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High-Dose Perinatal Folic-Acid Supplementation Alters Insulin Sensitivity in Sprague-Dawley Rats and Diminishes the Expression of Adiponectin

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ABSTRACT
The possible intake of folate in excess of the recommended upper levels is a matter of critical importance. This study was conducted to investigate the effects of prenatal and postnatal high folic acid supplementation (FAS) on glucose tolerance, insulin sensitivity, lipid metabolism, and expression of adiponectin in rats. The study included 20 female rats divided into two groups: control group and FAS group (receiving high folic acid supplemented diet). Both groups of female rats were mated and pregnancy confirmed. At parturition, the diet of 5 dams that were fed with control diet during gestation and their litters was changed to FAS diet and continued throughout lactation. Similarly, half of the dams that were previously fed with FAS diet during gestation and their litters were also changed to control diet. The remaining 5 dams in each group continued on their respective diets throughout lactation with their litters. Other dams remained on their respective diets throughout lactation. Food and water intake, body weight, lipid concentrations, insulin, and the expression of adiponectin were determined. Glucose tolerance and insulin sensitivity were also measured to evaluate glucose homeostasis. FAS significantly increased the postweaning food, water intake, triglyceride, and insulin levels but diminished insulin sensitivity in adult offspring. The expression of adiponectin in insulin-sensitive tissues was also significantly decreased and these were consistent with insulin resistance of FAS offspring. High-dose FAS may promote insulin resistance and dyslipidemia and disrupt glucose metabolism possibly by depressing adiponectin expression. Although this is an animal model and the effects of the diets cannot be directly transposed to humans, this study provides indications of the possible adverse effects of FAS maternal diet on glucose metabolism in the offspring.

KEYWORDS
Adiponectin; folate; glucose tolerance; insulin resistance; postnatal; prenatal

Introduction
The developmental origin of health and disease (DOHaD) hypothesis proposes that exposures during intrauterine and/or early life determine the risk of developing diseases in adulthood.
For example, there is consistent evidence for an association between low body weight (LBW) and insulin resistance, type 2 diabetes mellitus (T2DM), and coronary heart disease (CHD) (Newsome et al., 2003). The underlying reason behind this developmental plasticity can be explained by the “predictive adaptive response” hypothesis, which suggests that mismatch between in-utero (and early) life environmental exposures and the environment encountered postnatally (and in adult life) predisposes the offspring to increasing susceptibility of diseases (Gluckman and Hanson, 2004, 2005).

Much epidemiological and experimental evidence in support of this hypothesis indicates that altered nutritional environment during fetal and neonatal development leads to detrimental effects in offspring health later in life (Zambrano et al., 2005; Singhal and Lucas 2004). A sizeable number of experimental studies explored the impacts of micronutrient restriction on fetal origin of adult disease (Christian and Stewart 2010). In rats, prenatal and perinatal zinc restriction has been shown to result in impaired glucose-induced insulin secretion (Pamavathi et al., 2009) in adulthood. In other studies, maternal dietary restriction in zinc, calcium, and magnesium, individually or in combination, was found to result in varying degree of insulin resistance (Venu et al., 2008).

Evidence that maternal folate intake during pregnancy affects metabolic health of offspring abounds in literature, mainly regarding autism, asthma, insulin resistance, and cancer (Budge and Lillycrop, 2012; Castro et al., 2016). Stewart et al. (2009) reported that gestational folic acid supplementation lowers the risk of metabolic syndrome development in children. Conversely, a study in India found higher maternal erythrocyte concentration during pregnancy to be associated with higher offspring adiposity and insulin resistance (Yajnik et al., 2008).

Given the global reach of folate supplementation to women during pregnancy and the first 3 months postpartum, as recommended by WHO, the possible intake in excess of the recommended upper levels is a matter of critical importance, as has been recently expressed by several researchers (Bailey et al., 2010; Hoyo et al., 2011; Gomez et al., 2015). Therefore, this study was designed to investigate the effects of high folic acid supplementation (FAS) in early life on offspring health. We examined the offspring of female rats exposed to high folate intake during pregnancy and/or lactation to determine the effects on body weight, food intake, glucose tolerance, insulin sensitivity, lipid metabolism, and expression of adiponectin in the offspring.

