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N.B.: When citing this work, cite the original article.

Original Publication:
http://dx.doi.org/10.1016/j.tox.2012.09.013
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http://www.elsevier.com/
Postprint available at: Linköping University Electronic Press
http://urn.kb.se/resolve?urn=urn:nb:n:se:liu:diva-87627
Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats

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A R T I C L E   I N F O

Article history:
Received 2 May 2012
Received in revised form
18 September 2012
Accepted 20 September 2012
Available online 8 November 2012

Keywords:
MRI
Liver fat
Rat
Bisphenol A
Obesity

A B S T R A C T

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 hypotheses 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 fat 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.

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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
(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 (Ruzzini et al., 2010), and pharmaceuticals, such as the anti-diabetic drug rosiglitazone (ROS) 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 indicate 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 are measurable in about 98% of all subjects in a study of Swedish elderly persons (Ol森 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 (Vökel 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-glucuronidase enzyme present within many tissues, notably e.g. the placenta of animals and humans. β-Glucuronidase 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, C_{6}H_{10}O_{3}), ≥99% purity, fructose (C_{6}H_{12}O_{6}, ≥99% purity), N,N-dimethylformamide (DMF) (99% purity), and all other chemicals 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 SFS/1998:56) 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 pelleted RM1 diet (ad lib.) from NOVA-SCB, Soluntenna, Sweden. RM1 is a natural ingredient diet with a low level of phytosterogens (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. All cages were allocated to the different groups to achieve equality in weights in all groups. Food and liquid consumption in each cage and individual weights 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/mL, 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-hbmn.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/L – 0.029 mg/L; 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.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>BPA exposure, mean w 1–10 (µg/kg/day)</th>
<th>Control:water</th>
<th>Control:5% fructose solution</th>
<th>BPA 0.025 mg/L + 5% fructose solution</th>
<th>BPA 0.25 mg/L + 5% fructose solution</th>
<th>BPA 2.5 mg/L + 5% fructose solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA exposure, mean</td>
<td>0</td>
<td>0</td>
<td>5.1</td>
<td>5.6 (w 2)</td>
<td>61.6 (w 3)</td>
<td>595.3 (w 2)</td>
</tr>
<tr>
<td>Exposure highest (µg/kg/day)</td>
<td>0</td>
<td>0</td>
<td>4.6 (w 9)</td>
<td>46.3 (w 6)</td>
<td>400.3 (w 9)</td>
<td></td>
</tr>
<tr>
<td>BPA exposure lowest</td>
<td>0</td>
<td>0</td>
<td>28.1</td>
<td>30.1</td>
<td>24.7</td>
<td></td>
</tr>
<tr>
<td>(µg/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid, mean w 1–10 (g/rat and day)</td>
<td>11.6</td>
<td>28.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid w 1 (g/rat/day)</td>
<td>11.6</td>
<td>20.8</td>
<td>20.4</td>
<td>21.8</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>Liquid w 10 (g/rat/day)</td>
<td>10.8</td>
<td>32.0</td>
<td>33.0</td>
<td>36.8</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td>Food, mean w 1–10 (g/rat and day)b</td>
<td>10.2</td>
<td>8.5</td>
<td>8.3</td>
<td>8.3</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Food w 1 (g/rat/day)</td>
<td>10.8</td>
<td>10.1</td>
<td>10.0</td>
<td>9.6</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Food w 10 (g/rat/day)</td>
<td>10.0</td>
<td>8.7</td>
<td>7.9</td>
<td>8.3</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Fructose energy mean w 1–10 (kcal/rat/day)</td>
<td>5.6</td>
<td>5.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Energy total mean w 1–10 (kcal/rat/day)</td>
<td>28.9</td>
<td>24.6</td>
<td>24.1</td>
<td>23.5</td>
<td>24.6</td>
<td></td>
</tr>
</tbody>
</table>

Difference in mean caloric intake was less than 5% between the groups with highest and lowest caloric intake.

### 2.4. Magnetic Resonance Imaging and Post Processing

The MR imaging was performed on a 1.5 T clinical MR system (Achieva; Philips Healthcare, Best, Netherlands) using a quadrature knee coil. The rats lay in prone position. MR compatible pads were used to position the animal in the coil center. Two bottles of warm tap water were positioned next to the rats to help maintain their body temperature.

