Bisphenol-A and metabolic diseases: epigenetic, developmental and transgenerational basis

Paloma Alonso-Magdalena,1 Francisco J. Rivera,2,3 and Carlos Guerrero-Bosagna4,*

1Departamento de Biología Aplicada, Universidad Miguel Hernández, Elche, Spain, 2Laboratory of Stem Cells and Neuroregeneration, Institute of Anatomy, Histology and Pathology, Faculty of Medicine and Center for Interdisciplinary Studies on the Nervous System (CISNe), Universidad Austral de Chile, Valdivia, Chile, 3Institute for Molecular Regenerative Medicine and Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus University, Salzburg, Austria and 4Avian Behavioral Genomics and Physiology Group, IFM Biology, Linköping University, Linköping, Sweden

*Correspondence address. Avian Behavioral Genomics and Physiology Group, IFM Biology, Linköping University, Linköping, Sweden; Tel: +46-700895837; E-mail: carbo@ifm.liu.se

Abstract

Exposure to environmental toxicants is now accepted as a factor contributing to the increasing incidence of obesity and metabolic diseases around the world. Such environmental compounds are known as ‘obesogens’. Among them, bisphenol-A (BPA) is the most widespread and ubiquitous compound affecting humans and animals. Laboratory animal work has provided conclusive evidence that early-life exposure to BPA is particularly effective in predisposing individuals to weight gain. Embryonic exposure to BPA is reported to generate metabolic disturbances later in life, such as obesity and diabetes. When BPA administration is combined with a high-fat diet, there is an exacerbation in the development of metabolic disorders. Remarkably, upon BPA exposure of gestating females, metabolic disturbances have been found both in the offspring and later in life in the mothers themselves. When considering the metabolic effects generated by an early developmental exposure to BPA, one of the questions that arises is the role of precursor cells in the etiology of metabolic disorders. Current evidence shows that BPA and other endocrine disruptors have the ability to alter fat tissue development and growth by affecting the capacity to generate functional adipocytes, as well as their rate of differentiation to specific cell types. Epigenetic mechanisms seem to be involved in the BPA-induced effects related to obesity, as they have been described in both in vitro and in vivo models. Moreover, recent reports also show that developmental exposure to BPA generates abnormalities that can be transmitted to future generations, in a process called as transgenerational epigenetic inheritance.

Key words: BPA; obesity; metabolic diseases; epigenetics; mesenchymal stem cells; transgenerational
Introduction

The incidence of obesity has increased in the past few decades across age segments, in both genders and in both developing and developed countries [1]. Importantly, other diseases such as cardiovascular diseases, diabetes and reproductive disorders are also associated with the incidence of obesity [2–4]. Diseases correlated to obesity are generally integrated into the concept of metabolic diseases [2, 5, 6]. Interestingly, conditions associated with metabolic diseases, such as heart diseases and diabetes mellitus, are among the leading causes of morbidity and mortality in the modern world (Fig. 1).

The causes behind the alarming increase in the incidence of metabolic diseases are now known to be multifactorial and include excess calorie intake, food composition, physical inactivity and other factors [7, 8]. It is becoming increasingly evident that these ‘other factors’ include exposure to environmental contaminants, since many of them have been demonstrated to be involved in the etiology of obesity [9, 10] and diabetes [11, 12]. The importance of investigating these factors is such that the World Health Organization has set a high research priority on the effects of environmental contaminants [13].

Furthermore, developmental processes and epigenetic mechanisms are now known to be affected by the same environmental exposures that correlate with obesity, as well as the involvement of epigenetic mechanisms. A consensus statement was published highlighting the relevance of exposure to environmental contaminants for the etiology of metabolic diseases and the need for epigenetic research in order to unveil the molecular mechanisms involved in this connection [17].

Among the known developmental exposures that correlate with the incidence of obesity, exposure to bisphenol-A (BPA) is one of the best documented. BPA is used to make polycarbonate plastic and epoxy resins, which are in turn used in a variety of plastic items including water bottles, children toys, sports equipment, medical and dental devices, dental fillings and sealants, as well as household electronics. In humans, BPA has been detected in urine, amniotic fluid, neonatal blood, placenta, cord blood and breast milk at levels that are known to be of biological relevance [18].

Early developmental exposure to BPA is reported to trigger obesity that emerges later in life [19–22]. BPA-induced obesity is, however, accompanied by other disease phenotypes, which include insulin resistance, glucose intolerance [19] or reproductive impairments such as primordial follicle loss and polycystic ovaries in females, and testis and prostate abnormalities in males [21]. This review aims at summarizing the available evidence linking BPA exposure to obesity, as well as the involvement of epigenetic mechanisms.

