The Effect of Prenatal and Postnatal Exposure of Bisphenol A on Body Weight and Percent Body Fat in Rattus Norvegicus

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The Effect of Prenatal and Postnatal Exposure of Bisphenol A on Body Weight and Percent Body Fat in *Rattus Norvegicus*

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Natural Sciences at Carroll College, Helena, Montana

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April 14, 2009
This thesis for honors recognition has been approved for the Department of Natural Sciences by:

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Abstract

Bisphenol A (BPA) is an estrogenic compound that is used in the manufacturing and production of polycarbonate resins and plastics. BPA is commonly found in dental sealants, linings of tin cans, and some water and baby bottles. Human consumption may occur through the leaching of BPA from these products. Because BPA is an endocrine disruptor, it is suggested to have a negative effect on body energy levels, reproduction, and growth and development. Of particular concern is the effect of BPA on the growth and production of adipocytes (fat cells) in fetuses and the newborn. Three randomly selected Fischer 344 pregnant female rats were exposed to BPA through their drinking water at an average of 0.1 mg BPA/kg/day from day 11 of pregnancy until offspring were weaned. Once weaned, offspring were exposed to BPA through their drinking water at an average of 0.06 mg BPA/kg/day from the day of weaning (21 days after birth) to day 42 of development. Through the use of specific gravity, the percent body fat of each offspring was determined. Statistical analysis demonstrated that exposure to this low-dose BPA did not have a significant effect on an increase in rat percent body fat. A significant increase in body weight was demonstrated in male offspring, but not in female offspring.
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Introduction

Each year in the United States (US), a projected 300,000 adults die of causes related to obesity (Mokdad et al., 2001). As the population of obese individuals rises in the United States and across the globe, so too does the frequency of diseases such as cardiovascular disease, type 2 diabetes mellitus, stroke, and even some types of cancers (Must et al., 1999). Must et al. (1999) reported that 63% of men and 55% of women had a body mass index of 25 kg/m² or greater (Must et al., 1999). Must et al. (1999) confirmed that with the increasing number of people who are overweight and obese, the occurrence of the diseases mentioned also increased, with the exception of coronary heart disease in men and high blood cholesterol level in men and women. Must et al. (1999) concluded that more than half of all adults in the United States are considered to be overweight or obese. A more recent study performed by Mokdad et al. (2001) reported that the prevalence of obesity is continually rising in US adults.

With the constant increase in obesity found in US adults, research is now being directed toward examining numerous chemicals known as endocrine disruptors. Endocrine disruptors are chemicals that are thought to interfere with the activity or production of hormones within the endocrine system. These chemicals have the ability to mimic naturally occurring hormones. They also are capable of blocking endogenous hormones from binding to specific receptors within a cell. Examples of such chemicals include various pesticides (DDT) and a chemical known as Bisphenol A (BPA). These chemicals, which are commonly found in everyday products, may produce adverse affects in humans and other wildlife (“Endocrine Disruptors,” 2009).
Specifically, Bisphenol A (BPA) is an estrogenic compound commonly used in the production of plastics and resins; such products include can linings (Brotons et al., 1994), dental sealants (Olea et al., 1996), and some plastic bottles (Biles et al., 1997). In a study performed by Masuno et al. (2002), it was hypothesized that BPA combined with insulin could affect the formation of adipocytes (fat cells). Masuno et al. (2002) suggests that exposure to BPA may contribute to increased body fat by fat cell hyperplasia, fat cell hypertrophy, or possibly both. Moreover, Masuno et al. (2002) concluded that BPA triggered the conversion of pre-adipocytes to adipocytes (hyperplasia), along with enhancing their fat storage (hypertrophy). Although the effects of insulin and BPA were greater than the use of just BPA (Masuno et al., 2002), the mere prospect of BPA contributing to the obesity epidemic across the globe makes studies involving BPA and other endocrine disruptors essential.

Several researchers have begun to investigate the impact of BPA. Takahashi and Oishi (2000) examined BPA's placental transfers in Fischer 344 rat fetuses whose mothers were exposed orally to BPA while pregnant. The results from this study suggest that the transfer and absorption of BPA in maternal organs, along with fetal organs, is rapid and BPA is passed through to the fetus, signifying that the placenta is not a natural barrier to BPA (Takahashi and Oishi., 2000).

Further studies conducted using rodent models have produced inconsistent data concerning how BPA influences and affects different species of rodents (Rubin et al., 2001). Species type, dose, time of exposure, and course of exposure to BPA are thought to play key roles in its effects (Richter et al., 2007, vom Saal and Hughes, 2005). Current studies have shown Fischer 344 rats to be particularly sensitive to the exposure of BPA.
(Steinmetz et al., 1998). Rubin et al. (2001) concluded that Sprague Dawley rats perinatally administered 1 mg/L (low dose) of BPA had offspring with a noted increase in body weight. The term “low dose” is considered to be the lowest dose of BPA studied for risk assessment and is currently accepted at any amount lower than or equal to 50 μg/kg/day (vom Saal and Hughes., 2005). Rubin et al. (2001) further reported there was a greater difference in the mean body weight found in female offspring compared to male offspring. Although the males did show an increase in mean weight, the low dose females exposed to BPA retained a greater amount of that weight over an extended period of time compared to both the males and the non-BPA controls (Rubin et al., 2001).