Two different MR protocols were used. A whole-body single echo water–fat imaging protocol was used to analyze adipose tissue distribution. A 32-echo water–fat imaging protocol covering most of the liver was used to analyze liver fat content and the relaxation parameter R2* using model-based fitting to time domain data. This model-based determination of fat content and R2* is similar to quantification of resonance peak heights and widths, respectively, from the corresponding MR spectrum. The image data and the analysis used are illustrated in Fig. 2.

The whole-body imaging was performed using a volume of interest (100 mm × 100 mm × 150 mm, sagittal × coronal × axial) positioned to cover the volume from neck to tail, see Fig. 1a. A spoiled 3D single gradient-echo protocol with imaging parameters repetition time 8 ms, echo time 3.2 ms, and flip angle 12° was used. The acquired voxel size was 0.5 mm × 0.5 mm × 1.0 mm. The reconstructed voxel size was 0.45 mm × 0.45 mm × 1.0 mm. Fold-over direction was anterior–posterior. Total imaging time, using one signal average was 4 min 17 s. Water fat shift was 0.486 pixels. No parallel imaging was used.

Water and fat images were reconstructed from the complex single echo image data using a previously presented model-based method (Berglund et al., 2010). The possibility to separate water and fat signal from a single echo acquisition can be rather intuitively realized. The echo time used in the current protocol gives an approximate phase shift of 270° between water and fat. Hence, after correction for B0 inhomogeneity, the water and fat signal vectors are aligned along the positive real axis and negative imaginary axis, respectively. In brief, the algorithm determined the water and fat content in each voxel using these three assumptions. First, the majority of voxels were assumed to have one of two different water/fat signal ratios. The assumed ratios were 100:0, for muscles and organs, and 0:100, for adipose tissue. Second, the static magnetic field distribution was assumed to be smooth. Third, voxels with an equal amount of fat and water were located on interfaces between water-domain regions and adipose tissue. The first assumption left two possible alternatives for the static magnetic field in each voxel. Using the second assumption, the right alternative could be selected using optimization. In this study a multi-scale belief propagation approach was used (Felzenszwalb and Huttenlocher, 2006). To allow a continuous spectrum of water-fat ratios, the phase map was filtered using an averaging filter. The determination of the static magnetic field distribution allowed direct calculation of the water and fat components. Method feasibility has previously been demonstrated in whole-body scans of a human subject at both 1.5 T and 3.0 T (Berglund et al., 2010).

Volumes of total, visceral, subcutaneous adipose tissue, and lean tissue (TAT, VAT, SAT, and LT, respectively) were quantified using a semi-automated approach. Fat fraction images, defined by fat/fat + water, were calculated and adipose tissue and lean tissue were separated by thresholding at 50% fat fraction.

To reduce the effect of fat fractions originating from background and low signal regions in the analysis, the tissue of the rats was separated from background by clustering. The water and fat images were clustered into three classes (adipose tissue, lean tissue, and background) using a version of Fuzzy C-means that incorportates spatial continuity (Leung et al., 2005). Fat fractions originating from low signal regions were suppressed by multiplying by the background cluster inverse.

The VAT volume was segmented from the fat fraction image using a previously described semi-automated method (Malmberg et al., 2009). The operator manually placed foreground seeds in the VAT depot and background seeds in SAT, muscles, and in the background. The algorithm then determined the boundary between VAT and other tissues. The operator interactively added/removes seeds in a three-plane view until the segmentation was visually determined to be accurate. Two operator segmented the VAT depot in all animals. The mean VAT volume was used (mean CV was 1.40%). The subcutaneous adipose tissue volume was calculated as the difference between the TAT and VAT volumes.

