Original Article

Perinatal and Postnatal Exposure to Bisphenol A Increases Adipose Tissue Mass and Serum Cholesterol Level in Mice

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Aim: To investigate whether the perinatal and postnatal exposure of mice to bisphenol A (BPA) caused the development of obesity and/or hyperlipidemia.

Methods: Pregnant mice were exposed to BPA in drinking water at concentrations of either 1 µg/mL (LD group) or 10 µg/mL (HD group) from gestation day 10 and throughout the lactating period. After weaning, the pups were allowed free access to drinking water containing the appropriate concentrations of BPA. The body weight, adipose tissue weight, and serum lipid levels were measured in the offspring at postnatal day 31.

Results: In females, the mean body weight increased by 13% in the LD group (p<0.05) and 11% in the HD group (p<0.05) compared with the control group. The mean adipose tissue weight increased by 132% in the LD group (p<0.01). The mean total cholesterol level increased by 33% in the LD group (p<0.01) and 17% in the HD group (p<0.05). In males, the mean body weight and mean adipose tissue weight increased by 22% (p<0.01) and 59% (p<0.01), respectively, in the HD group compared with the control group. The mean triacylglycerol level increased by 34% in the LD group (p<0.05).

Conclusions: The continuous exposure of mice to BPA during the perinatal and postnatal periods caused the development of obesity and hyperlipidemia.


Key words: Bisphenol A, Adipose tissue mass, Serum cholesterol, Perinatal exposure

Introduction

Bisphenol A (BPA) is an estrogenic endocrine-disrupting chemical released by polycarbonate plastics, such as baby bottles¹, the lacquer coatings of food cans², and dental sealants³. A small amount of BPA is found in human serum⁴-⁶. Thus, BPA is present ubiquitously in the environment and ingested routinely by humans. Since BPA has been associated with reproductive abnormalities⁷, the alteration of patterns of estrous cyclicity⁸,⁹, and the development of embryos¹⁰ in rodents, there is increasing concern about the negative impact of BPA on public health in humans.

Obesity is one of the greatest concerns in public health, because it is associated with serious morbidities, including diabetes, hyperlipidemia, hypertension, cardiovascular disease and osteoarthritis. Obesity is the result of an increase in body adipose tissue mass produced by either an enlargement of adipocytes or an increased number of adipocytes or both. We previously found that BPA not only had the ability to trigger 3T3-L1 fibroblasts to differentiate into adipocytes¹¹, but also the ability to accelerate terminal adipocyte differentiation¹². Sakurai et al.¹³ reported that BPA stimulated glucose transport in 3T3-F442A adipocytes. These findings suggest that in vivo prolonged exposure to BPA may increase adipose tissue mass and promote the development of obesity. There are reports that the perinatal exposure of mice⁸ and rats⁹ to BPA...
Since it has been reported that obesity was induced in cellulose 40, milk casein 163, vitamin mixture 10, and energy of 4,353 kcal/kg. The composition of the diet (in as carbohydrate, and 15% kcal as protein with total energy) was as follows: lard 72.5, soybean oil 72.5, cholesterol 2, sucrose 109, dextrin 150, corn starch 340, cellulose 40, milk casein 163, vitamin mixture 10, mineral mixture 40 and choline chloride 1. We used glass water bottles to ensure that related compounds did not leach from plastic water bottles.

In the present study, we examined whether the perinatal and postnatal exposure of mice to BPA caused an increase in body adipose tissue mass, resulting in the development of obesity. Since obesity is often associated with hyperlipidemia in humans, the levels of serum lipids were also measured.

Materials and Methods

Animals and Materials

Pregnant ICR mice were purchased from Clea Japan Inc. (Tokyo, Japan). A 30% fat diet was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Triglyceride E-test Wako, cholesterol E-test Wako, NEFA C-test Wako and glucose CII-test Wako were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). An ELISA kit for mouse/rat leptin was purchased from B-Bridge International, Inc. (CA, USA).

Experimental Design

Mice were housed in a 12h light- dark cycle (lights on at 7 am) and allowed free access to food and water. Since it has been reported that obesity was induced in mice by exposure to a high-fat diet, we used a 30% fat diet. The diet contained 30% kcal as fat, 55% kcal as carbohydrate, and 15% kcal as protein with total energy of 4,353 kcal/kg. The composition of the diet (in g/kg) was as follows: lard 72.5, soybean oil 72.5, cholesterol 2, sucrose 109, dextrin 150, corn starch 340, cellulose 40, milk casein 163, vitamin mixture 10, mineral mixture 40 and choline chloride 1. We used glass water bottles to ensure that related compounds did not leach from plastic water bottles.

