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Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats

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ABSTRACT

Background: Prenatal exposure to bisphenol A (BPA) has been shown to induce obesity in rodents. To evaluate if exposure also later in life could induce obesity or liver damage we investigated these hypothesises in an experimental rat model.
Methods: From five to fifteen weeks of age, female Fischer 344 rats were exposed to BPA via drinking water (0.025, 0.25 or 2.5 mg BPA/L) containing 5% fructose. Two control groups were given either water or 5% fructose solution. Individual weight of the rats was determined once a week. At termination magnetic resonance imaging was used to assess adipose tissue amount and distribution, and liver fat content. After sacrifice the left perirenal fat pad and the liver were dissected and weighed. Apolipoprotein A-I in plasma was analyzed by western blot.
Results: No significant effects on body weight or the weight of the dissected fad pad were seen in rats exposed to BPA, and MRI showed no differences in total or visceral adipose tissue volumes between the groups. However, MRI showed that liver fat content was significantly higher in BPA-exposed rats than in fructose controls ($p = 0.04$). BPA exposure also increased the apolipoprotein A-I levels in plasma ($p < 0.0001$).
Conclusion: We found no evidence that BPA exposure affects fat mass in juvenile fructose-fed rats. However, the finding that BPA in combination with fructose induced fat infiltration in the liver at dosages close to the current tolerable daily intake (TDI) might be of concern given the widespread use of this compound in our environment.

1. Introduction

The prevalence of obesity (BMI > 30) has risen dramatically in the world over the past two decades. In 2009–2010, 35.5% of adult men and 35.8% of adult women in the US were obese (Flegal et al., 2012). Obesity causes negative effects on quality of life while also predisposing individuals to a number of diseases, including type 2 diabetes and cardiovascular diseases.

Many researchers consider obesity mainly as an unfavorable balance between a high energy intake and low energy expenditure due to poor diet and inadequate exercise habits. However, overweight early in life is a risk factor for overweight and obesity later in life, and paradoxically underweight is another risk factor due to a "catch up" phenomenon. Obviously there exists some sort of programming regarding weight development, at least in the earliest stages of life. Recent research has suggested that environmental contaminants could play an important role in modulating the balance between energy intake and expenditure, reviewed in

Abbreviations: apo A-I, apolipoprotin A-I; BMI, body mass index; BPA, bisphenol A; HDL, high density lipoproteins; IL-6, interleukin-6; LCAT, lecithin-cholesterol acyltransferase; LPS, lipopolysaccharide; LSI, liver somatic index; LT, lean tissue; MRI, magnetic resonance imaging; NOAEL, no adverse effect level; PPAR-γ, peroxisome proliferator activated receptor-gamma; SAT, subcutaneous adipose tissue; SRBI, Scavenger Receptor Class B-I; TAT, total adipose tissue; TDI, tolerable daily intake; TNF alpha, tumor necrosis factor-alpha; VAT, visceral adipose tissue; VLDL, very low density lipoproteins.

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(Janesick and Blumberg, 2011). In a study on mice it was found that prenatal exposure to tributyl tin (TBT) caused obesity later in life and the term "obesogens" was coined (Grun and Blumberg, 2006). This observation supports the hypothesis of fetal programming in humans as a source of certain disorders, such as obesity and diabetes, emerging many years later (Barker et al., 2002). In addition to fetal programming, exposure to certain chemicals in adulthood is also important. Adult rats given persistent organic pollutants (POPs) via crude salmon oil become obese (Ruzzin et al., 2010), and pharmaceuticals, such as the antidiabetic drug rosiglitazone (ROSI) acting on the important receptor peroxisome proliferator-activated receptor-gamma (PPAR-γ) increase body fat when administered to adult humans (Choi et al., 2010). Moreover, it was recently shown that thiazide antihypertensive agents induce visceral obesity when given to adult hypertensive patients (Eriksson et al., 2008). Taken together, these data indicates that exposure to chemicals not only in utero or early childhood could be of importance for the development of obesity.

Bisphenol A (BPA) was discovered to be an artificial estrogen as early as the 1930s (Dodds, 1936), but the synthesis of another chemical, diethylstilbestrol (DES), with more potent estrogenic properties precluded the use of BPA as a pharmaceutical agent. Today its main applications are as a hardener in plastic goods and as a monomer for production of polycarbonate plastics. As such, it is a high-volume chemical and circulating levels of this compound were measureable in about 98% of all subjects in a study of Swedish elderly persons (Olsen et al., 2012) confirming the National Health and Nutrition Examination Survey (NHANES) 2007–2008 where the urinary concentrations were measurable in 94% of the subjects (<LOD 6.1%) (LaKind et al., 2012).

BPA is almost completely absorbed in the gastrointestinal tract in humans and is highly conjugated to form the major metabolite bisphenol A glucuronide by first pass metabolism in the liver (Pottenger et al., 2000). The glucuronide, which is not estrogenically active, is then cleared from blood by elimination with urine. In rats the main route of elimination of conjugated BPA is by biliary and fecal elimination which enables enterohepatic recirculation (Völkel et al., 2002). These mechanisms indicate that the metabolism of BPA is faster and the conjugation more efficient in humans, where enterohepatic recirculation is negligible, than in rats. However, strain differences has been reported, and in female Fischer 344 (F 344) rats the excretion via urine was 42%, and twice as high as in CD rats (21%) (Snyder et al., 2000). The efficient conjugation and relatively low BPA-exposure are the main reasons why BPA is considered to be safe to humans despite a notable amount of animal studies demonstrating effects on various outcomes and in various doses. One mechanism to further evaluate is the action of the β-glucoronidase enzyme present within many tissues, notably e.g. the placenta of animals and humans. β-Glucoronidase deconjugates BPA to its active form which may lead to fetal exposure in the uterus (Ginsberg and Rice, 2009). There has been a focus on BPA as an endocrine disruptor because of its estrogenicity, while there also might be other mechanisms that explain the effects of BPA seen in various studies.