Mechanistic Actions of Endocrine Disruptors

The ‘International Programme on Chemical Safety’ from the World Health Organization defined in 2002 an endocrine-disrupting chemical (EDC) as ‘An exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations’ [23]. From a mechanistic perspective, most EDC effects are thought to result from their interaction with nuclear hormone receptors, including estrogen receptors (ERs), androgen receptors, progestosterone receptors, thyroid receptors and retinoid receptors among others. However, increasing evidence shows that the mechanism is much more complex and that some EDCs may also modulate nonnuclear steroid hormone receptors, orphan receptors, like the aryl hydrocarbon receptor, and many other pathways that converge upon endocrine and reproductive systems. From the structural point of view, EDCs are highly heterogeneous, although they have in common a phenol moiety that allows EDCs to interact with steroid hormone receptors as analogs or antagonists [24].

In particular, BPA has two phenolic and two (4, 4)-OH substituents that allow it to join into the ER-binding structure. Binding assays have demonstrated that BPA binds both ERα and ERβ although with higher affinity for the second. Despite its relatively low affinity for both receptors, increasing number of studies have demonstrated that BPA can promote estrogen-like activities that are similar or stronger than the ones elicited by
17β-estradiol. These low-dose responses result in part from the activation of rapid responses via non-classical ER pathways or by a different BPA recruitment of co-activators or co-repressors [25, 26]. In addition to its estrogenic activity, BPA has been shown in some studies to exhibit anti-thyroid [27] and anti-androgenic activity [28], to bind the human estrogen-related receptor [29] and pregnane receptor [30], as well as to stimulate the glucocorticoid receptor [31].

**Developmental Effects of EDCs Related to Metabolic Diseases**

Many studies to date have reported the link between developmental exposures to EDCs and the emergence of metabolic diseases. Pioneering studies by Grun and Blumberg [32] described the concept that some compounds have an ‘obesogenic’ action, showing that developmental exposure to such compounds is correlated with the generation of obesity. Organotins, such as tributyltin chloride and triphenyltin chloride, were the first class of compounds described to be obesogenic [32]. Since this initial description, numerous studies have reported other compounds as being obesogenic in humans and animal models. One of them is DDE, which is one of the degradation products of DDT. Indeed, most DDT degrades slowly to DDE and DDD; these compounds stick strongly to soil particles, make their way into rivers and lakes in their runoff and persist in the environment for hundreds of years [33]. The main route of exposure in humans is through food contaminated with these compounds [33]. Human exposure to DDE is still occurring nowadays even in the countries where DDT has been banned, due to their persistence in the environment [33]. Prenatal exposure to DDE in humans has been associated with rapid weight gain in children [34, 35]. Also, DDE is found at higher levels in the blood of pregnant mothers who gave birth to obese individuals [36]. Similarly, studies have also shown obesogenic effects of pollutants and toxicants such as mixtures of organochlorines [37] and hexachlorobenzene [35, 38].

In animal models, TBT exposure during gestation is associated with higher body weight and increased adipogenesis [39]. Not only synthetic but also natural EDCs such as genistein have been reported to be obesogenic. Female rats neonatally exposed to genistein present increased body weight, fat/lean mass ratio, fat mass and larger adipocytes in higher density [40].

In general, several compounds are now known to fit the category of obesogenic. These include polychlorinated biphenyls, dioxins, plastic compounds or pesticides, which have been associated with metabolic disturbances such as mitochondrial dysfunction, lipid disturbances, insulin resistance, diabetes and high blood pressure in both experimental animals and human studies [17, 41, 42].