Nine pregnant mice were individually housed in standard polypropylene mouse cages (282 × 157 mm) on postnatal day 157. They were divided into three groups (3 mice/group) and exposed to BPA in their drinking water at concentrations of either 1 μg/mL (LD group) or 10 μg/mL (HD group) beginning on day 10 of pregnancy. BPA was dissolved in absolute ethanol and the final concentration of ethanol was 0.2%. We gave the control group drinking water containing only 0.2% ethanol.

Water intake on days 13 and 16 of gestation was determined by measuring the difference in the amount of water placed in the water bottle each day and the amount remaining the following day, and the levels of BPA consumed daily were estimated. Daily water intake on gestation day 13 and 16 was 11.9 ± 5.8 and 12.6 ± 1.1 mL, respectively, in the control group, 10.5 ± 3.9 and 13.5 ± 3.7 mL, respectively, in the LD group and 10.8 ± 5.6 and 16.3 ± 0.9 mL, respectively, in the HD group. Body weight on gestation day 13 and 16 was 42.5 ± 2.0 and 53.0 ± 2.3 g, respectively, in the control group, 41.1 ± 0.2 and 51.5 ± 1.1 g, respectively, in the LD group, and 42.9 ± 3.2 and 53.1 ± 4.4 g, respectively, in the HD group. Based on these values, we estimated the levels of BPA consumed daily to be 0.26 ± 0.09 (gestation day 13) and 0.26 ± 0.08 mg/kg body weight (gestation day 16) in the LD group and 2.42 ± 1.12 (gestation day 13) and 3.01 ± 0.89 mg/kg body weight (gestation day 16) in the HD group. The average BPA intake was 0.26 mg/kg body weight/day in the LD group and 2.72 mg/kg body weight/day in the HD group. The numbers of newborn mice were 14.0 ± 0.6 pups/litter in the control group, 13.3 ± 0.3 pups/litter in the LD group, and 12.7 ± 1.5 pups/litter in the HD group. BPA exposure continued throughout the period of lactation. The sexes of the offspring were initially recorded on postnatal day 8. The numbers of females and males were 19 and 23 in the control group, 16 and 25 in the LD group, and 19 and 19 in the HD group, respectively. The numbers of both sexes per litter were as follows: 6.3 ± 0.9 females and 7.7 ± 0.9 males in the control group; 5.3 ± 1.5 females and 8.0 ± 1.5 males in the LD group; 6.3 ± 1.3 females and 6.3 ± 0.9 males in the HD group. All pups were transferred with their dam to larger polypropylene mouse cages (282 × 451 × 157 mm) on postnatal day 12. After weaning, the pups were separated from their dam and allowed free access to a 30% fat diet and water containing the appropriate concentrations of BPA. Thirty days after birth, the food was removed from the cage at 5 pm, and all mice were killed between 9 and 11:30 am on the following day under ether anesthesia. The parametrial adipose tissues of female mice were removed and weighed. The epididymal adipose tissues of male mice were removed and weighed.

Animals were treated humanely and with regard for alleviation of suffering, and the experimental protocols were reviewed and approved by the local animal ethics committee at Ehime Prefectural University of Health Sciences (approval no. 37).

Measurements of Serum Lipids, Glucose and Leptin

The levels of serum total cholesterol (TC), triacylglycerol (TG), non-esterified fatty acid (NEFA) and glucose were measured using kits for TC, TG, NEFA and glucose, respectively. Serum leptin was measured using a leptin ELISA kit.
Statistical Analyses

Significant differences among three independent groups were evaluated statistically using one-way analysis of variance, and subsequent comparisons were performed using the Tukey-Kramer test, which allows for unequal sample sizes. Significant differences between two independent groups were analyzed by Student’s t-test. Pearson r was used to calculate correlations between adipose tissue weight and body weight, and the levels of serum lipids and leptin. All statistical procedures were carried out using SPSS software (version 13.0). A p value of <0.05 was considered to indicate significant difference. All values were expressed as the mean ± S.E.