**Materials and methods**

**Animals, diet, and experimental design**

All experimental procedures were authorized by the institutional ethics committee and were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care & Use of Laboratory Animals, 2011). Twenty female Sprague-Dawley rats (140–160 g) and 10 males (160–180 g) were selected for this experiment. All animals received the standard rat diet two weeks before the beginning of the study for adaptation and were housed at 21°C–23°C with controlled humidity (50% ± 5%) under a 12 h light-dark cycle. After the adaptation period and before breeding, the female rats were randomly assigned to different dietary groups (n = 10 in each group) and fed with a diet containing either 2 mg or 5 mg folic acid. The AIN-93G control diet containing 2 mg folic acid/kg served as the control.
### Table 1. Composition of formulated control and FAS diets.

<table>
<thead>
<tr>
<th>Diet Component</th>
<th>Control</th>
<th>FAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>530</td>
<td>530</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soya bean</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TBHQ</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Fibre</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Fat (%Kcal)</td>
<td>17.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Carbohydrate (%Kcal)</td>
<td>63.9</td>
<td>63.9</td>
</tr>
<tr>
<td>Protein (%Kcal)</td>
<td>18.8</td>
<td>18.8</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>3840</td>
<td>3840</td>
</tr>
</tbody>
</table>

FAS = folic acid supplementation; TBHQ = tertiary butylhydroquinone.

The diet containing 5 mg folic acid/kg (modified AIN-93G) was considered the folic acid supplemented (FAS) group diet (compositions of the two diets are shown in Table 1). This dose level of folic acid in the FAS group is 2.5 times the normal requirement for rodents and is the equivalent of the tolerable upper intake level in humans (Huang et al., 2014). For breeding, two female rats were allowed to mate with one fertile, sexually active male rat per night. The next morning, the vaginal plugs were collected from the females. A vaginal smear was prepared and examined under the microscope for the presence of sperm. Presence of sperm served as the confirmation of pregnancy; as such, that day was recorded as “day zero” of pregnancy. At parturition, the diet of 5 dams and their litters (CF) from the control group was changed to FAS diet and continued throughout lactation; similarly, 5 dams and their litters (FC) from the FAS group changed to the control diet. The remaining dams and their litters (CC and FF) remained on their respective diets during lactation (see Figure 1 for schematic illustration of study design). Offspring were reduced to four pups per dam, nursed by birth mothers, and weaned on day 21, when they were separated according to sex. Subsequently, all female weanlings were removed to avoid potential variable nature of female data caused by hormonal fluctuations associated with the female reproductive cycle, while the male weanlings were retained, with a maximum of six rats per cage.

**Body weight, food and water intake**

The body weight, and food and water ingestion of the dams were measured daily from the beginning of the study till parturition and weekly thereafter. For the pups (both males and

![Figure 1. Schematic illustration of the study design. FAS = folic acid supplementation.](image-url)
females), body weight was measured weekly, while feeding and drinking were determined daily from birth till the end of the study. Food and water intake are reported as the average daily intake over a week.

**Glucose and insulin tolerance tests**

After eight weeks of diet exposure, the offspring were subjected to an oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) using standard methods as previously described (Morakinyo et al., 2016). Briefly, for OGTT, these rats \( (n = 6) \) were fasted overnight and then challenged with 2 g/kg D glucose (Sigma-Aldrich, St. Louis, MO, USA), followed by serial assessment of blood glucose from tail blood samples measured at 0, 30, 60, 120, and 180 min using a blood glucose level monitor (Accu Chek glucometer, Roche Diagnostics, Germany). For ITT, rats \( (n = 6) \) that were fasted for 4 hr were injected intraperitoneally (i.p.) with regular human insulin (Humulin, 0.75 U/kg body weight). Blood glucose concentrations were monitored before (0 min) and 15, 30, 60, 90, and 120 min after insulin injection. The area under the curve (AUC) for the blood glucose–time function (AUC-glucose) for both curves was calculated by the trapezoid rule (GraphPad Prism software).

**Lipid profile and insulin assay**

Trunk blood was collected into dry tubes immediately after rapid decapitation. Following centrifugation at 4°C, the resulting serum was separated and stored at –80°C for later analysis. Serum concentrations of triglyceride (TRIG), total cholesterol (CHOL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined with an automatic blood chemical analyzer (BT2000 Plus, Germany). Insulin was assayed using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Co., Wuhan, China) per the manufacturer’s instruction. All the reagents and samples were brought to room temperature before conducting the experiment.