The 32-echo water–fat liver imaging was performed using a 3D spoiled gradient echo acquisition with the following scan parameters: Field of view, 95 mm × 95 mm × 15.6 mm (sagittal × coronal × axial), acquired and reconstructed voxel size, 1.19 mm isotropic, repetition time, 55 ms, first echo time, 1,628 ms, inter echo spacing, 1.274 ms, flip angle, 35°. Imaging time 1 min 46 s. The water–fat tissue reconstruction was performed using a previously described method that employs a whole-image optimization approach (Berglund and Kullberg, 2012). A multi-peak triglyceride spectrum model derived from liver MR spectroscopy (Hamilton et al., 2010) and a common R2* for all peaks were used in the modeling. The R2* parameter can be thought of as the peak width in frequency domain and can be used to detect liver iron deposition (positive correlation). The study protocol, the R2* parameter was used as an additional biomarker of liver status. The liver fat content and R2* from the entire liver was analyzed by manual identification of the volume of interest and by fitting of a Gaussian function to the liver fat fraction and R2* histograms (see Fig. 1f and g). The center of the Gaussian function was used to sample robust estimates of liver fat content and R2*.

### 2.5. Tissue Sampling

At termination blood was collected from the abdominal aorta in EDTA-treated tubes (Greiner bio-one, Frickenhausen, Germany) and centrifuged for 10 min to prepare plasma. Aliquots were stored at –70 °C pending biochemical analyses of the following circulating markers: triglycerides, cholesterol, and apolipoprotein A-I (apo A-I). The liver and the left perirenal fat pad (see Fig. 2) were dissected and weighed. The liver weight was used to calculate the liver somatic index (LSI, liver weight × 100/body weight).

### 2.6. Biochemistry

The analysis of cholesterol and triglycerides was a standard laboratory technique and was performed on an Architect C 8000 analyzer (Abbott Laboratories, Abbott Park, IL, USA) and reported using SI units. Analysis of protein apo A-I: Prior to Western blot 1 µl of plasma from rats of all groups [W; n = 12, F; n = 12, BPA 0.025 mg/L; n = 11, BPA 0.25 mg/L; n = 8 and BPA 2.5 mg/L; n = 9] were separated on SDS-polyacrylamide gradient gels (T = 5–20%L, C = 5% with stacking gels (T = 5%, C = 1.5% for 1h (180V, 60 mA) in an electrode buffer (0.15% (w/v) Tris, 0.72% (w/v) glycine, 0.05% (w/v) SDS) using a Mini Protein Electrophoresis cell (BioRad). Samples were diluted in sample cocktail (4% (w/v) SDS, 200 mM DTT, 20% (w/v) sucrose) and boiled for 3 min. Plasma proteins separated by SDS PAGE were transferred to a PVDF membrane. After blocking 1 h (5% milk in TBS) and incubation overnight with primary antibodies 1:1000 (25 mL milk in TBS) against apo A-I (rabbit anti rat apoA-I, polyclonal, Ab24053, Abcam, UK), the membrane was incubated for 1 h with goat anti-rabbit HRP-conjugated secondary antibodies 1:40000 (25 mL in TBS). Proteins were visualized using an ECL plus western blotting detection system. Gel images were evaluated using Image Lab 3.0.1 (Bio Rad, Hercules, CA) and apo A-I levels were determined as intensity/mm².

### 2.7. Statistical Analysis

Differences between the fructose control group and the three BPA plus fructose exposed groups were evaluated by factorial ANOVA. When the three BPA groups...
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**Fig. 1.** Illustration of the MR image data and post processing. In (a) one coronal slice from a whole body fat image is shown with a red overlay of the segmented VAT depot. In (b–e) one axial slice from the liver scan is shown, where (b) shows the reconstructed water image, (c) the fat image, (d) the signal fat fraction image, and (e) the R$_2^*$ image. The manually segmented region of the liver is illustrated by the yellow delineation. Images (f) and (g) show the distributions, and the fitted Gaussian functions, of the fat signal fraction and R$_2^*$ data, respectively, from the delineated liver volume.

were analyzed vs the fructose control group one by one, a Bonferroni adjustment for 3 tests was used and $p < 0.0167$ considered significant ($p = 0.05/3 = 0.0167$).

In the secondary analysis, when the water control group was compared with the fructose control group $p < 0.05$ was considered as significant.

StatView (SAS Inc, USA) was used for calculations.

**3. Results**

3.1. Primary aim

No differences between the four fructose-fed groups were seen regarding the initial body weight recorded prior to the intervention ($p = 0.83$, Table 2). Neither did the weight at the time of termination of the experiment ($p = 0.84$), nor the weight gain during the intervention ($p = 0.68$), differ between the four groups. No differences were found between the four groups regarding the weight of the fat pad ($p = 0.32$), and MRI showed no differences in total or visceral adipose tissue volumes between the four groups (see Table 2 for details).

