Effect of Severe Maternal Dietary Restriction on Growth and Intra-Abdominal Adipose Tissue Weights in Offspring Rats

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Summary  In Japan, the number of low weight birth babies is increasing. The increase in the number of slim young women is considered to be associated with the rising number of low birth weight babies in Japan. In 1993, Barker et al. published highly influential findings indicating a relationship between low birth weight and increased risk of developing symptoms of metabolic syndrome. Here, we report on results that occur when dietary restriction is applied during all periods of pregnancy. It was shown that, at 5 d, the mean weight of pups in the dietary restriction group was lower than the mean weight of pups in the control group. Catch-up growth began when milk yields of the dietary restriction group pups attained the same levels as those of the control group pups. Intra-abdominal adipose tissue weights of the dietary restricted group were significantly higher than those of the control group in males at 280 d after birth. Intra-abdominal adipose tissue weights of the dietary restricted group had a tendency to be higher than those of the control group for female rats. In male rats, it is considered that increase in intra-abdominal adipose tissue is related to lean body mass but it is not related to the function of brown adipose tissue (BAT). In female rats, it is considered that the increase in intra-abdominal adipose tissue is related to the function of BAT and lean body mass.

Key Words  maternal, severe dietary restriction, intra-abdominal adipose tissue, brown adipose tissue, lean body mass

In 1993, Barker et al. published a highly influential hypothesis indicating a relationship between low birth weight and increased risk of developing symptoms of metabolic syndrome (1). In Japan, the number of low weight birth babies has been increasing recently. It is considered that slender young women are associated with the rise in low birth weight babies in Japan. Severe dietary restriction is increasing among young women who aim to be and remain slim.

Ravelli et al. reported that men who had suffered Dutch famine in the first two trimesters of intrauterine life had an increased incidence of obesity in adulthood (2).

Recent investigations of severe maternal dietary restriction in pregnancy have consecutively brought to light results reporting babies having lower birth weight or babies exhibiting evidence of disproportionate growth (3). Human epidemiological studies have shown that fetal under-nutrition increases the risk of cardiovascular disease and type II diabetes in adult life (4, 5). Jones and colleagues observed that rats subjected to intrauterine malnutrition during the first 2 wk following conception become hyperphagic and obese after 5 wk of age (6, 7). Experimental studies in humans and animals have documented many examples of fetal under-nutrition (8–11). It is known that malnutrition during each trimester of the gestation period has different effects. Maternal exposure to under-nutrition in early gestation induced adult obesity (1). Exposure to under nutrition during middle or late-stage gestation was associated with an increase in obesity and glucose intolerance (12, 13).

In this report, we present results for when there is dietary restriction throughout all periods of pregnancy.

MATERIALS AND METHODS

Experimental design of gestation and lactation.

Animals and diet: Male and female Wistar rats, aged 8 wk, were purchased from Charles River Japan. These rats were kept under controlled temperature (23 ± 2°C), humidity (60 ± 10%) and lighting (12 h light and 12 h dark cycle). After 14 d of acclimation, female rats were mated with a male for 1 d. The presence of spermatozoa in vaginal smears taken the following day was considered to be the first day of pregnancy. Following impregnation, female rats were divided into two experimental groups. Pregnant female rats were then transferred into individual plastic cages. The first group was the control group which was permitted free access to food throughout the gestation and lactation periods. The second group was the dietary restricted group which was permitted to intake 50% of the consumption had by the control group during gestation. However, the dams were allowed to eat ad libitum during lactation. The
Early gestation  
mid gestation  
late gestation  

Control group  
Dietary restriction group  

1st day  
21st day  
40th day  

Blood glucose response measurement  
Sacrifice  

Table 1. Average of body weight and body weight gain in pups.

