Early-life exposure of Bisphenol A and obesity


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EARLY-LIFE EXPOSURE OF BISPHENOL A AND OBESITY

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ABSTRACT

Bisphenol A (BPA) is used in the manufacturing of food and beverage containers. BPA can leach from the lining of the container to contaminate stored food and drinks, and can therefore be consumed by humans. Concerns have been raised that BPA acts as an endocrine disruptor of both developmental and reproductive processes. To date several nuclear hormone receptors have been shown to be able bind to and be activated by BPA. Recent data show that foetuses are more susceptible to BPA exposure, as BPA can accumulate in the placenta and high doses of BPA have been measured in umbilical cord and amniotic fluid. The potential of BPA exposure to influence body weight has been suggested from in vitro studies showing an effect of BPA on adipocyte differentiation and lipid accumulation and in vivo rodent studies, showing an association between BPA exposure in utero and increased body weight in adulthood. The mechanisms through which foetuses and infants are exposed to BPA and the consequences of BPA exposure and the onset of obesity are discussed in this chapter.

Keywords: Bisphenol A, development, obesity, adipogenesis

INTRODUCTION

BPA (2,2-bis(4-hydroxyphenyl)propane) is a monomer widely used in the plastics industry since the 1950s to manufacture polycarbonate plastics and epoxy resins which line
Today, global annual BPA production exceeds 6 billion tonnes [2]. BPA is among the most frequent organic wastewater contaminants detected in ground water in the USA [3]. In the United States, the median reported BPA concentration in surface waters was 0.14 μg/L (6.14x10^{-4} μM) whereas in Europe, mean values of 0.0047 μg/L (0.2x10^{-4} μM) and 0.0052 μg/L (0.23x10^{-4} μM) were observed in Germany and Netherlands respectively [4-6]. In water near processing facilities, BPA levels can be higher than 20 μg/L (877x10^{-4} μM) [7]. Humans are exposed to BPA mainly by consuming contaminated food and drink products. A tolerable daily BPA intake (TDI) of 50 µg/kgBW/day has been set by the Environmental Protection Agency (U.S. EPA) [8], Food Standards Australia and New Zealand (FSANZ) [9] and the European Food Safety Authority (EFSA) [10].

In the US, measurements of BPA in serum of adult men and women showed that the mean concentration of BPA in 2002 was 2.49 ng/mL (109x10^{-4} μM) [11]. In 2011 a study showed that subjects consuming a daily serving of canned vegetable soup for 5 days exhibited a 1200% increase in urinary BPA concentration compared with controls [12].

Almost everyone in the modern world is exposed to BPA. BPA was detected in urine samples from 92.6% of the US population studied (National Health and Nutrition Examination Survey (NHANES) 2003-2004) [13]. This includes neonates, as BPA is also detected in maternal and foetal plasma, in placental tissue in humans [14] and in the milk of nursing mothers [15].

BPA receives scrutiny because it is an estrogenic endocrine disrupting chemical and is therefore classified as endocrine disrupter (ED). Historically BPA was classified and only suspected to be an estrogenic compound: BPA has been shown in human tissue in vitro to bind to and activate the classical estrogen receptors [16] (Figure 1), ERα and ERβ, with a lower binding affinity than the natural ligand 17-β estrodial or E_2 (10-1000 times lower binding affinity) [17, 18] and to the non-classical membrane bound estrogen receptor (ncmER) [19] also known as GRP30 [20].

Although BPA action on insulin secretion via the ER pathway and the onset of diabetes has been extensively studied [1, 16, 21], the effects of BPA on obesity are not fully understood. Indeed, the available data may point to an antagonistic effect of the ER pathway for the onset of diabetes. Hewitt and colleagues investigated a mouse lacking a functional aromatase, a crucial enzyme for estrogen biosynthesis, and found that these mice display a very low level of estrogen and develop obesity [22].

This result was also observed in another estrogen deficient mouse lacking follicle-stimulating hormone (FSH) [23]. Implication of the estrogen pathway was further confirmed by the development of obesity in ERα knock-out adult male mice [24]. Interestingly, ERβ knock-out adult mice did not show signs of obesity pointing to ERα specificity for development of obesity [24]. Therefore it seems that reduced ER activation contributes to the development of obesity. As BPA is a known activator of the ERs [25, 26] other protein targets for BPA must be examined to understand the possible links between BPA exposure and obesity.

Excessive body weight can be detected very early in life and as pregnant women and babies are under constant BPA exposure, the idea that BPA might exert its negative effect during foetal life has become increasingly of interest. Recent studies have looked at the developmental effects induced by BPA in utero.
Early BPA Exposure and the Onset of Obesity

Figure 1. Chemical structure of BPA and estradiol. Chemical structure of BPA (A) and E2 (B). Interaction networks of E2 (C) and BPA (D) with LBP residues in ERα. Oxygen, nitrogen, sulfur, fluorine, and chlorine atoms are colored in red, blue, yellow, cyan, and green, respectively. Hydrogen bonds are indicated by black dashed lines. For clarity, not all protein–ligand interactions are depicted. The blue electron density represents a $F_o - F_c$ simulated annealing omit map contoured at 3σ. (C and D) [27].