Results

Effect of BPA on Body Weight and Adipose Tissue Mass

Pregnant mice were exposed to BPA from gestation day 10 and throughout the lactating period. After weaning, the pups were separated from their dams and exposed to BPA. Thirty-one days after birth, their body weights and adipose tissue weights were measured. In females, the mean body weight increased by 13% in the LD group and 11% in the HD group compared with the control group [F(2,51) = 5.306, p < 0.01] (Fig. 1A). The mean adipose tissue weight of the LD group increased by 132% compared with the control group, while the weight of the HD group did not differ significantly from the control group [F(2,51) = 9.883, p < 0.01] (Fig. 1B). The percentage of adipose tissue weight to body weight was significantly higher in the LD group, but not the HD group, than in the control group [0.36 ± 0.03% in the control group; 0.71 ± 0.09% in the LD group (p < 0.01, vs the control group); 0.50 ± 0.04% in the HD group; F(2,51) = 10.811, p < 0.001]. When the correlation between adipose tissue weight and body weight was examined in all females, adipose tissue weight showed a strong positive correlation with body weight (Fig. 1C). This result indicates that increased body weight resulted from an increase in adipose tissue mass.

In males, the mean body weight and mean adipose tissue weight of the HD group increased by 22% and 59%, respectively, compared with the control group, while these two weights in the LD group did not differ significantly from the control group [body weight, F(2,64) = 12.069, p < 0.001; adipose tissue weight, F(2,64) = 10.904, p < 0.001] (Figs. 2A and 2B). The percentage of adipose tissue weight to body weight was significantly higher in the HD group, but not the LD group, than in the control group [0.61 ± 0.03% in the control group; 0.79 ± 0.05% in the HD group (p < 0.01, vs the control group): F(2,64) = 6.505, p < 0.01]. As in females, the adipose tissue weight of males also correlated positively with body weight (Fig. 2C).

Effect of BPA on Serum Leptin Levels

Since leptin is a product of the ob gene and a hormone secreted by adipocytes with an important function in the regulation of the amount of body fat\(^7\), the level of serum leptin was measured. In females, the mean leptin level of the LD group increased by 123% compared with the control group, while the level of the HD group did not differ significantly from the control group [F(2,51) = 3.449, p < 0.05] (Fig. 3A).
leptin levels of females showed a positive correlation with adipose tissue weight (Fig. 3B).

In males, no differences in mean leptin levels were observed among the three groups (Fig. 3C), and leptin levels did not show any significant correlation with adipose tissue weight (Fig. 3D).

**Effect of BPA on Serum Lipid and Glucose Levels**

Table 1 shows the levels of serum TC, TG, NEFA and glucose. In females, the mean TC level increased by 33% in the LD group and 17% in the HD group compared with the control group [F(2,51) = 8.645, p < 0.001]. There were no differences in the mean TG, NEFA and glucose levels among the three groups.

In males, the mean TC level in LD and HD groups tended to be higher, but not significantly, compared with the control group. The mean TG and NEFA levels of the LD group increased by 34 and 29%, respectively, compared with the control group, while the levels of the HD group did not differ significantly from the control group [TG, F(2,64) = 9.674, p < 0.001; NEFA, F(2,64) = 18.171, p < 0.001]. The mean glucose level of the LD group, but not the HD group, decreased by 41% compared with the control group [F(2,64) = 4.667, p < 0.05].

**Correlation between Adipose Tissue Weight and the Levels of Serum Lipids and Glucose**

Table 2 shows the correlation coefficients between adipose tissue weight and the levels of serum lipids and glucose. In females, the levels of serum TC, TG and glucose correlated positively with adipose tissue weight, while the level of serum NEFA correlated negatively. As in females, the levels of serum TC and

![Fig. 2. Effect of BPA on body weight and adipose tissue weight in male offspring.](A and B) Thirty-one days after birth, overnight fasted male offspring were killed under ether anesthesia, and their body weight (A) and adipose tissue weight (B) were measured. The values given are the mean ± S.E. **p < 0.01 (compared with the value of the control group). C, control group (n=23); LD, LD group (n=25); HD, HD group (n=19). (C) Correlation between body weight and adipose tissue weight in all males (n=67). (○) control group; (●) LD group; (□) HD group.

![Fig. 3. Effect of BPA on the level of serum leptin.](A and C) Levels of serum leptin in offspring were measured using an ELISA kit for leptin. The values given are the mean ± S.E. Panel (A) shows the serum leptin level of female offspring. *p < 0.05 (compared with the value of the control group). C, control group (n=19); LD, LD group (n=16); HD, HD group (n=19). Panel (C) shows the serum leptin level of male offspring. C, control group (n=23); LD, LD group (n=25); HD, HD group (n=19). (B and D) Correlations between the leptin level and adipose tissue weight in female offspring (B) and male offspring (D) are shown. (○) control group; (●) LD group; (□) HD group. N.S., not significant.
Table 1. Serum TC, TG, NEFA and glucose levels in female and male mice

