Letter to the Editor

Effect of Perinatal Bisphenol A Exposure on Serum Lipids and Lipid Enzymes in Offspring Rats of Different Sex

GAO Liang¹, WANG Han Ning², ZHANG Ling¹, PENG Fang Yuan¹, JIA Yue¹, WEI Wei¹, and JIA Li Hong¹,*

Rats were exposed to 1 or 10 µg/mL bisphenol A (BPA) in water during pregnancy and lactation. Offspring rats were given normal water and a standard diet from weaning to postnatal day (PND) 50. Perinatal exposure to BPA resulted in significantly increased body weight, visceral adipose tissue, abnormal serum lipids, and lower adiponectin (ADP) levels in both female and male offspring rats. Liver adipose triglyceride lipase (Atg/l) mRNA levels and ADP protein in visceral adipose tissue were significantly decreased in BPA-exposed offspring rats. In both female or male offspring rats, obesity and dyslipidemia induced by perinatal exposure to BPA were associated with down regulation of Atg/l mRNA in liver and ADP protein in visceral adipose tissue.

Emerging data suggest that Bisphenol A (BPA) may be an important contributing factor to the obesity epidemic; especially early life exposure to BPA can alter developmental programming, increasing the risk of metabolic disorders[1-2]. However, the exact mechanism of BPA-mediated effects is not fully understood. Based on several studies showing BPA-mediated changes in serum lipids and lipid-related metabolic enzymes in rats of different sex, we explored the effect of perinatal BPA exposure on serum lipids. Specifically, we evaluated changes in fat synthetase (Fas) and adipose triglyceride lipase (Atg/l) mRNA in liver tissue and measured adiponectin (ADP) in serum as well as in visceral adipose tissue in male and female offspring rats that were exposed to BPA in utero.

Male (250-300 g) and female (200-220 g) Sprague-Dawley (SD) rats were purchased from the animal center of China Medical University [license number, SYXK (Liao) 2008-0005]. All animals were handled in accordance with the Guidelines for Animal Experimentation issued by the Chinese Association for Laboratory Animal Science. After a 1-week adaptation period in a room with standard temperature (22±2 °C) and illumination on a 12-h light-dark cycle, females were mated with males. A sperm-positive vaginal smear indicated the first day of pregnancy. Physiologically normal pregnant rats were individually housed and randomly allocated into three groups (n=7/group). Two groups were exposed to BPA (Sigma Aldrich, St. Louis, MO) with free access to water containing 1 µg/mL (low dose) or 10 µg/mL (high dose) BPA from gestation day 6 until the end of lactation. The third group comprised of control animals given water containing 1% ethanol, the vehicle for BPA solution. Pups were weaned on postnatal day (PND) 21 and given access to normal drinking water and fed with a standard diet until PND 50. At end of the experiment, blood samples were collected from abdominal aorta under aether anesthesia, and serum was separated by centrifugation and stored at -80 °C until subsequent analysis. Liver, perigonadal and perirenal adipose tissue were dissected, weighed, and stored at -80 °C until subsequent analysis of gene or protein expression to assess lipid metabolism.

Serum triglyceride (TC), cholesterol (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) levels were determined by commercially available reagent kits (Biosino Biotechnology and Science Beijing, China). Fas and Atg/l gene expression levels in liver specimens were measured by real-time (RT) polymerase chain reaction (PCR). Briefly, total RNA was isolated from frozen liver tissue using TRizol® (Takara Biotechnology, Dalian, China). All samples with an

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An antibody against glyceraldehyde-3-phosphate conjugated with horseradish peroxidase in TBSTM. with a rabbit anti-mouse IgG secondary antibody overnight. Membranes were then incubated for 1 h Santa Cruz Biotechnology, USA) in TBSTM at 4 °C monoclonal anti-human ADP (1:1000; sc-26496; milk (TBSTM), followed by incubation with a mouse ADP, membranes were blocked overnight at 4 °C