**Quantification of adiponectin level**

The adipose tissue (AT) and gastrocnemius muscle (GM) were dissected and homogenized in 9 volumes of ice cold 0.1 mM phosphate buffer saline (pH 7.4) to prepare 10% homogenate. The homogenate was then centrifuged for 5 min at 5,000 \( \times \) g to get the supernatant used for the measurement. AT and GM homogenate were used for the determination of adiponectin levels. This was determined using enzyme immunoassay (EIA) kit (Elabscience Biotechnology Co., Wuhan, China). The procedure specified in the manufacturer’s manual for the kits was followed. A 96-well microtiter plate was used to conduct the analysis.

**Statistical analysis**

Data are expressed as the mean ± standard error of the mean (SEM). Data were analyzed using one-way ANOVA followed by Tukey’s honest significant difference (HSD) test except for litter size, birth weight, male-to-female ratio, and maternal body weight, where Student’s \( t \) test was employed. All analyses were done using GraphPad Prism software version 6.00 for
Table 2. Morphometric data and pregnancy outcome.

<table>
<thead>
<tr>
<th></th>
<th>Litter size</th>
<th>Birth weight (g)</th>
<th>Male-to-female ratio</th>
<th>Maternal body weight (g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.2 ± 0.6</td>
<td>5.13 ± 0.47</td>
<td>0.72 ± 0.18</td>
<td>369.7 ± 10.2</td>
</tr>
<tr>
<td>FAS</td>
<td>11.5 ± 0.9</td>
<td>6.24 ± 0.53</td>
<td>0.80 ± 0.11</td>
<td>381.4 ± 11.6</td>
</tr>
<tr>
<td>*p value</td>
<td>.2571</td>
<td>.2186</td>
<td>.7124</td>
<td>.4663</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard error of the mean (SEM) (n = 6).
* Measured 3–4 days before delivery.

Results

Morphometric data

As shown in Table 2, the differences in litter size, body weight, male-to-female ratio, and maternal body weight observed between the two groups were not statistically significant even though these morphometric measures tend to be higher in the FAS group.

Feed and water intake

Postnatal feed intake was monitored in the offspring as shown in Table 3. Starting from postnatal week 4, the quantity of feed consumed by CF rats was significantly (p ≤ .05) higher than that of the CC throughout the 7-week period. Except for FC rats at weeks 8 and 10, as well as FF rats at weeks 7 and 10, the quantity of feed consumed by each of these groups was significantly higher than that of CC rats during the period. In comparison with CF rats, there was a significant (p ≤ .05) decrease in feed intake at weeks 6, 7, and 10 in the FC rats, while only the week 4 value was significantly (p ≤ .05) higher. The trend was the same for FF rats with lower (though not statistically significant [p ≥ .05]) feed intake at weeks 6, 7, 8, and 10 compared with CF rats, while only weeks 4 and 9 were significantly (p ≤ .05) higher. Interestingly, in all groups of rats, feed intake per 100 g body weight (relative feed intake) from week 4 to week 10 was significantly higher compared with CC. The relative feed intake of FC rats was significantly (p ≤ .05) higher compared with CF rats apart from week 7. The water intake was generally increased in all FAS rats (CF, FC, and FF) when compared to CC rats from week 4 to week 10 despite some inconsistencies as shown in Table 4. The data showed continuous dietary FAS exposure in FF rats produced the highest water intake among the experimental rats.

Postnatal body weight

Figure 2 shows the impact of prenatal FAS on body weight in the experimental rats. In comparison with CC rats, the increase in postnatal body weight of FC rats was significantly higher at weeks 1, 2, and 3. CF offspring, however, showed a significantly higher (p ≤ .05) body weight compared with the CC offspring from week 4 to week 10 of postnatal life. In addition, the data showed a significant (p ≤ .05) increase in the body weight of FF offspring as compared with CC throughout the 10-week period.
### Table 3. Absolute feed intake (per day per group) in the offspring after weaning.