Gibert et al. found that BPA exposure during early zebrafish embryogenesis causes otolith (a crystallized structure of the inner ear used for maintaining proper balance) malformations [17] and Dong Li et al. determined that BPA exposure during rat embryogenesis caused retarded growth in a dose dependant manner, with embryos exhibiting hydrocephaly, smaller midbrain, forebrain and forelimb bud, open neural tube and abnormal heart, optics and flexion [27]. While these effects found were detectable during development, it is hypothesised in regards to metabolic disease, that in utero exposure to BPA may cause long term effects that may not be detectable during development or at birth, that increase susceptibility to developing metabolic disease later in adulthood. This review will consider both the immediate and long term effects of in utero and early life BPA exposure and its long term effects on adipogenesis and obesity.

**Route of BPA Exposure during Early Life**

As mentioned, human BPA exposure occurs mainly via the consumption of contaminated food and drink. This contamination occurs via the hydrolysis of the ester bonds which link BPA to the polycarbonate, allowing for the migration of BPA from the polycarbonate into the food or drink it is storing [28]. The BPA migration rate increases with heating [28], prolonged storage time and polycarbonate degradation [29]. In addition to BPA exposure via consumption, high levels of BPA have also been found in cashiers who handle polycarbonate
receipts [30] and in BPA manufacturing workers [31] suggesting BPA exposure may also occur via skin contact the respiratory tract.

Upon oral consumption, BPA is absorbed in the gastrointestinal tract in its free form [32]. Some of the free BPA is conjugated to glucuronic acid by the enzyme UDP glucuronosyltransferase (UGT). This glucuronation of BPA forms the main BPA metabolite BPA-glucuronide, which exhibits no estrogenic activity. The remaining free BPA and BPA-glucuronide then enter the liver via the portal vein, where it is thought that the majority of BPA conjugation takes place by hepatic UGT isoforms [33, 34]. From the liver, free BPA and BPA-glucuronide enter the systemic circulation [33]. The conjugation process prior to entry to circulation results in low levels of free BPA in adult human plasma, with 17 studies between the years 1999-2009 finding serum BPA levels ranging from non-detectable level to a highest level of 2.5 ng/ml [35]. Both free BPA and BPA-glucuronide are later excreted via the urine [32]. This process of BPA consumption and excretion via the urine has been measured to take between 4-42 hours [32, 36]. Interestingly, fasting does not always result in rapid decrease in urinary BPA levels, suggesting BPA may be accumulating somewhere in the body preventing its rapid excretion [36]. Two tissues suggested to facilitate this BPA accumulation are adipose and placental tissue, which will be discussed in more detail later in this chapter.

**Highest Levels of BPA Exposure Occur from Foetal Development to Early Childhood**

Urinary BPA levels have been used to estimate total BPA exposure levels in different population subsets. In 2008, the European Food Safety Authority (EFSA) reported urinary BPA exposure levels of: 0.2-13 μg/kgBW/day in infants (3-6 months of age), 5.3 μg/kgBW/day in children (1.5 years of age) and 1.5 μg/kgBW/day in adults [37]. Chapin et al determined similar urinary BPA levels of 1-11 μg/kgBW/day in bottle fed infants (3-6 months age), reducing to 0.2-1 μg/kgBW/day in breast fed infants, 1.7-14.7 μg/kgBW/day in children and 0.008-1.5 μg/kgBW/day in adults [38]. These data indicate that the highest levels of BPA exposure are in infants and children and have recently prompted an increase in the number of cross sectional studies which are focusing solely on determining BPA exposure levels in children.

Enabling researchers to extend their study parameters, to not only look at urinary BPA levels and body mass index (BMI), but to also include analysis of environmental influences that promote obesity. To assist in determining if BPA exposure is associated with obesity independent of obesity promoting behaviour, or as a consequence of consuming increased levels of BPA contaminated foods. A cross sectional study using 2003-2008 NHANES data on children aged 6-19 years found a significant association between higher urinary BPA levels and obesity in children, consistent with other studies in adults [39]. Interestingly, this study no association between urinary BPA levels and the amount of time spent watching television or calorie intake, despite the fact these two factors are associated with obesity [39]. The obtained data were able to further support the association of BPA with obesity by finding no other environmental phenols other than BPA to be associated with obesity (with the exception of one phenol used in sunscreen benzophenone-3, in which they found a nonmonotonic relationship, suggesting association was by chance) [39].
Early BPA Exposure and the Onset of Obesity

What is of most concern and the focus of this chapter is early BPA exposure. As in utero foetuses are also exposed to BPA, however it is not as simple as determining the mother’s BPA exposure level in estimating the amount of BPA to which the foetus is exposed throughout development. There is concern that urinary BPA concentrations in pregnant women may underestimate the amount of BPA exposure to the developing foetus. As BPA is able to readily cross and accumulate in the placenta via binding to the estrogen related receptor (ERRγ) [41, 42], which estradiol cannot bind to and for which the natural ligands are unknown [43]. There are four ERRs in the human genome: α, β, γ and δ. BPA has the highest in vitro binding affinity to ERRγ, which is suggested to play a role in facilitating foetal exposure of BPA, as ERRγ is highly expressed in the placenta [41].

The strong binding affinity of BPA to ERRγ is suggested to reduce the excretion of BPA via the urine. Maternal plasma has been shown to contain 0.3-18.9 ng/ml of BPA (median 3.1 ng/ml), foetal plasma 0.2-9.2 ng/ml (median 2.3 ng/ml) and placental tissue 1-104.9 ng/ml (median 12.7 ng/ml) [14]. Balakrishnan et al used an ex vivo human placental fusion model to demonstrate that BPA can cross human placental tissue and that the majority of BPA that crosses the placenta is unconjugated [44]. They used an environmentally relevant dose of 10 ng/ml, similar to the mean BPA level found in placental tissue by Schonfelder et al in 2002 [14]. The BPA was added to the maternal compartment and within three hours 27% of the BPA had transferred to the foetal compartment, less than 10% of which was conjugated [44]. This transfer rate of 27% is consistent with knowledge that the median BPA level in placental tissue is 12.7 ng/ml and the range of BPA found in umbilical cord blood is 0.5 ng/ml [45] to 2.59 ng/ml [46].