**Metabolic Effects of a Gestational Exposure to BPA**

Laboratory animal work has provided conclusive evidence that early-life exposure to BPA is particularly effective in predisposing individuals to weight gain. Initial evidence came from studies performed by Howdeshel et al. in 1999, which revealed that in utero exposure to low doses of BPA (2.4 μg/kg) resulted in increased body weight on postnatal day 22 [43]. From that moment until now, many studies have evaluated the effects of the exposure to this endocrine disruptor during pregnancy or pregnancy and lactation in terms of lipid and energy balance. One of the most comprehensive studies was conducted by Angle et al. [20]. This study showed that mice exposed to dietary BPA at doses ranging from 5 to 50,000 μg/kg/day presented increased postnatal body weight, adipocyte number, and volume of abdominal fat, together with changes in insulin, leptin and adiponectin levels. The effects, that were maximal at the lowest doses from 5 to 500 μg/kg/day, confirmed the obesogenic effects of BPA [20]. Longer exposure times to BPA at doses from 0 to 3000 μg/kg/day also provoked a dose-dependent increase in body and liver weight in the male offspring, while adult females showed a reduction in body weight [44]. Similar experimental procedures in rats caused fatty acid accumulation in BPA offspring that contributes to the development of hepatic steatosis [45]. When the exposure to BPA occurred via drinking water similar results were found. Perinatal exposure to BPA (1 or 10 μg/mL) elicits increased body weight and adiposity [46]. Higher drinking BPA doses also resulted in accelerated body weight gain that was evident soon after birth and continue into adulthood [47]. Concomitantly, maternal oral BPA exposure altered the expression of several genes that are key in the regulation of adipogenesis including PPARγ, SREBP-1c, C/EBPα and C/EBPβ in the female offspring [22]. Although some studies in mice investigating perinatal exposure to BPA did not find weight changes in the 5-week-old adult offspring [48], most of the published studies have reported effects in body weight later in life. In any case, it is important to note that many of the responses reported are non-monotonic, so the lack of effect when only one dose is assayed should be considered with caution. Interestingly, decreased physical activity and enhanced carbohydrate metabolism have also been reported in female mice developmentally exposed to BPA [49].

Overall, we can conclude that perinatal exposure to BPA behaves as a metabolic stressor in the offspring. Importantly, when BPA administration is combined with a high-fat diet (HFD), there is an exacerbation in the development of metabolic disorders. This has been observed in a study by García-Arevalo et al. [50], in which pregnant mice were exposed to BPA at the dose of 10 μg/kg/day from day 9 to 16 of gestation and later. When pups reached 1 month of age, they were fed control or HFD during 13 or 24 weeks. The authors described an increase in body weight in BPA-treated animals to a similar extent to that observed in control animals fed HFD at the age of 20 weeks. Moreover, important changes were observed in the levels of expression of major genes implicated in fatty acid metabolism including PPARα, SREBP-1c, CPT1β and CD36 [50]. Higher doses (50 μg/kg/day) administered throughout gestation and lactation in combination with HFD after weaning aggravated the dyslipidemia and the body weight gain caused by BPA only [51]. In another study, male BPA offspring challenged with HFD weaning until PND110 showed an increased fat/lean ratio. This was accompanied by increased triglycerides (TG) and free fatty acid levels, as well as by marked changes in the expression of TG synthesis- and β-oxidation-related genes [52].

Prenatal exposure to BPA is a potential contributor to the onset of metabolic disorders, including not only obesity but also type 2 diabetes. The diabetogenic effect of BPA is consistent among different studies after prenatal or perinatal exposure. First evidence came from a study published in 2010 by Alonso-Magdalena et al. [19]. The treatment with BPA (10 or 100 μg/kg/day) from day 9 to 16 of gestation provoked marked glucose intolerance, insulin resistance and alterations in pancreatic β-cell function in male offspring at 6 months of age. No effects were observed neither in male mice at younger ages nor in females [19]. In a similar manner, lower doses of BPA 3.5 μg/kg/day induced glucose intolerance in male offspring at 3 months of
age but not in females [53]. When a more exhaustive range of BPA doses (5 to 50,000 µg/kg/day) was analyzed, the data demonstrated that the oral exposure to BPA in mice from days 9 to 18 of gestation led to impairment of glucose tolerance and insulin sensitivity with maximum effect at the dose of 5 and 50 µg/kg/day [20]. Oral exposure in pregnant rats also led to development of insulin resistance in the offspring at 21 weeks of age [54]. Preimplantation, fetal and neonatal periods of exposure to BPA were also studied. In all cases, glucose intolerance was observed in 3-month-old male offspring. At the age of 8 months, glucose intolerance persisted together with decreased insulin sensitivity and insulin release in the case of fetal BPA exposure [55]. As with lipid disturbances, glucose intolerance was aggravated when combining BPA treatment with later HFD [50, 51]. Interestingly disturbances of glucose metabolism due to maternal BPA exposure have been reported not only in F1 generation but also in F2 [56, 57].