Proteins were separated for 1.5 h at 100 V and loaded into the wells of 14% SDS-acrylamide gels. 95 °C for 5 min. Twenty μL of each sample was prepared from 5 × sodium dodecyl sulfate (SDS)-acrylamide gel loading buffer and incubated at 100 V and transferred to membranes. For immunodetection of ADP, membranes were blocked overnight at 4 °C with TBST containing 0.1% Tween-20 and 5% nonfat milk (TBSTM), followed by incubation with a mouse monoclonal anti-human ADP (1:1000; sc-26496; Santa Cruz Biotechnology, USA) in TBSTM at 4 °C overnight. Membranes were then incubated for 1 h with a rabbit anti-mouse IgG secondary antibody conjugated with horseradish peroxidase in TBSTM. An antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Autoradiographs were scanned for densitometric analysis to quantify changes in ADP expression using Phoretix 1D advanced software. The signal intensities of ADP bands were normalized to corresponding GAPDH bands. Five samples from each group were analyzed.

For statistical analysis, SPSS software (version 20.0 for Windows; Chicago, IL) was used. All data were presented as means±standard deviation. Differences between groups were analyzed by analysis of variance (ANOVA) followed by appropriate post-hoc tests, and P values of <0.05 were considered as statistically significant.

Our results showed that body weights of both female (Figure S1A see the www.besjournal.com) and male (Figure S1B see the www.besjournal.com) offspring rats born to dams exposed to 1 or 10 µg/mL BPA from PND 1 to PND 50 were significantly higher than those of offspring rats born to control dams (n=7 belonging to seven different litters in each group, P<0.05), as well as there was more weight perigonadal adipose tissue in BPA-exposed female offspring rats, and more weight perirenal adipose tissue in BPA-exposed male offspring rats compared controls (Figure S3 see the www.besjournal.com). However, the effect of perinatal BPA exposure on body weight was not statistically different between the low-dose (1 µg/mL BPA) and high-dose (10 µg/mL BPA) groups, suggesting that the effect of BPA on body weight was not dose-dependent. The ubiquity of BPA in environment necessitates careful assessment of exposure to low-dose BPA[3]. Numerous studies showed that BPA exposure during early development could result in higher body weight in female rats[3,4]. However, very few studies examined the effect of BPA exposure on body weight in male rats. Furthermore, conflicting results of epidemiologic studies investigating the effect of BPA exposure on human body weight might be associated with the dose, time and route of BPA exposure[5-6]. Our study also indicated that perinatal exposure to BPA resulted in the different distribution of visceral adipose tissue, such as more fat around uterus for female rats and around kidney for male rats. This effect of BPA on distribution of visceral adipose tissue was worth study further.

Serum lipid and ADP levels in female and male offspring are shown in Table 1. In female offspring rats, both low- and high-dose BPA exposure during perinatal period led to significant increases in serum
TG and TC levels ($P<0.05$ and $P<0.01$, respectively) and significant decreases in serum HDL and ADP levels ($P<0.05$ and $P<0.01$, respectively), compared to controls. Conversely, compared to control males, significantly higher levels of serum TC and significantly lower levels of serum ADP were observed only in the high-dose male offspring rats ($P<0.05$ and $P<0.01$, respectively); no significant differences were observed between the low-dose and the control male offspring rats. These findings suggested that dyslipidemia was more severe in female offspring rats than in male offspring rats following exposure to BPA during gestation and lactation. Previously, perinatal BPA exposure was shown to result in higher serum TG levels in adult offspring rats that were fed a high-fat diet after weaning\[7\]. Our results indicated that perinatal BPA exposure precipitated abnormal lipid levels in both female and male offspring rats despite being fed a standard diet after weaning.

