Environmental Research 166 (2018) 117–129

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Low-dose developmental bisphenol A exposure alters fatty acid metabolism in Fischer 344 rat offspring

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ARTICLE INFO

Keywords:
BPA, Bisphenol A
Metabolism disrupting chemical
Low-dose exposure
FA, Fatty acid
SCD-1, Stearoyl-CoA desaturase 1
Adipose tissue

ABSTRACT

Background: Bisphenol A (BPA) is an endocrine disruptor and also a suggested obesogen and metabolism-disrupting chemical. Accumulating data indicates that the fatty acid (FA) profile and their ratios in plasma and other metabolic tissues are associated with metabolic disorders. Stearoyl-CoA desaturase 1 (SCD-1) is a key regulator of lipid metabolism and its activity can be estimated by dividing the FA product by its precursor measured in blood or other tissues.

Objective: The primary aim of this study was to investigate the effect of low-dose developmental BPA exposure on tissue-specific FA composition including estimated SCD-1 activity, studied in 5- and 52-week (wk)-old Fischer 344 (F344) rat offspring.

Methods: Pregnant F344 rats were exposed to BPA *via* their drinking water corresponding to 0: [CTRL], 0.5: [BPA0.5], or 50 μg/kg BW/day: [BPA50], from gestational day 3.5 until postnatal day 22.

Results: BPA0.5 increased SCD-16 (estimated as the 16:1n-7/16:0 ratio) and SCD-18 (estimated as the 18:1n-9/18:0 ratio) indices in inguinal white adipose tissue triglycerides (iWAT-TG) and in plasma cholesterol esters (PL-CE), respectively, in 5-wk-old male offspring. In addition, BPA0.5 altered the FA composition in male offspring, *e.g.* by decreasing levels of the essential polyunsaturated FA linoleic acid (18:2n-6) in iWAT-and liver-TG. No differences were observed regarding the studied FAs in 52-wk-old offspring, although a slightly increased BW was observed in 52-wk-old female offspring.

Conclusions: Low-dose developmental BPA exposure increased SCD-16 in iWAT-TG and SCD-18 in PL-CE of male offspring, which may reflect higher SCD-1 activity in these tissues. Altered desaturation activity and signs of altered FA composition are novel findings that may indicate insulin resistance in the rat offspring. These aforementioned results, together with the observed increased BW, adds to previously published data demonstrating that BPA can act as a metabolism disrupting chemical.

1. Introduction

Bisphenol A (BPA) is a ubiquitous chemical mainly used as a monomer in the production of polycarbonate plastics and epoxy resins, but also as an additive in other plastics. BPA is present in numerous products used in our everyday life, resulting in long-term exposure of low levels of BPA in large populations. This exposure occur primarily through leaching of BPA from food and beverage containers, but also from other sources such as thermal papers and dental sealants (Vandenberg et al., 2007; Welshons et al., 2006). Notably, BPA is a major endocrine disruptor and a suggested obesogenic and metabolism-disrupting chemical (Heindel et al., 2017; Grun and Blumberg, 2009). In humans, BPA has been detected in maternal serum, amniotic fluid and cord blood taken at birth, as well as in placental tissues (Ikezuki et al., 2002; Zhang et al., 2013; Schönfelder et al., 2002). The detection of BPA in embryonic fluids and tissues is of particular concern for human exposure since the developmental period is particularly sensitive to environmental stressors such as endocrine disrupting chemicals (EDCs).

Mounting epidemiological studies have reported a link between urinary levels of BPA and metabolic disorders, including type-2-diabetes (T2D), cardiovascular disease (CVD), dyslipidemia, obesity, and

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https://doi.org/10.1016/j.envres.2018.05.023
Received 9 March 2018; Received in revised form 18 May 2018; Accepted 22 May 2018

metabolic syndrome (MetS) (as reviewed in (Rochester et al., 2013)). Numerous experimental studies have consistently reported various disturbances on multiple metabolic outcomes following low-dose BPA-exposure, *e.g.* augmented glucose-stimulated insulin secretion (Alonso-Magdalena et al., 2006), increased liver fat (Ronn et al., 2013) and elevated serum triglyceride and cholesterol levels in rodents (Moghaddam et al., 2015). In addition, developmental exposure to BPA has been shown to mimic the effects of a high-fat diet by impairing glucose metabolism and altering gene expression in male mice (García-Arevalo et al., 2014). *In vivo,* BPA-exposure has for example been shown to enhance adipogenic differentiation in human adipose stem cells (Gao et al., 2016; Ohlstein et al., 2014).