It was hypothesized, given our current understanding of the effects of BPA and its relation to increased body weight, that female Fischer 344 rats, prenatally and postnatally exposed to low dose BPA, will show an increased body weight due to a greater percentage of body fat. Percent body fat was determined by a method using specific gravity developed by Dahms and Glass (1982). All animals received BPA through oral administration to replicate the most common form of exposure that humans encounter (Rubin et al., 2001).
Materials and Methods

Animals

Six pregnant female Fischer 344 rats were received on their 11th day of pregnancy from Simonsen Laboratories, Inc. (Gilroy, CA). Rats were housed in separate metal cages in a room with controlled 12 hour lighting and a constant temperature of 25°C. They were given constant access to Mazuri Rat Chow and water. Water was measured and re-administered every 48-72 hours. Rats initially weighed between 202.3 and 237.4 grams. Drinking water was administered in glass bottles.

Treatment A

On the 12th day of pregnancy, three females were randomly selected and exposed to Bisphenol A (BPA) in concentrations of 1 mg/L in their drinking water (low dose BPA; Rubin et al., 2001). Exposure to BPA was continued until the day of weaning. The three control females were given water with 1% ethanol used as a diluent in the BPA solutions (Rubin et al., 2001). All water throughout the study was kept in glass bottles. The mean level of BPA consumed daily from drinking water by pregnant females was calculated to be 0.08 mg/kg/day. Measurements were determined by calculating the total amount of water consumed (in mL) during the gestation period (21 days). The total amount of water recorded was assumed to be completely from consumption (Rubin et al., 2001). Water lost from leakage and/or possible evaporation was not accounted for (Rubin et al., 2001).

Offspring A

On day 21 of development, offspring were separated based on sex. Offspring born to mothers exposed to BPA, received concentrations of BPA of 1 mg/L in their drinking
water until day 42 of development. Similarly, control offspring were given water containing 1% ethanol until day 42 development. Initially, a total of 43 rats were obtained, but due to lack of maternal care and cannibalistic behavior, only six offspring survived: three control females and three BPA (two females, one male). The mean level of BPA consumed daily by offspring from their drinking water was calculated to be 0.05 mg/kg/day. Measurements were determined by calculating the total amount of water consumed (in mL) during days 21-42. Once again, the total amount of water recorded was assumed to be completely from consumption.

Treatment B

Due to the low number of surviving offspring observed, one Fischer 344 control female and one Fischer 344 BPA exposed female were chosen to be impregnated by one Wister male rat. In separate metal cages, each female rat was housed for 14 days with a male rat. During this period, the rats had free access to food and water. Water was obtained from the faucet, and kept in glass bottles. After day 14 the male rat was removed and the pregnant BPA rat was started on a regimen of BPA (1 mg/L) in her drinking water. The pregnant control rat was given water with 1% ethanol. The mean level of BPA consumed daily by the pregnant female from her drinking water was calculated to be 0.12 mg/kg/day. Measurements were determined from the procedure seen in Treatment A.

Offspring B

Twenty one days after birth, offspring were separated from their mothers. All further exposure to BPA follows the Offspring A procedure. A total of 20 rats were kept; 8 controls and 11 BPA. The mean level of BPA consumed daily by offspring from their
drinking water was calculated to be 0.07 mg/kg/day. Measurements were determined as in treatment A.

Percent Body Fat Testing

All animals were euthanized by carbon dioxide (CO₂) on day 42 of development. The procedures of Dahms and Glass (1982) were followed for hair removal and dissection. An OHAUS (Florham Park, NJ) 700 Series Triple Beam Balance was placed at the edge of a laboratory table with its weighing hooks positioned, allowing for a metal wire to hang freely without being inhibited by the table edge. Located below the weigh hooks, was a seven liter Pyrex glass cylinder filled with deionized water (25°C). A small metal wire was fastened to the tips of the rats' tails and then secured to the weighing hook of the balance allowing the rat to hang freely. Rats were first weighed in air and then in water. Further methods used for weighing and calculating percent body fat using specific gravity followed those in Dahms and Glass, (1982).

Statistical Analysis

Analysis of variance (ANOVA) was used to analyze the body weight data as well as the percent body fat data. Graphs and figures were created using the Microsoft Excel program.
Results

Body Weight Testing
The body weights of the rats were measured following euthanasia using a CO₂ chamber. Using a Fisher EMD ES-210 scale, rats were weighed following hair removal and values were recorded in grams. Mean body weights of male and female rats (BPA exposed and controls (BPA not exposed)) are shown (Fig.1). A significant difference was observed in males exposed to BPA compared to controls (P<0.05) (Table 1).