<table>
<thead>
<tr>
<th></th>
<th>Initial body weight (g)</th>
<th>1 wk after weaning (g)</th>
<th>Final body weight (g)</th>
<th>Body weight gain rate (×100)%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male Control:</strong> n=5</td>
<td>53.97±2.16</td>
<td>91.00±3.57</td>
<td>864.46±60.12</td>
<td>15.16±1.36</td>
</tr>
<tr>
<td>Dietary restriction: n=5</td>
<td>30.47±0.10**</td>
<td>84.59±4.03</td>
<td>796.2±32.45</td>
<td>25.14±1.10**</td>
</tr>
<tr>
<td><strong>Female Control:</strong> n=6</td>
<td>49.73±2.55</td>
<td>83.05±2.17</td>
<td>519.40±42.87</td>
<td>9.40±0.52</td>
</tr>
<tr>
<td>Dietary restriction: n=6</td>
<td>48.84±2.05</td>
<td>77.08±1.84</td>
<td>569.91±42.21</td>
<td>10.64±0.60</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those for the controls; **p<0.01. Mean±SE.

restricted rats were given meals twice per day (at about 9:00 h and 18:00 h) in a manner in which they were unable to intake the total amount. Water was always available ad libitum for all experimental groups. Food intake was measured daily for gestation rats.

Gestation and lactation: Body weight was measured weekly and food intake was measured daily for pregnant mothers. The day of parturition was set as the first day of lactation. Body weight and naso-tail length of litters were measured and litter size was adjusted to 10 pups per dam on the 4th day after birth. Body weights of both dams and pups were measured daily from the 5th day to 20th day.

Milk yield measurement: Milk yield was measured daily from the 5th day to the 20th day. Dams were separated from litters for 3 h prior to milk yield measurement. The body weight of both dams and pups was measured immediately prior to milk yield measurement. Lactation was permitted for 1.5 h. Thereafter, body weights of dams and pups were measured after 1.5 h. The milk yield of dams and the intake by pups were calculated from the difference in body weight before and after the lactation period.

Experimental design after weaning. At day 21 after birth all pups were weaned and divided randomly into various groups for future studies. Next, the pups were sorted by gender. Body weights of pups were recorded at the time of weaning. Food intake was measured every 2 d and body weight every 7 d. The experimental diet consisted of AIN93G composition. After overnight fasting (16–18 h), glucose (4 mg per 1 kg in body weight) was orally administered on the 60th day after birth. Blood samples were drawn from the vein of the tail in: −15, 0, 15, 30, 60, 90, and 120 min for blood glucose measurement. The pups were bred up to 280 d after birth. All animals were killed after 280 d of experimentation. Animals were anesthetized by inhalant anesthesia and exsanguinated following 12 h of food deprivation. Immediately after sacrifice, white and brown adipose tissues were removed. Subcutaneous, perinephric, epididymal (female-perimetric) and mesenteric adipose tissue were counted as WAT (white adipose tissue). Furthermore, perinephric, epididymal (female-perimetric) and mesenteric adipose tissue were counted as intra-abdominal adipose. Inter scapular brown adipose tissue was removed. The experimental protocol is shown in Fig. 1.

Statistical analysis. Values are given as the mean±SE. The data was analyzed by the one-way ANOVA test. The blood glucose data was analyzed by two-way (time×group) ANOVA, followed by a post hoc test. Males and females were analyzed separately.
RESULTS

DAWS

Weight gain and food consumption during gestation and lactation. Table 1 shows that food restriction caused lower body weight gain in pregnant rats in comparison to animals fed ad libitum. The unrestricted rats gained weight and reached approximately 350 g on day 18 of gestation. Food-restricted dams still gained some body weight during day 12–day 18 of gestation (Fig. 2). Mean food intake during gestation was 22.1 ± 0.35 g in the control group and 10.8 ± 0.14 g in the dietary restriction group. All dams lost weight during lactation; the weight change was not different among the groups.

Food consumption of dams in the dietary restriction groups was significantly higher compared to that of the control groups 1 d after birth. The higher food consumption of the dietary restriction group was sustained until the 10th day of lactation (Fig. 3).

Number of offspring. Figure 4a shows the number of offspring. Dietary restriction did not affect the number of pups.

Offspring

Litter sizes and body weights during lactation. Litter weight at delivery was affected by maternal dietary restriction. Figure 4c shows that, at day 5, the mean pup weight from the dietary restriction group was lower than the mean pup weight from the control group. Naso-tail length of 4-d-old pups in the dietary restriction group was significantly shorter in comparison to control rats (Fig. 4b). Pups of the dietary restriction group showed remarkable catch up for growth (Fig. 5).