Remarkably, upon BPA exposure of gestating females, metabolic disturbances have been found both in the offspring and later in life in the mothers themselves. These effects have been recently described using a model for BPA-induced metabolic in which two doses of BPA were used (10 and 100 µg/kg bw/d). BPA-exposed pregnant females developed severe glucose intolerance and aggravated insulin resistance, which resembles gestational diabetes. Even though alterations disappeared after parturition (as happens in many cases of gestational diabetes), the remission was only temporary and metabolic perturbations appeared again months later. No changes were observed in non-pregnant-treated female mice, suggesting that both pregnancy and BPA exposure are necessary to cause the described phenotype [19]. The most pronounced effects were observed at 6–7 months after the exposure and included decreased pancreatic β-cell function and mass, reduced plasmatic insulin levels and marked glucose intolerance. Interestingly, these effects accompanied increased body weight gain and fat accumulation [58]. The fact that these effects were observed months after the exposure suggests that reprogramming of epigenetic mechanisms might be involved. Interestingly, this would imply that gestating females would be particularly susceptible to have their epigenome affected by environmental exposures in comparison to non-gestating females. Concordant with this hypothesis, a recent study in domestic pigs has shown that undernutrition in early pregnancy affects maintenance and de novo DNA methylation in the endometrium, as well as de novo DNA methylation in the myometrium [59]. These effects seem unrelated to major hormonal changes, since neither plasmatic and intrauterine progesterone levels nor plasmatic estradiol levels were affected [59].

BPA and the Role of Precursor Cells in the Etiology of Metabolic Diseases

When considering the metabolic effects generated by an early developmental exposure to BPA, one of the questions that arises is the role of precursor cells in the etiology of metabolic disorders. Since BPA-induced abnormalities are not restricted to metabolism but also extend to fat tissue development and growth, many cell types are expected to be involved in the etiology of metabolic diseases. Cells with a preponderant role include adipocytes, pancreatic beta cells, fibroblasts and osteoblasts, among others. Adipocytes, fibroblasts and osteoblast originate from mesenchymal stem cells (MSCs), which also differentiate to other cells types such as chondrocytes and myoblasts [60–62]. Therefore, understanding how MSCs are affected by EDCs, and particularly by BPA, is fundamental in order to unveil whether shifts occur in the differentiation rates between these cell types.

MSCs can be found in different stages of commitment depending on the tissue where they reside. For example, adult adipose tissue contains ‘committed pre-adipocytes’ with narrow differentiation capabilities, while vascular tissue contains multipotent precursor cells known as ‘MSC-pericytes’, according to in vitro data [63]. Studies suggest that exposure to EDCs alters the cell fate and the capacity of MSCs to generate adipocytes at the detriment of other cell types [63]. A very complete in vitro study in which many EDCs were analyzed for their potential to alter adipogenic differentiation revealed that differentiation of 3T3-L1 pre-adipocytes to adipocytes was increased after exposure to BDE-47, TBT or BPA, while inhibited by TCDD [64]. A further study has confirmed such increased adipogenic differentiation of 3T3-L1 cells triggered by BDE-47 [65]. In adipose-derived stromal stem cells, TBT is shown to increase adipogenic differentiation while concomitantly decreasing osteogenic [39]. In regards to the effects of EDCs on MSC-derived cell types other than adipocytes, in vitro exposure to the pesticides chlorpyrifos and carbofuran has been shown to inhibit the differentiation of cultured human MSCs to bone, although no effects were seen in adipogenesis [66].

For the specific case of BPA, perinatal exposure is shown to increase adipogenesis and is associated with adipocyte hypertrophy in weaned female pups, indicating the ability of BPA to interfere with the generation of new functional adipocytes in fat tissue [22]. Also, in vitro exposure to BPA-diglycidyl-ether has been shown to induce adipogenic differentiation in both pre-adipocytes and multipotent MSCs even at nanomolar concentrations [67]. BPA-glucuronide, which is the predominant metabolite of BPA after in vivo conversion, has been shown to induce lipid accumulation and mRNA expression of adipogenic markers in cultured 3T3-L1 preadipocytes [68]. In general, BPA accelerates terminal differentiation of 3T3-L1 cells into adipocytes, which takes place through the phosphatidylinositol 3-kinase pathway [69].

Moreover, combined effects of BPA with other EDCs have also been reported. Exposure to insulin has been reported to trigger differentiation of 3T3-L1 fibroblasts into adipocytes [70]. Interestingly, insulin exposure either together or after exposure to BPA considerably increased the cellular content of triacylglycerol, lipoprotein lipase and glycerol-3-phosphate dehydrogenase in comparison to insulin alone [70]. Such evidence suggests that metabolism is severely disrupted in adipocytes differentiated in the presence of BPA. This is also supported by evidence showing that prolonged exposure to low doses of BPA (at environmental levels) increases pre-adipocyte proliferation and interferes with their differentiation program, leading to the generation of functionally impaired hypertrophic adipocytes [71].