Accumulating data indicates that the fatty acid (FA) profile and their ratios in plasma and other metabolic tissues are associated with metabolic disorders, such as obesity and insulin resistance in humans and experimental animals (Alsharari et al., 2017a, 2017b; Sjogren et al., 2008; Cedernaes et al., 2013a, 2013b), and further T2D (Riserus et al., 2009) and MetS in humans (Warensjo et al., 2005). Especially when saturated fatty acids (SFA) replace polyunsaturated fatty acids (PUFAs) such as linoleic acid (18:2n-6), there is increased CVD risk as well as increases in liver and visceral fat, blood lipids, and incidence of MetS (Schwab et al., 2014; Rosqvist et al., 2014). The FA composition is, besides the diet, also influenced by desaturating enzymes. The activity of these enzymes can be estimated by dividing the FA product by its precursor measured in blood or other tissues (Peter et al., 2009). Stearoyl-CoA desaturase 1 ((SCD-1) 16:1n-7/16:0; SCD-16 and 18:1n-9/18:0; SCD-18), Δ–5-desaturase (D5D; 20:4n6/20:3n-6) and Δ–6-desaturase (D6D; 18:3n-6/18:2n-6) are, together with elongases, the main enzymes responsible for endogenous synthesis of monounsaturated FAs and PUFAs. Positive correlations have been reported for SCD-1, D6D and metabolic disorders, whereas D5D has been demonstrated to be inversely related (Warensjö et al., 2009; Bjermo and Riserus, 2010).

The possible implications of BPA in the development of obesity-related metabolic disorders have gained much attention in recent years (Heindel et al., 2017, 2015). However, studies investigating effects on FA composition following developmental BPA exposure are scarce and virtually limited to investigation of the total amount of free FAs (FFAs) (García-Arevalo et al., 2014; Strakovsky et al., 2015; Veiga-Lopez et al., 2015; Jiang et al., 2014). One study has included the 18:1n-9/18:0 ratio and observed an elevation in the liver of BPA-exposed mice. However, the 18:1n-9/18:0 ratio was not used as an index of SCD-1 activity in that particular study (Marmugi et al., 2012).

Recently, we reported that a low, environmentally relevant dose (0.5 μg/kg BW/day) of BPA, 8 times lower than the current preliminary European Food Safety Authority's (EFSA's) tolerable daily intake (TDI), elevated plasma triglycerides, increased adipocyte density and disturbed transcriptional levels of adipogenic genes in developmentally exposed 5-wk-old Fischer 344 (F344) rat offspring (Lejonklou et al., 2017). Given the important role of SCD-1 in lipid metabolism and metabolic disease, the primary aim of this present study was to study if BPA alters the SCD-16 and SCD-18 FA ratios, which are estimates of SCD-1 activity, in 5- and 52-wk-old F344 rat offspring. The secondary aim was exploratory, *i.e.* to examine if BPA exposure alters overall FA composition, de novo lipogenesis ((DNL) Lipogenic index; 16:0/18:2n-6), D5D and D6D in these animals.

2. Materials and methods

Part of the data from a subset of these animals have previously been analyzed and published (Lejonklou et al., 2017) https://ehp.niehs.nih.gov/ehp505/. This report adheres to the Science in Risk Assessment and Policy (SciRAP) criteria for toxicity studies (Molander et al., 2014). A complete SciRAP guidelines checklist is included in Supplemental Table 1.

2.1. Chemicals

BPA (purity ≥ 99%, CAS no. 80-05-7) (Sigma Aldrich) was dissolved in ethanol (1% of final solution) and diluted with well-flushed tap water to defined concentrations. Water containing 1% ethanol (vehicle) was given to control dams. BPA concentrations were analyzed and verified at the Division of Occupational and Environmental Medicine in Lund, Sweden, using the modified method described in (Bornehag et al., 2015). The division in Lund is a reference laboratory chosen for the European biomonitoring project [Consortium to Perform Human Biomonitoring on a European Scale (COPHES); www.eu-hbm.info/democophes].

2.2. Animals and housing

A total of 45 time-mated 9-wk-old female F344/DuCrl rats (Charles River) were delivered to our laboratory on gestational day (GD) 3.5. Unfortunately we did not have the opportunity to breed the animals in our facilities. Subsequently we could not control for the pre-pregnancy environment, which is a limitation of the study since there is a risk that this may have led to stress-responses in the animals. The dams were weighed and chip-marked directly upon arrival. The study was performed using seven blocks (separated by 1 wk), and all dose groups were equally distributed among blocks. The dams were randomly distributed into three dosing groups [0 (n = 17), 0.5 (n = 12) or 50 (n = 15) μg BPA/kg BW/d], with dams assigned per group aimed at retrieving 12 offspring per dose and sex. Dams were housed one per cage until postnatal day (PND) 22, and litters were adjusted to six animals per dam at PND4. To mimic the most likely route of human exposure, dams were exposed to BPA *via* their drinking water *ad libitum* from GD3.5 until PND22. Consumed water volume was recorded twice a week. Doses aimed for were 0.5 μg/kg BW/day: BPA0.5 and 50 μg/kg BW/day: BPA50. Actual average doses were obtained after calculating the amount of water the animals consumed and were 0.404 μg/kg BW/day and 40.1 μg/kg BW/day between GD3.5 and PND22. The concentration of BPA in the drinking water given to control animals was below limit of detection (0.2 ng/l) (For details see Supplemental Table 2 in (Lejonklou et al., 2017)).

Rats were kept under standard conditions in enriched polysulphone cages (Euro Standard IV). The polysulphone cages, glass water bottles and wood shelters (instead of polycarbonate) were used to minimize the risk of migration of BPA that potentially could confound the results. Food and water were available *ad libitum,* and intake was registered per cage. Rats were fed a standard breeding chow, RM3 (NOVA-SCB), until weaning, and a maintenance diet, RM1 (NOVA-SCB), after weaning. As it is important to characterize the phytoestrogen content of the diet,