Table 1. Results of Anova for effects of BPA and Sex on rat body weight.

<table>
<thead>
<tr>
<th>Anova Testing for the Effects of Treatment (BPA) and Sex on Body Weight</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
<td></td>
<td>Effect</td>
<td></td>
<td>Error</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPA</td>
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<td>0.7585</td>
<td>20</td>
<td>296.78</td>
<td>0.0025</td>
<td>0.9601</td>
</tr>
<tr>
<td>Sex</td>
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<td>2086.38</td>
<td>20</td>
<td>296.78</td>
<td>7.0299</td>
<td>0.0153</td>
</tr>
<tr>
<td>BPA x Sex</td>
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<td>20</td>
<td>296.78</td>
<td>0.3800</td>
<td>0.5445</td>
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Percent Body Fat Testing
Percent body fat was determined by calculating the specific gravity of each rat (Dahms and Glass, 1982). No significant difference (P > 0.05) was observed in average percent body fat for exposed versus control in either the males or the female rats (Fig. 2) (Table 2).

Table 2. Results of Anova for effects of BPA and Sex on rat specific gravity.

<table>
<thead>
<tr>
<th>Anova Testing for the Effects of Treatment (BPA) and Sex on Specific Gravity</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
<td></td>
<td>Effect</td>
<td></td>
<td>Error</td>
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<tr>
<td>BPA</td>
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<tr>
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<td>BPA x Sex</td>
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<td>20</td>
<td>0.0022</td>
<td>0.0093</td>
<td>0.9239</td>
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Figure 1. Average body weights for each group exposed and not exposed to BPA. Error bars represent standard errors. A total of 24 for rats were studied: six control females, five control males, four BPA females, and nine BPA males. A significant difference was seen in males exposed to BPA compared to controls. The Y-axis represents body weight in grams.

Figure 2. Average specific gravity for each group exposed and not exposed to BPA. Error bars represent standard errors. A total of 24 for rats were studied: six control females, five control males, four BPA females, and nine BPA males. No significant difference was seen in either males or females. The Y-axis represents specific gravity.
Discussion

The results in this experiment show no link between pre and post natal exposure to low-dose BPA and an increase in percent body fat in male or female rats, but do suggest a link to increased body weight in male rats. The original hypothesis that female rats exposed in utero to low-dose BPA would have a greater increase in both percent body fat and body weight than male rats exposed in utero was not supported. An increase in body weights of both males and females exposed to BPA was observed compared to controls, but only the males demonstrated a significant difference.

These findings do not agree with those found by Rubin et al. (2001) who concluded that BPA did increase body weight in male and female rats born to exposed mothers. That particular study also found that the weight increase persisted longer in females and a lower dose of BPA, similar to the amount in my study, had a greater effect (Rubin et al., 2001). A similar study performed by Howdeshell et al. (1999) reported that daily oral administration of BPA to pregnant mice lead to an increased body weight in female offspring born to exposed mothers. A more recent study performed by Miyawaki et al. (2007) exposed pregnant mice to BPA through drinking water at concentrations of 1 \( \mu g/mL \) (low dose) or 10 \( \mu g/mL \) (high dose) from gestation day 10 to the lactating period. The percentage of adipose tissue weight compared to body weight was significantly higher in the low dose group compared to controls (Miyawaki et al., 2007). The correlation between adipose tissue weight and body weight indicates that increased body weight resulted from an increase in adipose tissue (Miyawaki et al., 2007). All three studies displayed an increase in body weight when pregnant mothers are exposed to BPA.
During my study, tested rats were unintentionally subjected to a stressful environment which may have led to cannibalistic behavior. Due to the low numbers of offspring resulting from this behavior, females were re-impregnated to increase offspring number. Even with the added offspring, the low sample size may have affected the final data. The final numbers were not significant, but males and females exposed to BPA exhibited an increase in body weight compared to controls. In addition, a more precise method for calculating percent body fat may be required to produce a more accurate calculation for each rat. Moreover, the Fischer 344 rats chosen for this study were reported by Richter et al. (2007) and vom Saal and Hughes (2005) to have the greatest susceptibility to treatment with BPA. However, this may have accounted for smaller litters of viable offspring. BPA exposed mothers may have cannibalized if they perceived offspring to be non-viable.

The continued research in understanding BPA and its effects might be directed at the different areas of the body where the apparent weight gain is taking place. For instance, based on existing evidence reported by Richter et al. (2007), BPA has been reported to have an extensive effect on enzymatic activity, growth, and metabolism, developmental and adult effects on the male reproductive system, and developmental effects on the brain and behavior. Different sensitivities of rat strains to estrogen and endocrine disruptors is another fertile field for future research.
Literature Cited:


Takahashi O, and Oishi S. "Disposition of orally administered 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A) in pregnant rats and the placental transfer to fetuses." *Environmental Health Perspectives*. 2000; 108: 931.