The placenta has also been found to contain high levels of the enzyme β-glucuronidase, which can free BPA which was previously conjugated to form BPA-glucuronide [47, 48], increasing the amount of free BPA that can cross to the foetus (Figure 2). The foetus is more vulnerable to the effects of BPA than the mother, as it has an impaired ability to inactivate BPA. The BPA conjugating enzyme UGT is present at low levels, in inactive forms or not present at all during foetal development, with one study finding no UGT transcripts in the foetus up to 20 weeks gestation [49] (Figure 2). BPA exposure is also suggested to reduce UGT function, with male Wister rats exposed to 1 mg of BPA for 2 days showing a reduced mRNA expression of UGT isoforms [50]. The impaired ability of the foetus to inactivate BPA continues through early infant development. The main isoform responsible for conjugation of BPA, UGT2B7 increases in activity with age [51]. Edginton and colleagues found UGT2B7 to have 5% of maximal activity in newborns, 30% activity at 3 months of age and an equal activity to adults by 1 year of age [51]. The difference in the ability of infants, children and adults to conjugate BPA before it reaches their plasma was estimated later by the same research team of Edginton and Ritter using a physiologically based toxicology model scaled to children < 2 years of age. It was estimated an oral dose of 1 μg/kgBW/day of BPA would result in newborns to have an 11 fold greater level of free BPA in their plasma and children (< 2 years of age) a 5 fold greater level [33] compared with adults. This study highlights that the differences between the absorption, distribution, metabolism and secretion of BPA in children compared with adults can result in increased plasma levels of BPA in children.

After birth infants continue to be exposed to BPA from potentially every aspect of their diet, with BPA found in the mother’s breast milk [1] (up to 1-11μg/kgBW/day [52]), formula containers and plastic baby bottles [1] (Figure 2).
Figure 2. Route of exposure of BPA to the developing foetus and infant. Adult hepatic conjugation of BPA is not sufficient to protect a developing foetus from exposure to free BPA. Due to the placental tissue facilitating both the accumulation of BPA via ERRγ and the de-conjugation of BPA via an enzyme expressed in placenta tissue, β-glucuronidase. The foetus is particularly vulnerable to the effects of BPA due to an impaired ability to conjugate and thus inactivate BPA. After birth the infant can then be exposed to BPA via every aspect of its diet, with BPA found in breast milk, formula containers and baby bottles. These multiple routes of exposure result in the risk for BPA exposure throughout all stages of foetal development and infant life.

This data showing increased risk of infant compared with adult BPA exposure has recently made several countries act in restricting the use of BPA for younger individuals. In 2012, the US Food and Drug Administration (FDA) banned the use of BPA in the manufacturing of baby bottles and sippy cups. This same year the French government banned the use of BPA from any products related to infants less than 3 years of age with an extension of this ban to cover all food containers from 2015. In the meantime, the plastics industry needs to come up with an alternative to BPA. Currently two other bisphenols are being studied to replace BPA; bisphenol S and bisphenol AF, which are supposedly less toxic than BPA. One of the main issues with these two bisphenols is the lack of data regarding their impact on human health. If these two bisphenols are indeed chosen by the plastic industry to replace BPA, we can expect more studies in the near future to address this lack of data.

**BPA Exposure Is Significantly Linked to Obesity**

Epidemiological studies show that significantly higher urinary BPA levels have been found in obese adults compared to adults of a healthy weight [53]. This was also observed in obese children compared with children in the healthy weight range [39]. Obesity is characterised by increased size of adipocytes (hypertrophy) and increased number of adipocytes (hyperplasia) [54]. It has been argued that these higher levels of BPA in obese individuals may be due to the consumption of more high energy foods which are packaged in
BPA products, and that BPA is not a direct cause of increased adipocyte hypertrophy and hyperplasia. However, it is also possible that early life exposure to increased levels of BPA could contribute to the development of obesity. To assist in elucidating if BPA can in fact contribute to obesity, the known effects of BPA on adipose tissue are discussed below.

**BPA Negatively Effects Adipose Function**

BPA is a lipophilic compound, with the ability to accumulate in fat [54]. Higher levels of BPA have been found in adipocytes (fat cells) of children compared with adults [45]. The expression of ERα, ERβ, ERRγ and GPR30 in adipose tissue may facilitate the accumulation of BPA in adipose tissue [54]. Adipose tissue functions to store energy and to act as an endocrine organ. The main type of adipose tissue in humans is white adipose tissue (WAT). WAT is composed of adipose derived stem cells such as MSC [55], adipocytes, fibroblasts (preadipocytes fated to adipose cell lineage [56]) and immune cells [57]. Adipocytes originate from pluripotent mesenchymal stem cells and produce hormones and cytokines involved in lipid and glucose metabolism such as; adiponectin, resistin, leptin, TNF-α and IL-6 [58]. Disruption of the normal production of these hormones and cytokines is seen in obesity (described below) [59].