However, MRI revealed a greater fat infiltration in the liver of BPA-exposed rats than in the fructose-fed control rats. In the medium-dose and the high-dose group of BPA exposed rats the liver fat content was higher when compared with the fructose control group ($p = 0.011$, medium dose; $p = 0.012$, high dose). The lowest dose of BPA did not significantly influence liver fat content (Fig. 3).

Also the MRI liver R$_2^*$ analysis showed an effect on the liver by BPA, being significant in all three groups when compared one by one to the fructose control group (low-dose; $p = 0.0008$, middle-dose; $p = 0.0001$, high-dose; $p = 0.0161$, Table 2).

A similar picture emerged, although not as pronounced as for the R$_2^*$ signal, when the liver somatic index (LSI) was investigated.

**Fig. 2.** Illustration of the location and shape of the left fat pad (see arrow).
Table 2
Details of initial body weight and results of weight gain, adipose and lean tissue volumes, liver weight and circulating markers in juvenile female Fischer 344 rats in a study with two control groups given either water or a 5% fructose solution and three exposed groups given bisphenol A (BPA) – 0.025, 0.25 or 2.5 mg/L – dissolved in 5% fructose solution for ten weeks. Numbers of observations are 12 if not otherwise stated. The p-values represent the ANOVA p-value for a difference between the four fructose-fed groups (control group with only fructose and the three groups given fructose plus BPA). All values are given as mean ± SD.