Milk yield. Milk yields of the dietary restricted group were no different in comparison to the control group.
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during the 11–20th day. However, milk yields of the dietary restriction group tended to be lower in comparison to the control group during the 5–10th day (Fig. 6).

**Body weight and food consumption after weaning**

**Females and males.** At the time of weaning mean body weights in the dietary restriction group showed no difference in comparison to the control group for female pup rats. However, mean pup weight in the dietary restriction group was lower than the control group in male rats. Mean body weights of male rats in the dietary restriction group caught up with those of the control rats 1 wk after weaning. Thereafter, the mean body weight of male rats in the dietary restriction group did not overtake those of the control group. The rate of body weight gain for the dietary restriction group was significantly higher than that of the control group. Females in the dietary restriction group tended to grow slightly larger in comparison to those in the control group after the 280th day post-birth (Table 1). Food consumption of male rats in the dietary restricted group showed a tendency to be lower in comparison to that of the control group throughout the 280 d. Food consumption of female rats was similar to that of male rats. Data is not shown.

**Blood glucose response.** There were no significant differences in blood glucose concentrations after fasting or blood glucose concentrations after an oral glucose administration among the groups during the experimental period (Fig. 7).

**Adipose tissue weight.** After 280 d of the experimental period, subcutaneous, perinephric, mesenteric and epididymal (female-perimetric) adipose tissue weights were measured. As shown in Table 2, the mean weight of white adipose tissue increased with dietary restriction during gestation. Perinephric adipose tissue weights of the dietary restriction group were significantly higher than those of the control group for male and female rats. Subcutaneous adipose tissue weights of the dietary restricted group had a tendency to be higher than those of the control group for female rats. Intra-abdominal adipose tissue weights of the dietary restriction group were significantly higher than those of the control group in male rats. Intra-abdominal adipose tissue weights of the dietary restricted group had a tendency to be higher than those of the control group for female rats. Thereby, brown adipose tissue weights of the dietary restriction group were lower than those of the control group in female rats. However there were no

![Fig. 5. Body weight of pups (10 in a mass) during lactation. Values are means±SE. Control dams: n=4, DR dams: n=4.](image)

![Fig. 6. Milk yield of dams. Values are means±SE and have been given per 1.5 h. Control dams: n=4, DR dams: n=4.](image)

![Fig. 7. Effect of dietary restriction during gestation on blood glucose levels in males (D) and females (E) after glucose administration. Values are means±SD. Control male: n=5, Control female, DR male and DR female: n=4. The data was analyzed by two-way (time×group) ANOVA, followed by a post hoc test.](image)
differences between the control group and the dietary restriction group in male rats. Lean body mass of the dietary restricted group was significantly lower than those of the control group for male rats (Table 2).

**DISCUSSION**

Food consumption was significantly higher in the dietary restriction group in comparison to the control group 1 d after birth (at time of ad libitum feeding for the maternal animals). Furthermore, this increase in food consumption continued until the 10th day of nursing. This phenomenon was considered to be a rebound reaction to the dietary restriction.

The naso-tail length of 4-d-old pups was shorter in the dietary restriction group in comparison to the control rats. Figure 4 shows that the mean pup weight on the 5th day in the dietary restriction group was lower than that of the control group. Milk yields of dietary restricted group dams tended to be lower than those of the control group dams during the 5th day–10th day. Catch-up growth began when milk yields of dietary restriction group pups attained the same levels as those of control group pups. Pups in the dietary restriction group showed remarkable catch-up growth to the control group. Catch-up growth is associated with adulthood obesity in mice as well as in humans (14, 15). Yura et al. demonstrated that the onset of leptin surge is advanced in fetal under-nutrition offspring during the catch-up period and that this premature leptin surge contributes to conversion to an obesity-prone phenotype in fetal under-nutrition offspring. Furthermore, they suggest that the premature leptin surge alters energy regulation by the hypothalamus and contributes to "developmental origins of health and disease" (16–18). No significant differences were found between the two groups in body weight change during 280 d of gestation in either males or females. However, intra-abdominal adipose tissues, especially perinephric adipose tissues weights of the dietary restricted group, were significantly higher than those of the control group in male rats. Intra-abdominal adipose tissue weights of the dietary restricted group had a tendency to be higher than those of the control group for female rats. In addition, food consumption in pups of the dietary restriction dam group tended to be lower than that of the control group throughout the 280 d in both males and females. In other words, despite food consumption in pups from the dietary restriction dam group tending to be lower than those of the control group, body weight gain proportion and intra-abdominal adipose tissue were remarkably higher than those of the control group in male rats.