A chronic state of low grade inflammation is seen in obese adipose tissue, with an increased presence of IL-6 and TNF-α producing macrophages [60]. In obese adipose tissue there are higher levels of free fatty acids (FFAs) released from adipocytes, due to the increased number and increased size of adipocytes. These FFAs bind the toll-like receptor-4 on the surface of macrophages [61], activating the NF-κB pathway [61] and inducing the release of a pro inflammatory cytokine TNF-α [62]. TNF-α has been shown to directly cause insulin resistance in adipocytes by inhibiting the insulin signalling pathway [63]. The release of TNF-α also promotes increased infiltration of macrophages into adipose tissue by activating adipocytes. Activated adipocytes produce IL-6 and monocyte chemoattractant protein-1. IL-6 further increases lipolysis [64] and decreases the production of lipoprotein lipase [64, 65] (LPL, an enzyme which releases FFAs from lipoproteins for storage in adipocytes) and adiponectin [64, 66] (a hormone that increases insulin sensitivity [67]). MCP-1 attracts monocytes from the blood into adipose tissue, which differentiate into macrophages. BPA has been shown to have similar effects on adipose tissue as obesity. Increasing the production of IL-6 and TNF-α in human adipose tissue in vitro [68] and increasing the production of a pro inflammatory hormone leptin in vivo in mice [69]. Leptin production is significantly increased in obese adipocytes [70] and increased leptin production in adipocytes is associated with increased IL-6 and TNF-α [71]. The consequences of increased presence of IL-6 and TNF-α in adipose tissue may be the development of inflammation [72]. Inflammation of adipose tissue is associated with the increased risk of developing insulin resistance and type 2 diabetes [73, 74].

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BPA has been shown to reduce adiponectin release from human adipocytes in vitro [54]. Unlike leptin, adiponectin provides protection against inflammation [75], inhibiting the activation of NF-κB in macrophages and subsequent IL-6 production. Decreased adiponectin levels are associated with obesity [70]. Using mouse 3T3-L1 cells in vitro, Kidani et al. showed that BPA may reduce adiponectin release by down regulating the PI3K-Akt signalling pathway [76] (insulin activated pathway, which when inhibited results in reduced adiponectin
production [77]). Reduced adiponectin release is suggested to contribute to the development of insulin resistance as a result of a decrease in the protection it provides against inflammation [75, 78].

**BPA Action on PPARs Leads to Obesity**

In addition to negatively affecting adipose function with similar end points as obesity, BPA has been shown to increase adipogenesis. Adipogenesis normally occurs in the fed state, in which excess free fatty acids (FFAs) from the diet are stored in adipocytes as triglycerol. Adipogenesis is promoted by transcription factors such as the peroxisome proliferator-activated receptor (PPAR)γ, which targets genes involved in fatty acid transport and adipocyte differentiation. FFAs are released from lipoproteins (which they are packaged in for transport in the blood) by PPARγ activated lipoprotein lipase (LPL). FFAs are then re-esterified to form triglycerol and transported into adipocytes [79]. In a starved state the triglycerol can be a source of energy, released from the adipocytes by lipolysis. As well as excess FFAs from the diet causing hypertrophy of adipocytes, the presence of preadipocytes [80, 81] and mesenchymal stem cells in adipose tissue suggests that these FFAs may also be stored in newly differentiated adipocytes, which increase the number of adipocytes, contributing to the development of obesity.

Known natural ligands for PPARγ include the unsaturated fatty acids; oleate, linoleate, eicosapentaenoic and arachidonic acids [82]. Activated PPARγ, acts by forming an obligate heterodimer with retinoid X receptor (RXR). This heterodimer binds peroxisome proliferator-activated response elements (PPREs) which are present in the promoter region of genes involved in promoting adipogenesis and fatty acid transport such as; PEPCK, LPL and α2 [83], a fatty acid binding protein involved in fatty acid transport [84]. PPARγ also increases activity of acetyl-CoA synthetase and the re-esterification of fatty acids [79].

Recently, BPA has been shown to be unable to act as a ligand of PPARγ. Riu et al showed that BPA is unable to activate PPARγ in HGLEN-PPAR cells [85] and Chamorro-Garcia and colleagues showed that BPA cannot activate PPARγ in COS7 cells [86]. However, BPA has been shown to increase PPARγ activity in vitro in human adipocytes [87] and in vivo in rats [69]. It is hypothesised that BPA may increase PPARγ activity levels by activating components of the MAPK pathway [88]. Adipose tissue from children aged 3-13 years treated with 10 nM BPA in vitro showed increased mRNA expression of PPARγ and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme which activates cortisone and promotes adipogenesis [87]. Sprague-Dawely rats exposed to BPA via their mothers placenta and breast milk from gestational day 6 to the end of lactation showed significantly increased levels of WAT at postnatal day 21 that expressed greater levels of PPARγ mRNA compared with control offspring [69]. These data suggest that although BPA cannot bind PPARγ, it is able to increase PPARγ production and adipogenesis thus highlighting the potential for BPA to act as an obesogen, increasing the risk of developing obesity.