<table>
<thead>
<tr>
<th>Control: water (g)</th>
<th>Control: 5% fructose solution (g)</th>
<th>BPA 0.025 mg/L + 5% fructose solution (g)</th>
<th>BPA 0.25 mg/L + 5% fructose solution (g)</th>
<th>BPA 2.5 mg/L + 5% fructose solution (g)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>92.6 ± 10.5</td>
<td>90.1 ± 9.0</td>
<td>88.7 ± 11.1</td>
<td>88.7 ± 10.3</td>
<td>88.3 ± 11.7</td>
<td>0.83</td>
</tr>
<tr>
<td>Weight gain, week 1-10 (g)</td>
<td>86.2 ± 14.4</td>
<td>86.3 ± 7.7</td>
<td>82.6 ± 8.9</td>
<td>86.4 ± 13.9</td>
<td>0.68</td>
</tr>
<tr>
<td>Body weight at sacrifice (g)</td>
<td>171.7 ± 7.2</td>
<td>173.7 ± 9.0</td>
<td>172.9 ± 11.8</td>
<td>175.1 ± 8.2</td>
<td>0.84</td>
</tr>
<tr>
<td>Estimated body weight, MRI (g)</td>
<td>148.4 ± 4.7</td>
<td>141.1 ± 5.9</td>
<td>151.5 ± 9.2</td>
<td>149.6 ± 9.5</td>
<td>0.63</td>
</tr>
<tr>
<td>Total adipose tissue (cm³)</td>
<td>28.2 ± 3.4</td>
<td>29.8 ± 5.7</td>
<td>29.6 ± 4.9</td>
<td>28.9 ± 5.2</td>
<td>0.60</td>
</tr>
<tr>
<td>Visceral adipose tissue (cm³)</td>
<td>13.9 ± 1.5</td>
<td>14.3 ± 3.0</td>
<td>13.9 ± 2.4</td>
<td>13.8 ± 2.6</td>
<td>0.57</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue (cm³)</td>
<td>14.4 ± 2.0</td>
<td>15.4 ± 2.8</td>
<td>15.6 ± 2.6</td>
<td>15.1 ± 2.9</td>
<td>0.65</td>
</tr>
<tr>
<td>Lean tissue (cm³)</td>
<td>95.5 ± 2.9</td>
<td>95.2 ± 4.3</td>
<td>96.6 ± 5.0</td>
<td>95.4 ± 6.0</td>
<td>0.92</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td>5.5 ± 1.0</td>
<td>5.6 ± 0.86</td>
<td>6.4 ± 1.2</td>
<td>7.0 ± 1.7</td>
<td>0.037</td>
</tr>
<tr>
<td>Liver R²* (1/s)</td>
<td>45.7 ± 3.3</td>
<td>46.5 ± 3.6</td>
<td>51.6 ± 3.8**</td>
<td>53.6 ± 4.1***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat pad (g)</td>
<td>0.74 ± 0.15</td>
<td>0.86 ± 0.21</td>
<td>0.77 ± 0.14</td>
<td>0.76 ± 0.14</td>
<td>0.32</td>
</tr>
<tr>
<td>Fat pad/bodyweight ratio (%)</td>
<td>0.43 ± 0.08</td>
<td>0.50 ± 0.11</td>
<td>0.44 ± 0.07</td>
<td>0.44 ± 0.08</td>
<td>0.28</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>4.8 ± 0.29</td>
<td>5.1 ± 0.20</td>
<td>5.6 ± 0.86</td>
<td>5.6 ± 0.68</td>
<td>0.19</td>
</tr>
<tr>
<td>Liver somatic index (LSI)</td>
<td>2.8 ± 0.16</td>
<td>3.0 ± 0.10***</td>
<td>3.2 ± 0.32**</td>
<td>3.2 ± 0.34**</td>
<td>0.08</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.0 ± 0.17</td>
<td>3.1 ± 0.23</td>
<td>3.2 ± 0.16</td>
<td>3.1 ± 0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.88 ± 0.22</td>
<td>1.3 ± 0.36**</td>
<td>1.7 ± 0.62</td>
<td>1.7 ± 0.79</td>
<td>0.48</td>
</tr>
<tr>
<td>Apo A-I (intensity/mm²)</td>
<td>5648 ± 1249</td>
<td>5907 ± 1714</td>
<td>7622 ± 2968</td>
<td>11271 ± 3049***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose week 9 (mmol/L)</td>
<td>4.6 ± 0.79</td>
<td>4.7 ± 0.70</td>
<td>4.5 ± 0.45</td>
<td>4.4 ± 0.36</td>
<td>0.40</td>
</tr>
<tr>
<td>ASAT</td>
<td>0.95 ± 0.052</td>
<td>0.96 ± 0.22</td>
<td>1.24 ± 1.3</td>
<td>1.32 ± 1.29</td>
<td>0.75</td>
</tr>
<tr>
<td>ALAT</td>
<td>0.82 ± 0.097</td>
<td>0.73 ± 0.087</td>
<td>0.82 ± 0.41</td>
<td>0.80 ± 0.19</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* n = 11.  
† n = 8.  
‡ n = 9.  
¥ p < 0.05.  
** p < 0.01.  
*** p < 0.001.  

When given after values in the fructose control group this indicates a difference vs the water control group. When given after values in any of the three BPA groups this indicates a difference vs the fructose control group.

LSI was increased in the low-dose (p = 0.043, not significant following Bonferroni adjustment) and middle-dose group (p = 0.018, not significant following Bonferroni adjustment), but not significantly so in the high-dose group when compared with the fructose-fed control rats (Table 2).

Both the medium-dose and high-dose of BPA groups showed significantly higher levels of plasma apo A-I, when compared with the fructose control group (p < 0.0001, medium dose; p < 0.0001 high dose). The lowest dose of BPA did not cause any significant difference in apo A-I (Fig. 4). Plasma cholesterol and plasma triglycerides were not significantly altered by the BPA exposure. Neither was blood glucose at week 9, or ASAT and ALAT altered by BPA exposure.

3.2. Secondary aim

Of all variables studied (see Table 2), only plasma triglycerides and LSI were significantly increased by fructose feeding alone when compared to the water-fed control p = 0.0011 and p = 0.0031, respectively.

![Fig. 3](image_url)  
**Fig. 3.** Liver fat content (%) (mean ± SEM, water; n = 12, fructose; n = 11, BPA 0.025 mg/L; n = 12, BPA 0.25 mg/L; n = 12 and BPA 2.5 mg/L; n = 12) in juvenile female Fischer 344 rats given water, 5% fructose solution or bisphenol A (0.025, 0.25 or 2.5 mg/L) dissolved in 5% fructose solution for ten weeks.