Prenatal exposure to BPA in rodents has previously been shown to induce obesity (Miyawaki et al., 2007; Somm et al., 2009; Wei et al., 2011), and the effect of exposure to BPA later in life has recently been studied by e.g. Marmugi et al. (2012). But there is an inconsistency regarding BPA exposure and weight gain since other studies show no significant effects despite exposure over generations in the environmentally relevant doses (Ema et al., 2001; Tyl et al., 2008, 2002).

In order to study effects of BPA in doses in the range of tolerable daily intake (TDI) we have used three exposure levels, the medium dose being close to TDI as established by the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA) at 50 μg/kg and day. The low dose was 10 times lower and the high dose 10 times higher than the medium dose.

The primary aim of this study was to test the hypothesis that exposure to BPA in combination with carbohydrates after the sensitive prenatal and perinatal periods also could affect fat mass or liver fat content. Since exposure to BPA only, later in life (Marmugi et al., 2012) and perinatal exposure to BPA in combination with high fat diet later in life (Wei et al., 2011) have been reported, this study will focus on exposure to BPA in combination with a diet supplemented with carbohydrates. As fructose is a widely used sweetener in processed food and has been suggested to contribute to unfavorable metabolic alterations (Bocarsly et al., 2010; Bremer et al., 2012) juvenile rats were exposed to BPA in combination with a 5% fructose solution, which is about the same fructose concentration as in common soft drinks (9–13% sucrose). Effects on adipose tissue volume and liver fat content in the BPA-exposed groups were evaluated by magnetic resonance imaging (MRI) and compared with a control group also given fructose solution. As a secondary aim, we investigated whether obesity parameters and the liver were affected by fructose feeding alone, using water-fed rats as a control group.

2. Material and methods

2.1. Chemicals

Bisphenol A (BPA), (80-05-7, $(CH_3)_2C(C_6H_4OH)_2$, ≥99% purity), fructose ($C_6H_{12}O_6$, ≥99% purity), Griess modified reagent, $ZnSO_4$, and VCl_3 were purchased from Sigma–Aldrich, St. Louis, MO. $NaNO_3$ was purchased from Merck chemicals, Darmstadt, Germany.

2.2. Animals

The animal study was approved by the Uppsala Animal Ethical Committee and followed the guidelines laid down by the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998:56) and European Union Legislation (Convention ETS123 and Directive 86/609/EEC).

Sixty female F 344 rats at 3 weeks of age were purchased from Charles River International, Salzfeld, Germany, and housed 3 rats/cage at Uppsala University Hospital animal facility in a temperature-controlled and humidity-controlled room with a 12-h light/dark cycle. To minimize background BPA exposure Polysulfone IV cages (Eurostandard IV) and glass water bottles were used. The rats were fed a standard pellet RM1 diet (ad lib.) from NOVA-SCB, Sollentuna, Sweden. RM1 is a natural ingredient diet with a low level of phytoestrogens (100–200 μg/g) (Jensen and Ritskes-Hoitinga, 2007; Odum et al., 2001). During the two-week acclimatization period preceding the ten-week intervention all animals were given water to drink and during the intervention water or 5% fructose solution (see Section 2.3). At 5 weeks of age the rats were assigned to five groups (12 rats/group); water control (W), fructose control (F), low dose BPA (0.025 mg/L), medium dose BPA (0.25 mg/L) or high dose BPA (2.5 mg/L). To avoid unnecessary stress no cage-mates were separated, but the cages were allocated to the different groups to achieve equality in weights in all groups. Food and liquid consumption in each cage and individual weight of the rats were determined once a week.

Before MRI exam, the rats were anesthetized with Ketalar 90 mg/kg bw (Pfizer, New York, NY) and Rompun 10 mg/kg bw (Bayer, Leverkusen, Germany). Immediately after the scanning they were killed by exsanguinations from the abdominal aorta while still under anesthesia.

2.3. Exposure

To prepare BPA exposure solutions (0.025, 0.25 and 2.5 mg/L), three stock solutions of BPA in 1% ethanol (2.5 mg/L, 25 mg/L and 250 mg/L) were diluted 1:100 in 5% fructose solution. The low dose was chosen to be well below the recommended TDI, the medium dose corresponding to TDI (50 μg/kg and day), while the highest dose was ten times this level. The BPA was analyzed by liquid chromatography–tandem mass spectrometry by the Division of Occupational and Environmental Medicine in Lund, Sweden. The division is a European reference laboratory in the DEMOCOPHES EU project (www.eu-hbm.info/democophes) for analysis of BPA. The BPA concentrations in analyzed samples of the solutions were: water control – 0.00020 mg/L; fructose control – 0.00011 mg/L; BPA 0.025 mg/mL – 0.029 mg/mL; BPA 0.25 mg/L – 0.25 mg/L and BPA 2.5 mg/L – 2.7 mg/L.

The exposure solutions were given ad lib. for ten weeks and exposure levels are presented in Table 1. The water control rats and the fructose control rats had free access to water containing 1% ethanol, and 5% fructose solution containing 1% ethanol, respectively. Groups given fructose solution drank more than the water control rats, and also raised their liquid consumption during the experiment, but ate less. The control group given water had an almost constant food and liquid intake.

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