BPA has also been shown to increase adipogenesis by increasing insulin induced triglyceride accumulation [89]. Combined treatment of 20 ng/ml BPA (875x10^-4 µM) and insulin (5 ng/ml) accelerated the conversion of mice 3T3-L1 cells into adipocytes in vitro [89], increasing the percentage differentiated cells from 28% in insulin treated to 83% in insulin plus BPA treated [89]. These differentiated cells showed a 13,000% increase in
triglyceride (TG) content, a 190% increase in LPL activity and a 3.3-fold increase in the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [89]. This effect of BPA on increasing LPL activity and adipocyte differentiation in 3T3-L1 cells was not supported in a study using Human Adult Stem Cells (hASC) [90], in which treatment with 80 μM BPA had the opposite effect, reducing LPL expression by 70% [90]. This reduction in LPL was not due to decreased cell viability from the treatment and did not alter the percentage of hASCs which committed to the adipose cell lineage [90]. The clinical implications of reduced LPL production in humans could be an increase in circulating FFAs in the blood stream which may accumulate in other organs such as liver and skeletal muscle instead of being deposited into adipocytes leading to a higher risk of insulin resistance and type 2 diabetes [90] (Figure 3).

![Figure 3](image)

Bisphenol A exposure may increase body weight via three main mechanisms; decreasing normal adipose function, resulting in increased TG storage. Promoting adipogenesis, resulting in increased adipocyte numbers and therefore increased ability for TG storage. And via altering metabolism, decreasing insulin sensitivity and glucose tolerance, resulting in hyperinsulinemia and an inability to sufficiently lower blood glucose levels.

Halogenated forms of BPA are able to bind and activate PPARγ. Halogenated BPAs have been detected in human tissues; human plasma [91], urine [92], umbilical cord blood [93] and breast milk [93, 94]. Riu et al demonstrated the flame retardants Tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA) can act as a ligand for PPARγ in vitro, and that 10 μM of these halogenated BPA analogues increased adipocyte differentiation and PPARγ mRNA expression levels in 3T3-L1 preadipocytes [85]. Co treatment with a PPARγ antagonist CD5477, prevented the halogenated BPAs from increasing adipocyte differentiation in 3T3-L1 preadipocytes, suggesting TBBPA and TCBPA are acting via PPARγ [85]. While the ability for TBBPA and TCBPA to activate PPARγ was found to be 100 fold less than rosiglitazone, TBBPA and TCBPA are 10-100 times more potent at activating PPARγ than other well-known PPARγ ligands; PFOA, PFOS and MEHP [85].

Another BPA halogen, Bisphenol A diglycidyl ether (BADGE) used in the production of epoxy resins, has also been shown to increase adipogenesis in 3T3-L1 preadipocytes, as well
as in mice and human mesenchymal stromal stem cells (MSCs) [86]. Unlike TBBPA and TBBPA, BADGE cannot activate PPARγ, having no agonistic or antagonistic activity to PPARγ [86]. Hypothesising instead that BADGE is acting downstream or parallel to PPARγ [86] to increase adipogenesis. In this study, BPA like BADGE, also induced adipocyte differentiation in the 3T3-L1 preadipocytes, consistent with previous studies [89,95,96], however unlike BADGE, BPA was unable to induce adipogenesis in the human and mice MSCs [86]. This suggests that BPA may only induce adipocyte differentiation in cells already fated to the adipocyte cell lineage. This finding is consistent with a previous study which found BPA to have no effect on adipocyte differentiation in hASC [90], which are also yet to be fated to the adipocyte cell lineage.

The ability of BPA and halogenated BPAs to increase the activity of PPARγ indirectly and directly respectively highlights the ability of these environmental contaminants to increase the rate of adipogenesis. In addition, the ability of BADGE to act on MSCs which have yet to commit to a cell lineage, suggests that exposure to halogenated forms of BPA during early development may increase the number of preadipocytes which can store triglycerol later in life, contributing to the development of obesity.

BPA and Glucocorticoid Receptor in Obesity

Recently a new binding target has emerged in the world of BPA, the glucocorticoid receptor (GR). Using in silico analysis, Prasanth and colleagues showed that BPA could successfully dock into the ligand binding pocket of human GR [97]. By comparing to the 3D structure of cortisol and dexamethasone bound to the GR the authors found that BPA had a similar mode of interaction and binding energy as these two known GR agonists. Therefore Prasanth et al concluded that BPA is able to bind GR as an agonist and may be able to produce similar biological effects as natural or synthetic glucocorticoids [97]. Although this study was in silico, the data support the idea that BPA can bind to and activate the human GR and therefore BPA exposure might reproduce the glucocorticoids induced phenotype.

Activation of the GR plays an important role in adipocyte differentiation [98]. Glucocorticoids (GCs) are also well known for their role in appetite regulation. GCs can stimulate food intake via different mechanisms: they stimulate the action of two orexigenic (appetite stimulant) peptides: NPY and agouti related peptide (AGRP) [5]. Moreover GCs also reduce the sensitivity of the brain to leptin, an anorexigenic (appetite suppressor) peptide, leading to general leptin resistance [99]. As it has recently been shown that appetite regulating gene expression can be modulated during embryonic development in absence of active feeding [100], it is easy to speculate the activation of the GC pathway by circulating BPA either in the mother’s blood or in the foetus might impact the expression of these appetite regulating genes and affect the feeding behaviour of the new born baby. Several observations tend to confirm this hypothesis. For example prenatal stress is associated with long term susceptibility to obesity [101]. This is most likely achieved through altered corticosterone levels in the adult offspring [102] and reduced hippocampal levels of mineralocorticoid receptor and GR [103]. Therefore if, as suspected, BPA is an agonist of GR [97], foetal exposure to BPA will have long term consequences in the adult life of the exposed foetus. It should to be noted however that these observations came from rodents and have not yet been proven in humans.
What is known about human obesity and GR comes predominantly from diseases associated with altered GC levels. Cushing’s syndrome patients have high levels of cortisol [104]. One of the main symptoms associated with this disease is the rapid weight gain, particularly in the upper body and the face. On the other hand, patients suffering from Addison’s disease, which is characterized by hypocortisolism (low level of cortisol), experience weight loss [105]. Therefore the link between obesity and the GR pathway is clear in humans. It has now to be investigated if BPA exposure acting through the GR could lead to weight gain in humans. As more and more data points to the fact that BPA target a wider range of nuclear receptors, future studies will help to confirm the putative link between BPA and the GR pathway in weight gain and obesity.