![Fig. 4](image_url)  
**Fig. 4.** Apolipoprotein A-I (INT/mm²) (mean ± SEM, water; n = 12, fructose; n = 12, BPA 0.025 mg/L; n = 11, BPA 0.25 mg/L; n = 8 and BPA 2.5 mg/L; n = 9) in juvenile female Fischer 344 rats given water, 5% fructose solution or bisphenol A (0.025, 0.25 or 2.5 mg/L) dissolved in 5% fructose solution for ten weeks.
4. Discussion

4.1. BPA, fructose and lipid metabolism

The present study disclosed no evidence that BPA exposure in juvenile female fructose-fed F 344 rats would increase fat mass, despite the use of both weights and MR imaging based detailed quantification of different adipose tissue compartments. However, the observed increase in liver fat infiltration, detected by MRI in parallel with increase in LSI, although in the latter case not significant following strict Bonferroni correction for multiple testing, even at dosages close to TDI, is a finding that warrants further investigations.

Interestingly, an increase in liver fat infiltration appeared at the middle dose, but was not further increased at the highest BPA dose. This finding confirms a previous in vivo study on mice by Marmugi et al., using the same dose range of BPA as in the present study, but without fructose. The Marmugi study showed an impact on the hepatic transcriptome, particularly on genes involved in lipid synthesis and that various transcription factors followed a non monotonic dose–response curve (Marmugi et al., 2012). In addition, also in line with the Marmugi study, the most significant effects were observed within one magnitude around the current TDI. However, Marmugi et al. used mice and did not combine BPA with fructose, so our study is not entirely comparable with theirs.

Low-dose effects of BPA are currently highlighted and under discussion worldwide (Rhomberg and Goodman, 2012; Richter et al., 2007; Ryan et al., 2010; Vandenberg et al., 2012) and therefore three dosages were used, of which the medium dose corresponded to the defined human TDI, as established by the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA) at 50 μg/kg and day. TDI is equal to NOAEL (5000 μg/kg and day, this is the highest dose which did not induce any adverse effect in animal testing), divided by a factor of 100 to compensate for possible species differences in sensitivity. The current TDI is assumed to be considerably higher than the calculated human exposure. However, in the present study and others, effects are seen in rats and mice at doses close to the current TDI and even at lower doses (Richter et al., 2007). Low dose effects of environmental contaminants have previously been suggested based on epidemiological studies, as well as in experimental settings using BPA (Lee et al., 2011; Marmugi et al., 2012; Rubin et al., 2001; Soriano et al., 2012). Also non monotonic relationships are suggested in e.g. a study by Wei et al. where pregnant Wistar rats were exposed to BPA (250, 250 or 1250 μg/kg and day) and their offspring given normal or high fat diet after weaning. Only the lowest dose (50 μg/kg and day) resulted in such effects as increased body weight, elevated serum insulin and impaired glucose tolerance in adult offspring. In the rats fed a high fat diet the effects were exacerbated and included metabolic syndrome (obesity, dyslipidemia, hyperlpeptinemia, hyperglycemia, hyperinsulinemia and glucose intolerance). Rats perinatally exposed to the higher doses did not show any of the adverse effects regardless of diet (Wei et al., 2011). A similar study has been performed with CD-1 mice by Ryan et al. but with a different conclusion. In this experiment the mice exposed to BPA (approximately 0.25 μg/kg bw and day via the diet) during gestation and lactation had heavier and longer pups at weaning than pups from the control groups, but the differences did not persist until adulthood, regardless of a high fat or low fat diet given from 9 weeks of age. As in our study MRI was used to determine body composition and no increase in body fat was seen in the BPA exposed rats (Ryan et al., 2010). However, these studies are not fully comparable due to differences regarding doses and species and the time point when the modified diet was introduced. Further Ryan et al. housed their mice in cages made of polycarbonate and used water bottles also made of polycarbonate, which might have been sources of BPA contamination in the control groups masking subtle effects, though it was otherwise a very sound study.