Interestingly, it seems that BPA effects on pre-adipocytes differ from those observed in multipotent MSCs. For example, in vitro exposure to BPA has been shown to decrease MSC differentiation towards the adipocyte lineage [72], which differs from the effects observed in pre-adipocytes. Nevertheless, a combined exposure of BPA and other EDCs, such as bis(2-ethylhexyl)phthalate (DEHP) and tributyltin (TBT) is shown to increase MSC adipogenesis [72]. This suggests that effects of BPA alone might drastically differ from those effects observed when the exposure is in combination with other EDCs. Besides influencing differentiation, BPA have also been shown to induce cytotoxicity in human-derived MSCs in a dose- and time-
dependent manner [73]. Therefore, while it appears that BPA promotes differentiation of abnormal pre-adipocytes, it also seems that BPA decreases adipogenesis and survival of MSCs. Although the exact mechanisms are currently unknown, current evidence certainly shows that BPA and other EDCs have the ability to alter fat tissue development and growth by affecting the capacity of pre-adipocytes and MSCs to generate functional adipocytes, as well as their rate of differentiation to specific cell types.

Epigenetic and Transgenerational Effects Induced by BPA

Epigenetics involve accessory chemical modifications to the DNA, which are mitotically stable and are able to regulate gene expression [74]. These modifications include DNA methylation or hydroxymethylation of CG dinucleotides, chemical modifications of histones, interaction of DNA with small RNAs or states of chromatin condensation [75–77]. Epigenetic patterns can be altered in organisms due to environmental exposures, especially when this occurs within sensitive developmental windows [78]. Epigenetic effects related to obesity and derived from exposure to EDCs have been described in both in vitro and in vivo models [79]. In pre-adipocytes decreased DNA methylation has been reported at 3 CpG sites in the PPARc promoter region after exposure to BDE-47 [65]. Hypomethylation has also been observed in the promoter of (Fapb4), which is target of PPARγ, after adipose-derived stromal cells from white adipose tissue were exposed to TBT [39]. Neonatal exposure to genistin generates differential DNA methylation effects between sexes in the WNT10B gene in white adipose tissue [40]. As for BPA, a perinatal exposure alters DNA methylation near the transcription start site of CPT1 and induces many histone modifications in liver cells of the male offspring. Also, the Bartolomei group has shown that gestational exposure to BPA disrupts genomic imprinting in the placenta and embryonic tissue of the offspring [80].

In addition, recent reports show that developmental exposure to BPA generates abnormalities that can be transmitted to future generations, in a process known as transgenerational epigenetic inheritance (TEI) [21, 81]. TEI involves environmental exposure of a gestating mother, which affects the germline epigenome of the developing embryo [74]. This altered germline epigenome has the ability to influence the somatic and germ-line epigenomes of the descendants [82, 83], which will then interfere with their phenotype (Fig. 2). Evidence demonstrating TEI has emerged from a variety of model organisms, including rodents, fish and invertebrates [84]. Also, reports of disease phenotypes being transgenerationally transmitted in humans [85] make TEI of wide interest for current and future human health [86]. Consideration of the current evidence on TEI leads to the reasoning that past exposure to certain environmental contaminants may have contributed to current increases in metabolic diseases, and in the same way, current exposures will influence future disease trends [86].

In rats, exposure to pharmacological doses of a variety of environmental toxicants during embryonic days 8–14 generates transgenerational effects such as reproductive abnormalities and changes in sperm DNA methylation [81]. In particular, embryonic exposure to a mixture of plastics that includes BPA increases the incidence of male and female reproductive abnormalities [21, 81, 87] and obesity [21]. The incidence of animals with augmented visceral adiposity increased from 0% in controls to >8% in the unexposed F3 generation [21]. The effects were observed in both genders. Two doses of BPA (in combination with other plastic compounds) were tested, with the lower doses generally producing larger effects. Moreover, a number of changes in DNA methylation (197 regions) were observed in the sperm three generations after the exposure [81]. Network analyses of these genes revealed that five of them had been previously shown to have direct connections to obesity, such as TNFRSF12A, ESRRA, FGF19, WNT10B and GDNF [21]. In addition, six other genes (ENOPH1, ATF3, NCAM2, NTF3, PITX3 and DPYSL2) were correlated to these directly connected genes and therefore represented indirect connections to obesity [21]. In summary, it is becoming increasingly evident that developmental exposure to BPA alters the epigenetic machinery to generate transgenerational effects related to metabolic diseases.