<table>
<thead>
<tr>
<th>Week 4 (g)</th>
<th>Week 5 (g)</th>
<th>Week 6 (g)</th>
<th>Week 7 (g)</th>
<th>Week 8 (g)</th>
<th>Week 9 (g)</th>
<th>Week 10 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC 22.2 ± 1.5 (9.0 ± 0.35)</td>
<td>27.4 ± 3.7 (9.08 ± 1.09)</td>
<td>41.5 ± 5.7 (9.65 ± 0.81)</td>
<td>41.7 ± 6.5 (7.71 ± 0.7)</td>
<td>61.0 ± 3.4 (7.81 ± 0.66)</td>
<td>82.1 ± 2.2 (9.28 ± 0.31)</td>
<td>104.1 ± 6.9 (10.82 ± 0.31)</td>
</tr>
<tr>
<td>CF 39.8 ± 1.8 (12.48 ± 1.26)</td>
<td>59.8 ± 4.3 (15.65 ± 1.81)</td>
<td>69.4 ± 4.3 (13.53 ± 1.54)</td>
<td>104.7 ± 8.2 (12.35 ± 1.49)</td>
<td>115.8 ± 5.9 (10.76 ± 1.02)</td>
<td>113.7 ± 6.4 (9.80 ± 0.87)</td>
<td>118.6 ± 12.8 (9.22 ± 0.92)</td>
</tr>
<tr>
<td>FC 101.1 ± 2.0 (35.53 ± 3.11)</td>
<td>114.8 ± 3.1 (33.53 ± 3.54)</td>
<td>88.3 ± 4.9 (39.55 ± 2.34)</td>
<td>93.1 ± 4.1 (41.41 ± 1.72)</td>
<td>155.4 ± 4.9 (18.9 ± 2.81)</td>
<td>141.7 ± 3.9 (15.33 ± 2.05)</td>
<td>127.1 ± 5.8 (11.18 ± 1.42)</td>
</tr>
<tr>
<td>FF 113.7 ± 2.5 (30.06 ± 2.78)</td>
<td>103.4 ± 5.0 (23.1 ± 2.33)</td>
<td>78.8 ± 3.3 (13.58 ± 1.88)</td>
<td>106.3 ± 4.7 (11.11 ± 1.6)</td>
<td>153.7 ± 7.4 (13.2 ± 1.78)</td>
<td>155.8 ± 6.4 (12.33 ± 1.05)</td>
<td>138.8 ± 8.2 (9.85 ± 1.42)</td>
</tr>
</tbody>
</table>

Relative feed intake (per 100 g body weight) shown in parentheses. Values expressed as mean ± standard error of the mean (SEM) (n = 6). CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate. a*p ≤ .05, b*p ≤ .01, c*p ≤ .001 versus CC; d*p ≤ .001 versus CF; *p ≤ .05, ‡p ≤ .01, §p ≤ .001 versus FC.
Table 4. Water intake in the offspring.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 4 (ml)</th>
<th>Week 5 (ml)</th>
<th>Week 6 (ml)</th>
<th>Week 7 (ml)</th>
<th>Week 8 (ml)</th>
<th>Week 9 (ml)</th>
<th>Week 10 (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>8.15 ± 2.34</td>
<td>10.50 ± 2.11</td>
<td>15.84 ± 0.91</td>
<td>14.68 ± 2.44</td>
<td>18.04 ± 2.14</td>
<td>16.89 ± 1.78</td>
<td>10.78 ± 1.93</td>
</tr>
<tr>
<td>CF</td>
<td>13.83 ± 1.22</td>
<td>16.19 ± 3.48</td>
<td>22.46 ± 1.53</td>
<td>27.03 ± 3.33</td>
<td>21.90 ± 2.42</td>
<td>24.71 ± 2.21</td>
<td>16.65 ± 4.91</td>
</tr>
<tr>
<td>FC</td>
<td>14.03 ± 2.12</td>
<td>18.71 ± 2.03</td>
<td>19.29 ± 2.79</td>
<td>19.83 ± 3.02</td>
<td>28.43 ± 5.66</td>
<td>30.00 ± 3.80</td>
<td>17.5 ± 2.63</td>
</tr>
<tr>
<td>FF</td>
<td>14.27 ± 1.96</td>
<td>20.10 ± 4.53</td>
<td>23.16 ± 1.58</td>
<td>23.37 ± 1.72</td>
<td>23.87 ± 3.49</td>
<td>39.59 ± 5.99</td>
<td>29.65 ± 6.15</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard error of the mean (SEM) (n = 6). CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate.

Glucose tolerance

Oral glucose tolerance at postnatal age 10 weeks in the offspring is shown in Figure 3. The CF offspring had lower baseline fasting glucose values (2.6 ± 0.21 versus 4.2 ± 0.17 mmol/L, p ≤ .05) compared with CC. However, the postloading blood glucose responses of CF, FC, and FF offspring as demonstrated by the AUC values (897 ± 58.3, 985.5 ± 89.7, 987 ± 102.6 versus 924 ± 72.1 mmol/L × 120 min, p ≥ .05) were not significantly different compared with the CC group.

Figure 2. Postnatal body weight of the offspring. Values expressed as mean ± standard error of the mean (SEM) (n = 6). b p ≤ .01, c p ≤ .001 versus CC; d p ≤ .05, e p ≤ .01, f p ≤ .001 versus CF. CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate.