In Utero Exposure to BPA in Rodents Has Variable Effects on Offspring Body Weight

It is hypothesised that the effects of BPA are not limited to immediate changes in adipose function and that short term exposure to BPA in utero may increase susceptibility to obesity later in adulthood [106]. Studies to date using rodents have shown variable effects on the body weight of offspring from pregnant rodents exposed to BPA throughout gestation and/or lactation. Some offspring exhibited increased [69, 107-110], decreased [108, 109, 111] or no changes [110, 112-114] in body weight compared with control offspring (Table 1). Effects of in utero exposure on the expression of adipogenesis markers and adipose function have also been variable. The variability in these studies may be due to the choice of animal model used, the litter size and the dose, route and duration of BPA exposure [115].

Somm et al. found that Sprague-Dawley rats exposed to BPA during gestation and lactation developed metabolic abnormalities that were detectable from birth to 6 months of age [69]. In their study pregnant Sprague-Dawley rats were exposed to 1 ng/ml of BPA via their drinking water from gestational day 6 (GD6) to postnatal day 21 (PND21) [69]. While the average level of maternal BPA exposure was estimated to be 70 μg/kgBW/day, this level is still considered a low dose and the route of exposure used in this study mimics the human route of exposure to BPA. Studies using subcutaneous injection result in BPA entering circulation before being partially inactivated by UGT in the liver, resulting in an increased plasma level of unconjugated BPA than would have been present using the same dose via an oral route [116]. Another advantage of the study design was the exposure of BPA throughout gestational day 6 until the end of lactation, as it is known that BPA exposure is not limited to in utero development, with BPA exposure continuing after birth via both breast milk and formula containers.

Somm et al. found both male and female pups exposed to BPA throughout gestation showed a significantly higher birth weight than control pups (p< 0.05, n=47-50) [69]. After weaning only females showed a significantly higher body weight than controls (p< 0.001, n=26-32) [69].

These females at postnatal day 21 had increased WAT mass with hypertrophy of adipocytes, and had up to two fold increase in mRNA expression levels of transcription factors that promote adipogenesis; C/EBP-α, PPAR-γ, SREBP-1C (p< 0.002) and significantly increased mRNA expression levels of LPL (p< 0.05) (Figure 3).
## Table 1. BPA exposure and change in weight

<table>
<thead>
<tr>
<th>Reference</th>
<th>Strain of animal</th>
<th>Dose and route of exposure</th>
<th>Duration of BPA exposure</th>
<th>Diet after weaning</th>
<th>BPA exposed offspring weight compared to control</th>
<th>Change in mothers weight</th>
</tr>
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<tbody>
<tr>
<td>[69]</td>
<td>Sprague-Dawley rats</td>
<td>Orally 1 mg/ml BPA in drinking water, 70 μg/kgBW/day</td>
<td>GD6 – end of lactation</td>
<td>n/a</td>
<td>BPA exposed males increased birth weight 7.33 ± 0.12 g, n = 45 compared to control 6.91 ± 0.15 g, n = 55, p=&lt; 0.05. BPA exposed females increased birth weight 7.03 ± 0.11 g, n = 50 compared to control 6.47 ± 0.12 g, n = 47, p =&lt; 0.001 and increased weight at PND21, 53.73 ± 0.65 g, n = 32 compared to controls 47.79 ± 1.44 g, n = 26, p=&lt; 0.001.</td>
<td>No change during or 6 months after pregnancy compared to control</td>
</tr>
<tr>
<td>[107]</td>
<td>CRI mice</td>
<td>Orally 1 μg/ml BPA in drinking water, 0.26 mg/kgBW/day</td>
<td>GD10 – PND31</td>
<td>Calories from protein; 15%, fat; 30%, carbohydrate 55% + continued BPA in drinking water</td>
<td>BPA exposed males (n=25) 22% increased weight compared to control (n=23) at PND31 (p=&lt;0.01). BPA exposed females (n=16) 13% increased weight compared to control (n=19) at PND31 (p=&lt;0.05).</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orally 10 μg/ml BPA in drinking water, 2.72 mg/kgBW/day</td>
<td>GD10 – PND31</td>
<td>Calories from protein; 15%, fat; 30%, carbohydrate 55% + continued BPA in drinking water</td>
<td>BPA exposed males (n=19) 59% increased body weight compared to control (n=23) at PND31 (p=&lt;0.05). BPA exposed females (19) 11% increased body weight compared to control (n=19) at PND31 (p=&lt;0.05)</td>
<td>Not specified</td>
</tr>
<tr>
<td>[108]</td>
<td>ICR mice</td>
<td>Orally via injection, 50 mg/kgBW/day</td>
<td>GD6-PND21</td>
<td>Soy free diet</td>
<td>BPA exposed males increased weight compared to control on PND21 (n=10 per group, p=0.05)</td>
<td>Not specified</td>
</tr>
<tr>
<td>Reference</td>
<td>Strain of animal</td>
<td>Dose and route of exposure</td>
<td>Duration of BPA exposure</td>
<td>Diet after weaning</td>
<td>BPA exposed offspring weight compared to control</td>
<td>Change in mothers weight</td>
</tr>
<tr>
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</tr>
<tr>
<td>[109]</td>
<td>OF-1 mice</td>
<td>Subcutaneous injection , 10 μg/kgBW/day</td>
<td>GD6-GD16</td>
<td>Calories from protein; 20%, fat; 13%, carbohydrates; 67%</td>
<td>BPA exposed male and female offspring had increased weight at birth and weaning compared to controls 3% and 7% respectively (n=25-60 per group, p&lt;0.05).</td>
<td>Increased weight measured at 3 and 4 month after pregnancy</td>
</tr>
<tr>
<td>[110]</td>
<td>Wister rats</td>
<td>Oral gavage, 50 μg/kgBW/day</td>
<td>GD0-PND21</td>
<td>Normal diet, calories from protein; 25%, fat 12%, carbohydrates; 63%</td>
<td>BPA exposed males increased weight compared to control from 19 weeks (n=10 per group, p&lt;0.05). BPA exposed females increased weight compared to control from 17 weeks (n=10 per group, p&lt;0.05).</td>
<td>Not specified</td>
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<td>High fat diet, calories from protein; 22%, fat 29%, carbohydrates; 49%</td>
<td>BPA exposed males increased weight compared to control from 7 weeks (n=10 per group, p&lt;0.05). BPA exposed females increased weight compared to control from 9 weeks (n=10 per group, p&lt;0.05).</td>
<td>Not specified</td>
</tr>
</tbody>
</table>