Previous studies using the fungicide vinclozolin as the environmental exposure during the developmental period of the
germline epigenetic reprogramming have demonstrated trans-generational (i.e. F3 generation after the exposure) changes in both DNA methylation and gene expression in Sertoli \cite{82} and granulosa \cite{83} cells. These are somatic cells fundamentally involved in the processes of spermatogenesis or oogenesis, respectively. These studies suggest that an environmentally altered germ line epigenome induce somatic epigenetic and transcriptomic changes several generations after the exposure.

Interestingly, not only BPA is reported to generate such trans-generational obesogenic effects. Other studies show that intraperitoneal exposure to DDT \cite{88} or Jet Fuel \cite{89} during the same developmental window previously described for the plastics mixture (i.e. embryonic days 8–14), generates increased incidence of obesity in the unexposed F3 generation but not in the developmentally exposed F1 generation. This is an important observation that has repeatedly been observed concerning transgenerational effects. A plausible explanation is that F1 effects are the result of epigenetic effects on somatic cells directly affected during the developmental exposure, while the effects observed in the F3 generation derive from an altered gametic epigenome (e.g. sperm epigenome), which will then influence the epigenome of the derived somatic cells (Fig. 3).

Conclusions

The concept of obesogens is nowadays well supported by experimental evidence in both animal and human studies \cite{17}. Among obesogens, BPA is one of the most widespread and ubiquitous compounds affecting humans and animals \cite{18,26}. An important fact emerging from studies investigating the obesogenic effects of BPA is that such effects are dose, age and generation dependent, with non-monotonic responses being commonly reported.

In addition, discernable windows of susceptibilities for BPA exposure exists, which also occurs with exposure to other EDCs. Well-described windows of exposure include early development and gestation. Importantly, gestational exposures generate long-lasting metabolic disturbances both in the exposed mother and in the developmentally exposed offspring.

Investigations also show that obesogenic effects are associated with epigenetic changes, of which changes in DNA methylation are the most studied. However, altered histone modifications have also been reported. DNA methylation changes related to a BPA exposure have been described in both somatic and germ cells, in \textit{in vivo} and \textit{in vitro} studies.

\begin{table}[h]
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\begin{tabular}{|l|l|}
\hline
\textbf{Gene} & \textbf{Gene Name} \\
\hline
ATF3 & Activating transcription factor 3 \\
CD36 & Cluster of Differentiation 36 \\
CPT1β & Carnitine palmitoyltransferase-1 beta \\
C/EBPα & CCAAT/enhancer binding protein alpha \\
DPYSL2 & Dihydropyrimidinase like 2 \\
ENOPH1 & Enoase-phosphatase 1 \\
ESRRA & Estrogen related receptor alpha \\
FGF19 & Fibroblast growth factor 19 \\
GDNF & Gial cell line derived neurotrophic factor \\
NCAM2 & Neural cell adhesion molecule 2 \\
NITF3 & Nutrotophin 3 \\
PITX3 & Paired-like homeomain transcription factor 3 \\
PPARα & Peroxisome proliferator activated receptor alpha \\
PPARγ & Peroxisome proliferator activated receptor gamma \\
SCD-1 & Stearoyl-Coenzyme A desaturase 1 \\
SREBP-1C & Sterol regulatory element-binding protein-1c \\
TNFRSF12A & TNF receptor superfamily member 12A \\
WNT10B & Wingless-related MMTV integration site 10b \\
\hline
\end{tabular}
\caption{Gene abbreviations}
\end{table}
An additional aspect of concern regarding the obesogenic effects of BPA is that the incidence of obesity is shown to increase in unexposed generations that descend from individuals developmentally exposed to BPA or other EDCs. This suggests that BPA and other EDCs are able to induce an ‘obesogenic transgenerational’ effect, as well as gametic epigenomic alterations being transmitted to future generations.

The evidence summarized in the present review points towards an urgency in investigating knowledge gaps related to BPA- and other EDC-induced metabolic disturbances. These include the exact physiological mechanisms involved and how they integrate with the epigenetic machinery. Also, a fundamental piece of information to understand is how precursor cells are affected by BPA exposure in such a manner that their differentiation capability is altered for life. More research is also needed on the effects generated by environmentally relevant doses of BPA exposure, given that many of the studies performed so far aimed at describing biological mechanisms but have not focused on risk assessment.

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