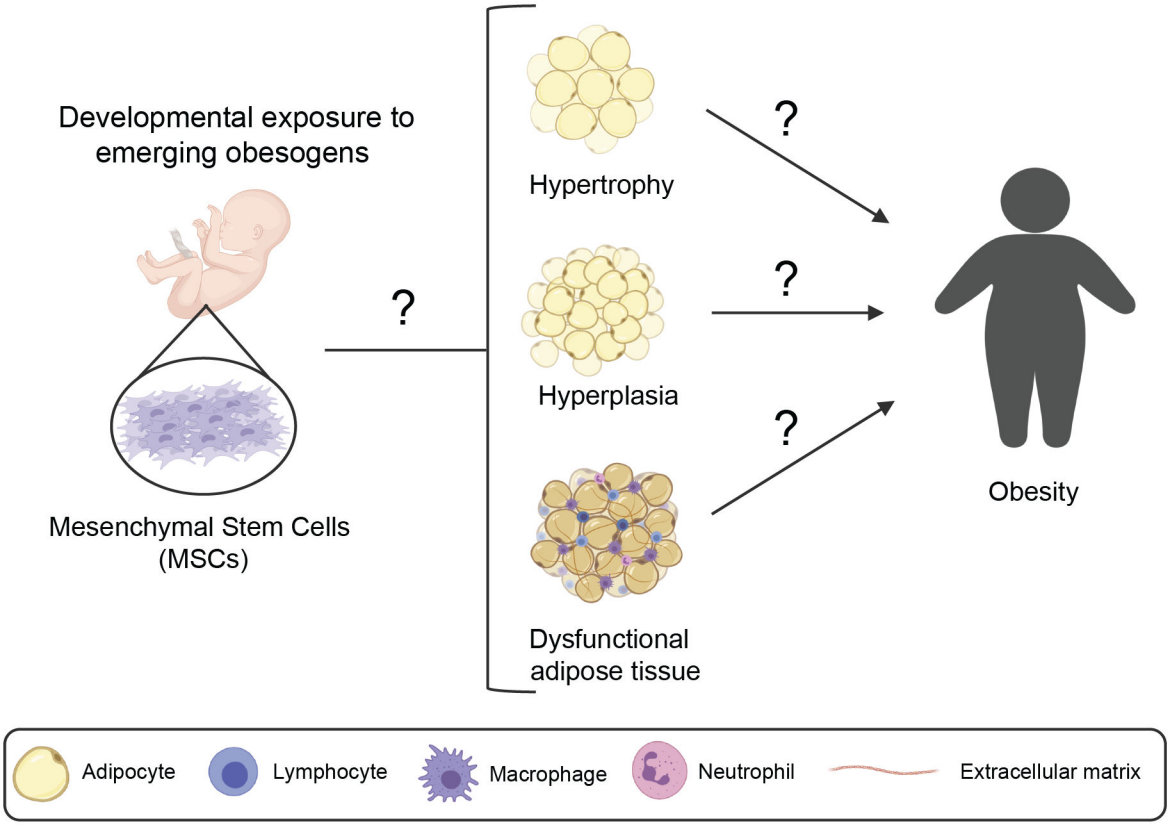

Figure 2. Model representing the effect of developmental exposure to emerging obesogens in obesity later in life.

Emerging obesogens could reprogram mesenchymal stem cells (MSCs) during early stages of development to favor their differentiation into larger adipocytes (hypertrophy), more adipocytes (hyperplasia) or dysfunctional adipocytes. These alterations would lead to obesity and other metabolic disorders during adulthood. Question marks point to gaps in knowledge. These gaps include: 1) understanding if emerging obesogens alter adipose tissue development leading to adult obesity (*left*) and 2) the underlying mechanisms by which emerging obesogens can lead to adipose tissue hypertrophy, hyperplasia, and/or dysfunctional adipose tissue and ultimately obesity.

Table 1.

Emerging obesogens and chemical mixtures

	Short name	Use	*Cell model*	Mechanism	*Animal model*
1. Food additives					
Dioctyl sodium sulfosuccinate	DOSS	Thickener Solubilizer Emulsifier Flavoring	3T3-L1	PPARγ	Mice
Sorbitan monooleate	Span 80	Emulsifier	3T3-L1	PPARγ RXRα	—
Polyoxyethylene (20) sorbitan monooleate	Tween 80 Polysorbate 80	Emulsifier	3T3-L1	RXRα	—
3-tertbutyl-4-hydroxyanisole	3-BHA	Antioxidant	3T3-L1	PPARγ activators	Mice
2. Alkylphenols					
Ethoxylated alkylphenols	EOAP	Non-ionic surfactant	3T3-L1	TRβ	—
4-hexylphenol	4-HP	Surfactant	3T3-L1 HepG2	PPARγ	—
3. Sunscreens					
Avobenzone	AVB	UVA filter	hMSCs	PPARγ	—
Benzonphenone 3 and 8	BP-3 BP-8	UV filter	hMSCs	PPARγ	—
4. EDC mixtures					
Unconventional oil and gas chemicals *Acrylamide, Alkylphenols, Benzenes, Bronopol, Diethanolamine, Ethanols, Ethylene glycol, Propylene glycol, Styrene, Toluene, Xylens*	UOG	—	3T3-L1	PPARγ independent	—
Oil sands process-affected water *See* Headly et al., 2013 *and* Pereira et al., 2013 *and* 2015 *for chemical mixture*	OSPW	—	3T3-L1	PPARγ	—
EDC mixture #1 *Phthalates, Triclosan, Perflorinated substances*	—	—	—	—	Zebrafish
EDC mixture #2 *Di(2-ethylhexyl) phthalate, Bisphenol A, 2,3,7,8-tetrachlorodibenzo-dioxine (TCDD), polychlorinated biphenyl 153 (PCB153)*	—	—	—	—	Mice