**Decrease in offspring weight**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Strain of animal</th>
<th>Dose and route of exposure</th>
<th>Duration of BPA exposure</th>
<th>Diet after weaning</th>
<th>BPA exposed female offspring decreased weight at PND22, 15.6 ± 0.8 g, compared to control 17.3 ± 0.5 g, n=10 per group, p&lt;0.05. BPA exposed males decreased weight at PND1, 1.81 ± 0.03 g, compared to control 1.91 ± 0.01 g, p&lt;0.05 and at PND60, 40.9 ± 0.9 g, compared to control 43.3 ± 0.6 g, n=10 per group, p&lt;0.05. Females decreased weight at PND22, 16.1 ± 0.8 g, compared to control 17.3 ± 0.5 g, p&lt;0.05 and at PND60, 32.3 ± 1.1 g, compared to control 33.7 ± 0.9 g, n=10 per group, p&lt;0.05.</th>
<th>Not specified</th>
</tr>
</thead>
<tbody>
<tr>
<td>[109]</td>
<td>OF-1 mice</td>
<td>Subcutaneous injection , 10 μg/kgBW/day</td>
<td>GD6-GD16</td>
<td>Calories from protein; 20%, fat; 13%, carbohydrates; 67%</td>
<td>BPA exposed female offspring 3% decreased weight compared to controls at 3 months of age (n=25-60 per group, p&lt;0.05)</td>
<td>Increased weight measured at 3 and 4 month after pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subcutaneous injection , 100 μg/kgBW/day</td>
<td></td>
<td></td>
<td>BPA exposed male and female offspring had decreased weight at birth and weaning compared to controls of 4.5% (p&lt;0.05). BPA exposed female offspring 2.5% decreased weight compared to controls at 3 months of age (n=25-60 per group, p&lt;0.05)</td>
<td>Increased weight measured at 3 and 4 month after pregnancy</td>
</tr>
</tbody>
</table>
### Table 1. (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Strain of animal</th>
<th>Dose and route of exposure</th>
<th>Duration of BPA exposure</th>
<th>Diet after weaning</th>
<th>BPA exposed offspring weight compared to control</th>
<th>Change in mothers weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>[108]</td>
<td>ICR mice</td>
<td>Orally via injection, 0.05 mg/kgBW/day</td>
<td>GD6-PND21</td>
<td>Soy free diet</td>
<td>BPA exposed males decreased weight on PND21 and PND56 compared to controls (n=10 per group, p=0.05)</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orally via injection, 0.5 mg/kgBW/day</td>
<td></td>
<td></td>
<td>BPA exposed males decreased weight PND56 compared to controls (n=10 per group, p=0.05)</td>
<td></td>
</tr>
<tr>
<td>[112]</td>
<td>Sprague dawley rats</td>
<td>Orally via diet 0.017, 0.17 and 1.7 mg/kgBW/day</td>
<td>GD6-PND21</td>
<td>Normal diet ad libitum</td>
<td>No overall changes (temporary changes in weight seen for only 1 or 2 weeks compared to controls, measurements taken from birth to 13 weeks).</td>
<td>No change during gestation or lactation</td>
</tr>
<tr>
<td>[113]</td>
<td>Wister rats</td>
<td>Orally 10 mg/L in drinking water, 1.2 mg/kgBW/day</td>
<td>GD6- day 21 of lactation</td>
<td>Not specified</td>
<td>No change (measurements taken from birth to 3 months of age).</td>
<td>Not specified</td>
</tr>
<tr>
<td>[110]</td>
<td>Wister rats</td>
<td>Oral gavage, 250 and 1250 μg/kgBW/day</td>
<td>GD0-PND21</td>
<td>Normal diet, calories protein; 25%, fat 12%, carbohydrates; 63%</td>
<td>No change (measurements taken from birth to 28 weeks)</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High fat diet, calories protein; 22%, fat 29%, carbohydrates; 49%</td>
<td>No change (measurements taken from birth to 28 weeks)</td>
<td></td>
</tr>
<tr>
<td>[114]</td>
<td>Long-Evans rats</td>
<td>Oral gavage 2, 20 and 200 μg/kgBW/day</td>
<td>GD7-PND18</td>
<td>Purina rat show 5001</td>
<td>No change (measurements taken from PND2)</td>
<td>No change during gestation or lactation</td>
</tr>
</tbody>
</table>