The above mentioned studies were carried out with rodents which are said to be poor models for BPA in humans due to different toxicokinetics. According to a study by Tominaga et al. using nonhuman primates: chimpanzees (Pan troglodytes versus) and cynomolgus monkeys (Macaca fascicularis), there are differences also among different primate species. In rodents the BPA T/5 is longer, primarily explained by enterohepatic recirculation in rodents but not in primates. The conjugation rate in the liver is faster in rodents than in primates, primarily explained by a higher hepatic blood flow-rate in rodents (Tominaga et al., 2006). However, there seem to be no differences in the metabolites formed e.g. it is a question of rate and time and not in the fate of BPA. The calculated mean exposure in humans is well below the TDI, but there are still uncertainties about the exact sources of exposure. Further, based on the WHO report: “Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A Summary Report” (http://www.who.int/foodsafety/chem/chemicals/BPA_Summary2010.pdf), the most sensitive individuals — newborn babies — are also the ones with highest exposure. According to this report the highest estimated exposure occurs in infants 0–6 months of age who are fed with liquid formula out of PC bottles: 2.4 μg/kg bw per day (mean) and 4.5 μg/kg bw per day (95th percentile), which is very close to the lowest dose used in the present study. In children, teenagers and adults the mean exposure was <0.01–0.40 μg/kg bw per day.

Prenatal exposure to BPA has been shown to increase expression of lipogenic genes and adipocyte size in rodents (Marmugi et al., 2012; Somm et al., 2009). Studies on isolated cells have shown BPA to induce production of proinflammatory cytokines, such as IL-6 and TNF-alpha (Yamashita et al., 2005), and to induce expression of adipogenic transcription factors (Phrakonkham et al., 2008), including PPAR-gamma activation (Kwintkiewicz et al., 2010). How these in vitro findings relate to the present finding of an increase in liver fat infiltration in combined exposure to fructose and BPA is not understood.

The above-mentioned study by Marmugi et al. further suggests that exposure to low BPA doses may influence de novo fatty acid synthesis and thereby contributing to hepatic steatosis in mice (Marmugi et al., 2012). Interestingly, fructose has also been pointed out as a possible contributor to similar effects on the liver by its interaction with the Glut5 receptor (Lustig, 2010). In line with Lustig, it has been suggested by others, summarized in a review by Yilmaz (2012) that high fructose diet (60–70% of total energy intake) may contribute to non-alcohol fatty liver disease, metabolic syndrome, formation of advanced glycated end products as well as direct dysmetabolic effects on liver enzymes.

In the present study, it can be concluded that 5% fructose alone or in combination with BPA results in unfavorable metabolic alterations. There are three possible sources of increases in liver fat; de novo lipid synthesis, decreased degradation or increased transport of cholesteryl esters to the liver. According to our data the most likely mechanisms behind the lipid accumulation in the liver are a combination of de novo lipid synthesis and increased reversed transport (also Section 4.2). The individual contribution from fructose and BPA can only be postulated, but according to the liver fat accumulation in the fructose group and further increase accompanied by the increase of plasma apo A-I (Fig. 4) after BPA exposure, we suggest that fructose is the main contributor to the de novo lipid synthesis while BPA is the main contributor to the increased reverse transport. The decrease in plasma apo A-I and thereby LSI at the highest BPA dose may be a negative feedback response on apo A-I synthesis but has to be further investigated.
In addition, in the three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats, by Tyl et al. (2002) rats of both sexes were exposed to BPA in six different concentrations between 0 and 7500 ppm for three generations and analyzed for many different outcomes. The study is consistent with ours regarding the weight gain of the rats, which was not significantly different in the doses used in either study. The only consistent effects of BPA in the three-generation study are toxic effects in the highest doses seen as e.g. decreased body weights. The results of the histopathology are somewhat hard to interpret because of aberrances in the control groups. One of the variables that did show significant effects in the second generation was the liver weights in female rats exposed to BPA in about the same actual dose range (0.7–30 μg/kg/day) as ours (5, 54, 487 μg/kg/day). One can argue that the effect was not consistent between generations and sexes, but also notice the reappearance of similar results in different studies. We assume that there are differences in vulnerability for BPA between sexes, different species and strains of rats, periods in life and also between individuals of the same species, e.g. humans, thus explaining the results.