ADP protein expression in visceral adipose tissue of female offspring rats was significantly lower in both low- and high-dose groups than in control offspring rats, whereas a similar change was only observed in male offspring rats in the high-dose group ($P<0.05$ and $P<0.01$, Figure S2 see the www.besjournal.com). As a vital adipokine, ADP is primarily expressed in and released from adipose tissue. It is important to note that ADP was demonstrated to positively correlate with serum HDL and negatively correlate with serum TG in mice; ADP was also found to confer protection from metabolic syndrome in humans\[8\]. A recent study found that BPA at environmentally relevant doses inhibited ADP release from human adipose tissue explants and adipocytes\[9\]. In the present study, serum ADP levels in both BPA-exposed female and male offspring rats were significantly lower; in addition, ADP protein expression was also lower in visceral adipose tissue. This decrease in ADP protein expression in tissue coincided with the increased body weight of offspring rats. These findings provide evidence to support our hypothesis that decreased ADP might be involved in abnormal lipid metabolism induced by BPA.

We also assessed changes in the expression of Fas and Atgl, two rate-limiting enzymes catalyzing fat synthesis and lipolysis, respectively, in liver. As shown in Figure 1, the levels of liver Fas mRNA were not significantly different between female and male offspring rats perinatally exposed to BPA and control rats ($P>0.05$). In contrast, liver Atgl mRNA expression was significantly lower in female offspring rats in both the low-dose and high-dose groups ($P<0.05$). Importantly, liver Atgl mRNA levels were decreased only in male offspring rats perinatally exposed to 10 μg/mL BPA ($P<0.05$). ATGL is a major hepatic lipase that regulates TG turnover and initiates the breakdown of intracellular TGs into fatty acid monomers; thus, lower Atgl expression indicates TC accumulation in hepatic cells\[10\]. Our results suggested that hyperlipidemia induced by perinatal BPA exposure in offspring rats was associated with down regulation of Atgl mRNA expression in liver. Furthermore, sex-specific changes in Atgl mRNA suggested that female offspring rats were more susceptible than male offspring rats to perinatal exposure to BPA.

In conclusion, perinatal exposure to low- as well as high-dose BPA induced obesity and dyslipidemia in both female and male offspring rats, which was likely due to the downregulation of Atgl in liver and ADP protein in visceral adipose tissue.

<table>
<thead>
<tr>
<th>Table 1. The Effect of Perinatal Exposure to BPA on Serum Lipids and ADP Levels in Female and Male Offspring Rats</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Lipids</td>
<td>Control</td>
<td>BPA (1 μg/mL)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.25±0.08</td>
<td>0.34±0.09 $^*$</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.50±0.25 $^*$</td>
<td>1.86±0.33 $^*$</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>0.21±0.09 $^*$</td>
<td>0.18±0.20 $^*$</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.99±0.24</td>
<td>0.57±0.15 $^**$</td>
</tr>
<tr>
<td>ADP (µg/L)</td>
<td>99.12±9.47</td>
<td>85.94±9.53 $^*$</td>
</tr>
</tbody>
</table>

Note. Data are shown as means±standard deviation, $n=7$ offspring rats/group. $^*P<0.05$ vs. control, $^**P<0.01$ vs. control.
Figure 1. Effect of perinatal exposure to BPA on levels of FAS and ATGL mRNA in liver of female (A) and male (B) offspring rats on postnatal day 50 by RT-PCR. Data are reported as means±SD (n=5). Significantly different from control by one-way analysis of variance (ANOVA) (∗ P<0.05).

REFERENCES
Figure S1. Effect of perinatal exposure to BPA on body weight of female (A) and male (B) offspring rats from on postnatal day 1 (PND1) to PND50. Values represent the mean±SD (n=7, one from each group). *P<0.05 compared with control.

Figure S2. Effect of perinatal exposure to BPA on levels of adiponectin (ADP) protein in visceral fat of female (A) and male offspring rats (B) on postnatal day 50 by Western blotting Western blotting. Data are reported as means±SD (n=5). Significantly different from control by one-way analysis of variance (ANOVA) ( *P<0.05 and **P<0.05).

Figure S3. Effect of perinatal exposure to BPA on the weight of perigonadal or perirenal adipose tissue in female and male offspring rats on postnatal day 50. Data are reported as means±SD (n=7, one from each group). Significantly different from control by one-way analysis of variance (ANOVA) ( *P<0.05 and **P<0.01).