No change in offspring weight

No change during gestation or lactation
These data are consistent with the *in vitro* increase in LPL activity in BPA treated 3T3-L1 cells [69] and in human adipocytes [87], and the *in vitro* increase in PPARG mRNA expression in BPA treated human adipocytes [87]. From weeks 4-14 the pups were then fed either a normal or high fat diet containing no BPA. There were no differences in energy intake between pups exposed to BPA in *utero* and control pups on either diet [69]. Male pups exposed to BPA in *utero* showed a significant increase in body weight compared with controls on the high fat diet [69]. Female pups exposed to BPA in *utero* showed significant increases in body weight compared to controls on both normal and high fat chow diets [69]. Therefore, *in utero* exposure to BPA may have long term effects on the susceptibility of developing obesity.

The pregnant Sprague-Dawley rats drinking the BPA water exhibited no change in body weight or metabolic profile throughout gestation and lactation [69]. This was an important factor to consider to eliminate defects in the mothers ability to maintain glucose homeostasis which may affect the offspring’s development throughout gestation and lactation as well as later in life. Offspring from obese or glucose intolerant mothers have significant increased risk of developing obesity later in life [117]. This study suggests that although adult human exposure levels to BPA are low, exposure to BPA exclusively in *utero* and during early infant development via a healthy mother’s placenta and breast milk may be sufficient to increase the susceptibility to developing obesity later in life.

**In Utero Exposure to BPA in Humans Is Significantly Linked to Decreased Birth Weight**

In humans the birth weight of infants from parents who work in BPA manufacturing and in a non BPA industry were compared [118]. Those parents working in BPA manufacturing had a higher level of BPA exposure compared with parents working in a non BPA industry [118]. Data from a previous study showed a mean 48-fold increase in urinary BPA levels in BPA manufacturing workers compared to workers from non BPA industries [31]. Infants from mothers with the higher levels of BPA exposure (n=46) had significantly lower birth weight compared with infants from mothers with lower BPA exposure (p=0.02) [118]. These findings were consistent with another study which used ultrasonography to measure foetal development, finding BPA exposure to be correlated with decreased foetal head, femur and abdominal circumference [119]. However another study found no correlation between infant birth weight from mothers exposed to higher BPA levels ranging from 0.5 to 22.3 ng/ml [120]. Therefore it is still unclear if pregnant mothers exposed to BPA will give birth to infants with a quantifiable decreased birth weight.

The first prospective study in humans looking at *in utero* BPA exposure and the development of obesity later in life has been published [121]. Data from this study shows a significant association between higher levels of *utero* BPA exposure and lower body weight in females at ages 6-9 years [121]. Contrary to the hypothesis that *in utero* BPA exposure is promoting obesity. However, it will be of interest to see the outcome of this study once participants have reached adulthood. As it is difficult to compare the current collected data from this study with available data from animal studies, due to the fact many rodent studies looking at *in utero* BPA exposure only see an onset of increased body weight in adulthood, which may also be true for humans.
Determining any long term effects of in utero BPA exposure is made difficult not only by the strong environmental influence of diet and lifestyle in the development of obesity regardless of any presence of BPA, but also the lack of available long term data showing levels of BPA exposure from utero to adulthood. While animal studies therefore play an important role in determining the extent of the effect, if any BPA may have in the development of obesity. The variability seen in rodent studies makes it difficult not only to determine the role BPA may have in the development of obesity in rodents, but to also then correlate these findings to humans.

**CONCLUSION**

The data discussed in this chapter suggest that BPA acting early in life may have implications on obesity and adipose capacity later in life, for example by increasing the number of adipocyte precursors. Although BPA is one of the most studied endocrine disrupters, a lot remains to be elucidated regarding this compound, especially when dealing with obesity, but with more and more studies in silico, in vitro, in several animal models, and in epidemiological studies, the years ahead should give answers regarding the effects of early-life exposure to BPA and their links with metabolic syndromes.

**REFERENCES**


Hewitt KN, Boon WC, Murata Y, Jones ME, Simpson ER. The aromatase knockout mouse presents with a sexually dimorphic disruption to cholesterol homeostasis. *Endocrinology* 2003;144:3895.


[37] EFSA. The EFSA Journal 2006;428.


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[77] Pereira RI, Draznin B. Inhibition of the phosphatidylinositol 3'-kinase signaling pathway leads to decreased insulin-stimulated adiponectin secretion from 3T3-L1 adipocytes. *Metabolism* 2005;54:1636.


[87] Wang J, Sun B, Hou M, Pan X, Li X. The environmental obesogen bisphenol A promotes adipogenesis by increasing the amount of 11beta-hydroxysteroid dehydrogenase type 1 in the adipose tissue of children. *Int. J. Obes* (Lond) 2012.


[96] Sargis RM, Johnson DN, Choudhury RA, Brady MJ. Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. *Obesity* (Silver Spring) 2010;18:1283.