4.2. BPA, apo A-I and immunity

Plasma Apo A-I, the dominating protein in high-density lipoproteins (HDL), is by its interaction with lecithin-cholesterol acyltransferase (LCAT) a crucial component in the cholesterol transport to the liver. In addition, apo A-I has anti-inflammatory properties via interactions with the immune system (Henning et al., 2011; Smoak et al., 2010; Yu et al., 2010) and in a recent study Karlsson et al. have also shown binding between apo A-I and nano-sized metal oxides (Karlsson et al., 2012). The mechanisms behind elevated plasma apo A-I levels in response to BPA exposure has to be further investigated and there are at least four different possibilities; (i) induced apo A-I gene expression by BPA, as has been reported regarding Aspirin (Jaichander et al., 2008), (ii) increased apo A-I expression in response to (pro-)inflammatory effects caused by BPA, (iii) that BPA, due to its structural similarities to cholesterol is in fact recognized as free cholesterol and (iv) that BPA causes oestrogenic effects on apo A-I gene expression (Duvillard et al., 2009). As shown in Fig. 4, apo A-I is also slightly increased in the fructose group. This is in line with a study by Koo et al., where rats fed with high doses of fructose (63%) showed altered lipid metabolism and increased apo A-I levels (Koo et al., 2008). The increased expression of apo A-I may result in BPA elimination from the plasma together with cholesteryl esters via the Scavenger Receptor Class B-I (SR-BI) in the liver. The interaction between apo A-I and SR-BI may thereby result in non-endocytotic hepatocytic uptake of hydrophobic compounds, such as cholesteryl esters and also possibly BPA. This would explain the inverted plasma cholesterol levels, albeit not significant, compared to apo A-I levels and also the increased fat infiltration in livers of BPA-exposed rats (Table 2 and Fig. 3). Interestingly, and in line with our findings, cholesteryl ester accumulation in the liver of mice exposed to BPA has previously been observed by Marmugi et al. (2012). The fate of BPA in the liver is not entirely known but elimination via bile or reemission into the circulation via very-low-density lipoproteins (VLDL) are options that need to be further investigated. Less is known about impacts of BPA on the liver and there are only a few other animal studies carried out, showing e.g. formation of DNA adducts and impaired mitochondrial functioning (Izzotti et al., 2009; Moon et al., 2012). However, due to disparities in e.g. doses and exposure route these studies are not comparable with our study.

4.3. Strengths and limitations

The strength of our study is that both fat pad weights and liver weights and extensive MR imaging-based techniques were used to quantify different fat depots, and the liver fat content. The 32-echo MR liver scan had relatively low spatial resolution. This resolution was high enough, however, for delineation of the liver tissue and the collection of 32 echoes allowed robust estimation of liver fat fraction and R2* values. We believe that the delineation of the entire liver volume imaged in combination with the analysis of the data distributions gave robust estimates of the liver tissue properties. It is possible that the higher R2* values measured in the exposed groups are due to iron infiltration of the liver tissue. Other possible causes might be due to changes in tissue structure. Another strength of the study is that LSL, liver fat. Apo A-I and R2* increased in parallel showing an internal consistency of the observations.

An obvious limitation of the present study is that only female rats were investigated. As BPA is an estrogenic-acting compound it cannot be taken for granted that different effects would not be seen in males. Unfortunately, we do not have reproducibility data on the methods used in the paper. No detailed histopathological examinations of the livers were performed.

The study was performed during 10 weeks of exposure. A longer exposure period might result in effects on the obesity measures used.

5. Conclusions

In the present study we found no evidence that BPA exposure affects fat mass in fructose-fed juvenile Fischer 344 rats. We also suggest that the increase in liver fat infiltration and apo A-I may result from combination effects of fructose and BPA exposure, and eventually may lead to more severe metabolic consequences. The present findings would motivate future studies regarding these more long term metabolic consequences. If so, the finding that fructose fed rats exposed to BPA induced fat infiltration in the liver at dosages close to the current TDI might be of concern given the widespread use of this compound in our environment and since a great proportion of the human population is exposed to both BPA and fructose daily.

Conflict of interest

None declared.

Acknowledgements

We thank Raiili Engdahl for excellent technical assistance, Katrina Cvek for expert advice about animal experiments, and Martin Ahlström for assistance with the MR image segmentation and Erik Lampa for statistical support.

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