In male rats, the lean body mass of pups from the dietary restriction group was significantly lower than those of the control group. It is suggested that factors for an increase of white adipose tissue include intra-abdominal adipose tissue, causes a decrease in basal metabolism. The lean body mass of the dietary restricted group had a tendency to be lower than that of the control group for female rats. Moreover the weight of brown adipose tissue (BAT) of pups from the dietary restriction group was lower than that of the control group in female rats. In male rats, an increase in intra-abdominal adipose tissue is considered to be related to the lean body mass but it is not related to the function of BAT. In female rats, it is thought that the increase in intra-abdominal adipose tissue is related to the function of BAT and lean body mass.

It is suggested that factors for an increase of white adipose tissue differ between males and females.

Differences between males and females were observed in the weights at the time of weaning. The mean male pup weight in the dietary restriction group was lower than that of the control group. At the time of weaning the mean pup weight in the dietary restriction group was no different from that of the control group for female rats. It has been understood that dietary restrictions influence males more than females up until the weaning period. However, we did not select for gen-

### Table 2. White and brown adipose tissue weight of pups at the 280th day.

<table>
<thead>
<tr>
<th></th>
<th>Subcutaneous</th>
<th>Perirenal</th>
<th>Epididymal</th>
<th>Mesenteric</th>
<th>Intra-abdominal</th>
<th>BAT</th>
<th>Mean pup weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>n=5</td>
<td>10.66±1.08</td>
<td>3.99±0.39</td>
<td>3.12±0.24</td>
<td>0.52±0.04</td>
<td>7.63±0.42</td>
<td>0.11±0.028</td>
</tr>
<tr>
<td>Dietary restriction</td>
<td>n=5</td>
<td>11.74±0.79</td>
<td>5.67±0.36#</td>
<td>3.28±0.20</td>
<td>0.54±0.08</td>
<td>9.71±0.52#</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>n=6</td>
<td>7.01±1.46</td>
<td>3.79±0.20</td>
<td>4.21±0.36</td>
<td>0.57±0.08</td>
<td>8.57±0.56</td>
<td>0.20±0.036</td>
</tr>
<tr>
<td>Dietary restriction</td>
<td>n=6</td>
<td>11.38±1.84</td>
<td>5.19±0.44#</td>
<td>4.61±0.39</td>
<td>0.46±0.04</td>
<td>10.53±0.58</td>
<td>0.08±0.016#</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those for the controls; #p<0.05.

Adipose tissue weight, Lean body mass: g/100 g BW. Mean±SE.
under at birth. In future it is necessary to investigate the different responses to food restriction between males and females.

We investigated blood glucose tolerance at 60 d after birth. Fasting blood glucose concentrations were not significantly different between the groups during the experimental period. Blood glucose tolerance in both males and females amongst groups showed no differences 60 d after birth.

Exposure to under nutrition during middle or late-stage gestation was associated with an increase in obesity and glucose intolerance (12, 13). Dietary restriction throughout all periods of pregnancy includes not only the middle and late period but also the early period. Therefore, it is thought that the dietary restriction in the middle period and late period and the influences of dietary restriction throughout all periods of pregnancy are different.

It would be desirable to confirm changes in serum insulin level. Dietary restriction during gestation did not affect blood glucose tolerance.

An increase in the weight of adipose tissue is a major risk factor for metabolic syndrome.

Therefore severe dietary restriction in the gestation period is a very important issue for the prevention of metabolic syndrome. The mechanism by which severe dietary restriction during gestation causes obesity in pups is a challenge for the future.

REFERENCES