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<tr>
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<th>Female mice</th>
<th>Male mice</th>
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<tr>
<td></td>
<td>Control group (n = 19)</td>
<td>LD group (n = 16)</td>
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<tr>
<td>Serum TC (mg/dL)</td>
<td>165 ± 8 b</td>
<td>219 ± 11 **</td>
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<tr>
<td>Serum TG (mg/dL)</td>
<td>87 ± 5</td>
<td>98 ± 12</td>
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<td>Serum NEFA (mEq/L)</td>
<td>2.23 ± 0.05</td>
<td>2.36 ± 0.18</td>
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<tr>
<td>Serum glucose (mg/dL)</td>
<td>86 ± 12</td>
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TC, total cholesterol; TG, triacylglycerol; NEFA, non-esterified fatty acid
bNumbers of parentheses show the number of mice.

Discussion

The aim of this study was to determine whether BPA is involved in the development of obesity and/or hyperlipidemia. In this study, we gave pregnant mice drinking water containing either 1 µg/mL or 10 µg/mL of BPA from gestation day 10 and throughout the lactating period, and then gave the pups drinking water containing appropriate concentrations of BPA after weaning. The measurement of body weight at postnatal day 31 showed that continuous exposure to BPA caused an increase in body weight in both female and male offspring. This agrees with the finding of Rubin et al. that both female and male offspring born to rats exposed to BPA exhibited an increase in body weight at postnatal day 28. They gave pregnant rats drinking water containing the same concentrations of BPA as those used in this study from gestation day 6 to postnatal day 21, and then gave the pups unadul-tered drinking water after weaning.

Since obesity is characterized by the accumulation of excessive adipose tissue mass in the body, we measured the parametrial/epididymal adipose tissue weight. Our data on adipose tissue weight demonstrated that BPA caused a nonmonotonic and inverted-U-shaped dose-response increase in the mean adipose tissue mass in female offspring, while this chemical caused a monotonic dose-response increase in the mass in male offspring. The ratios of adipose tissue mass to body weight were also increased in similar dose-response manners. Moreover, simple regression analysis showed that, in both female and male offspring, body weight increased with the increase in adipose tissue mass. These results suggest the involvement of BPA in the development of obesity in both female and male offspring. This interpretation raises the question of whether BPA affects other parameters of obesity.

Most cases of obesity in rodents and humans are associated with high circulating leptin levels. For example, the serum leptin levels of obese children correlated highly with arm fat and body mass index; therefore, to explore the above-mentioned question, we measured the serum leptin levels. In females, serum leptin levels were significantly higher in pups born to mice exposed to BPA than in those born to control mice. As for adipose tissue mass, mean leptin levels increased in a nonmonotonic and inverted-U-shaped dose-response manner, resulting in a positive correlation between serum leptin levels and adipose tissue mass. Based on these findings, we concluded that the continuous exposure of mice to BPA during the perinatal and postnatal periods caused the development of obesity in female offspring. To our knowledge, this is the first report demonstrating the increasing effect of BPA on adipose tissue mass and serum leptin level. Takeuchi et al. reported that serum BPA concentrations were higher in young obese women.
than in young non-obese women and correlated positively with body mass index. This finding suggests that BPA might be associated with the development of obesity in young women.

In males, BPA failed to increase serum leptin levels, resulting in no correlation between the serum leptin level and adipose tissue mass. Two possibilities for this failure were considered. One possibility is the lower increasing effect of BPA on adipose tissue mass in males than in females. For example, BPA caused a 132% increase in mean adipose tissue weight in the LD group of females, while it caused only a 59% increase in weight in the HD group of males. Another possibility is the gender differences in leptin production in adipose tissue. We observed in the control group that adipose tissue was heavier in males than in females [males (n = 23), 95.2 ± 5.6 mg]; females (n = 19), 56.1 ± 4.5 mg; p < 0.01], whereas there were no differences in serum leptin levels between genders [males (n = 23), 1.36 ± 0.21 ng/mL; females (n = 19), 1.51 ± 0.26 ng/mL]. Frederich et al. [2004] also reported that when fed the high-fat diet, equivalent levels of plasma leptin were observed in female and male mice despite female mice having less total body lipid. They described that if female mice produced more leptin at any fat cell size, and the hypothalamus responded to leptin in an identical manner in both sexes, reduced body fat content in the presence of equivalent leptin levels would result. Ahrén [2004] reported that the plasma leptin level displayed a nocturnal increase in mice and that this increase was 2.6-times greater in females than in males. Thus, the ability to produce leptin in adipose tissue was lower in males than in females.

In humans, obesity often develops secondarily to hyperlipidemia and cardiovascular disease. Cardiovascular disease is the major cause of morbidity and mortality, and the risk of coronary heart disease is associated with high circulating TC levels [2004-2006]. To our knowledge, there are only two reports on the effect of BPA on serum lipid levels. Dodge et al. [2009] reported that the 4-day oral administration of BPA (0.1, 1.0 or 10 mg/kg body weight/day) to ovariectomized rats (6 months old) did not change the serum TC level, but BPA at the highest dose (30 mg/kg body weight/day) decreased it. Seidlová-Wuttke et al. [2005] reported that the 3-month administration of pellet food containing BPA (0.033 or 0.333 mg/kg body weight/day) to ovariectomized rats (3 months old) did not change serum TC and TG levels. We recently observed that the 30-day administration of a 30% fat diet containing BPA (approximately 0.19 or 18.9 mg/kg body weight/day) to male ICR mice (4 weeks old) did not cause any changes in serum TC and TG levels (H Masuno - unpublished data). Thus, the administration of BPA at doses of <20 mg/kg body weight/day during adulthood did not affect serum lipid levels. In the present study, we administered BPA (0.26 or 2.72 mg/kg body weight/day) to mice during the perinatal and postnatal periods and measured serum lipid levels on postnatal day 31. In females, BPA increased the serum TC level in a nonmonotonic and inverted-U-shaped dose-response manner, but did not cause a significant increase in the serum TG level. The serum TC level showed a positive correlation with adipose tissue weight. This is compatible with the observation that obesity is often accompanied with hyperlipidemia. In males, BPA increased the serum TG level in a nonmonotonic and inverted-U-shaped dose-response manner, but did not cause a significant increase in the serum TC level. Surprisingly, the serum TG level correlated negatively with the adipose tissue weight. This is inconsistent with the above description. Since the serum TG level correlated positively with the serum NEFA (r = 0.913, p < 0.0001) and negatively with the serum glucose level (r = -0.541, p < 0.0001), this suggests that the high level of serum TG might be due to the overproduction of TG from NEFA, but not glucose. Based on these findings, we concluded that the continuous exposure of mice to BPA during the perinatal and postnatal periods caused the development of hypercholesterolemia in female offspring and of hypertriglyceridemia in male offspring.

Although the mechanism by which perinatal and postnatal exposure to BPA elicits increasing effects on the adipose tissue mass and serum lipids differently in female and male offspring is unclear, there have been some reports on the differences in responses to BPA between female and male offspring born to exposed dams. Rubin et al. [2005] reported that the increase in body weight of rats born to dams exposed to BPA persisted longer in female offspring than in male offspring after BPA administration ceased. Farabollini et al. [2006] described that perinatal exposure of rats to BPA in the period of sexual differentiation of the brain influenced adult behavior differently in females and males.

Our present data showed that low-dose BPA was more effective than high-dose BPA in altering some parameters of obesity and hyperlipidemia. This is consistent with the finding of Rubin et al. [2005] that, in female rats exposed perinatally to BPA, the lower of the two BPA doses (0.1 and 1.2 mg/kg body weight/day) produced a larger effect on body weight relative to the higher dose. Similar nonmonotonic and inverted-U-shaped dose-response curves of BPA were observed in measurements of preputial gland weight and testis weight in rodents. vom Saal et al. [2007] described that responses to weak environmental estrogens, including...
BPA, cannot be assumed to be monotonic across a wide dose range. Moreover, they found that estradiol and diethylstilbestrol also increased prostate weight in an inverted-U-shaped dose-response manner in mice.

BPA is used in the food-packaging industry and in dentistry, and leaches from the lining of tin cans into foods and from dental sealants into saliva. For example, microgram amounts of BPA (4-23 μg/can) were found in both extracted foods and water from autoclaved cans. Olea et al. detected 90-931 μg of BPA in the saliva during the first 1-h after the application of dental sealant (50 mg). The maximum amount (931 μg) was reported to represent 0.0133 mg/kg body weight for a person weighing 70 kg and 0.0372 mg/kg body weight for a child weighing 25 kg. The low-dose BPA used in the present experiment was close to that used by Rubin et al., but was about 10-times higher than a dose equivalent to that found in saliva. Howdeshell et al. reported that the perinatal exposure of mice to very low-dose BPA (0.0024 mg/kg body weight/day) increased body weight on the weaning day in female offspring. This finding suggests that BPA at lower doses than those used in the present study might increase body adipose tissue mass.

In conclusion, the perinatal and postnatal exposure of mice to BPA increased body adipose tissue mass and serum TC and TG levels. The increasing effect of BPA on adipose tissue mass was more pronounced in females than in males.

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