Figure 3. Glucose tolerance response and the related AUC in rat offspring. Data expressed as mean ± standard error of the mean (SEM). AUC = area under the curve; CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate.
Table 5. Lipid variables in the offspring.

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>FC</th>
<th>CF</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>2.23 ± 0.18</td>
<td>2.1 ± 0.20</td>
<td>2.28 ± 0.17</td>
<td>2.15 ± 0.19</td>
</tr>
<tr>
<td>TRIG</td>
<td>0.73 ± 0.04</td>
<td>1.30 ± 0.06</td>
<td>1.30 ± 0.05</td>
<td>1.38 ± 0.06</td>
</tr>
<tr>
<td>LDL</td>
<td>0.90 ± 0.16</td>
<td>0.43 ± 0.13</td>
<td>0.74 ± 0.06</td>
<td>0.43 ± 0.10</td>
</tr>
<tr>
<td>HDL</td>
<td>0.98 ± 0.06</td>
<td>1.08 ± 0.14</td>
<td>0.94 ± 0.09</td>
<td>1.07 ± 0.11</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard error of the mean (SEM) (n = 6). Unit of all measurements is mmol/l. CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate; CHOL = cholesterol; TRIG = triglyceride; LDL = low-density lipoprotein; HDL = high-density lipoprotein. *p ≤ .05, †p ≤ .001 versus CC.

**Insulin sensitivity**

FAS affected post–insulin loading glucose response in the 10-week-old offspring. The CF, FC, and FF offspring all had significantly higher blood glucose levels at the 180 min timepoint after insulin loading. The postloading blood glucose responses to insulin in CF, FC, and FF offspring as demonstrated by the AUC values (781 ± 26.6, 785.3 ± 27.5, 794.3 ± 23.0 versus 627 ± 25.1 mmol/L × 180 min, p ≤ .05) were significantly higher compared with CC (Figure 4).

**Lipid variables**

Table 5 shows the effect of FAS on the serum lipid profile of offspring. Plasma triglyceride and LDL levels but not CHOL and HDL were significantly (p ≤ .05) increased in CF and FF offspring when compared with the control. There was no difference in cholesterol levels when compared with control.

**Adiponectin and insulin level**

Adiponectin in the adipose tissue (AT) and gastrocnemius muscle (GM) of animals was quantified. There was a significant (p ≤ .05) decrease in adiponectin levels of AT and GM samples in the CF and FC groups when compared with the CC group. However, there was no difference in the adiponectin level of the FF group in both samples when compared with the CC group (Figure 5a). Serum insulin levels were significantly elevated in FC and FF offspring when compared with control offspring. The insulin level in CF, however, was comparable to that in CC offspring and significantly (p ≥ .05) lower than in both FC and FF offspring (Figure 5b).

![Figure 4](image-url)  
**Figure 4.** Insulin sensitivity response and the related AUC in rat offspring. Data expressed as mean ± standard error of the mean (SEM). AUC = area under the curve; CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate.
Figure 5. (A) Adiponectin level in the adipose tissue and skeletal muscle and (B) serum insulin in rat offspring. Data expressed as mean ± (SEM). CC = control-control; CF = control-folate; FC = folate control; FF = folate-folate. b*p = 0.01, c*p = 0.001 vs CC; 1p = 0.05, 3p = 0.001 vs CF; *p = 0.05 vs FC.

Discussion

In this study, we assessed the effect of prenatal and/or postnatal folate supplementation on glucose tolerance, insulin sensitivity, lipid metabolism, and adiponectin level in the offspring. Our findings reveal that gestational, lactational, and postweaning folic acid supplementation reduce the availability of adiponectin as well as promote insulin resistance in the offspring in their adult life. In addition, insulin level was elevated but glucose tolerance was unaffected and comparable to FAS offspring compared to the control diet–fed rats. The present study, therefore, showed that prenatal and postnatal FAS may alter glucose metabolism in offspring at adolescence by modulating the expression of adiponectin.

Our data show that the litter size, birth weight, male-to-female ratio, and maternal body weight of FAS rats were comparable with control rats. An earlier report suggested that maternal consumption of low folic acid and folate deficiency status are associated with adverse pregnancy outcomes such as low birth weight and stillbirth (Molloy et al., 2008). Nonetheless, we observed a slightly increased birth weight in FAS rats even though it was not statistically different from the control. This is consistent with previous studies with similar morphometric indices except for a lower female body weight in those studies (Keating et al., 2015).

The postweaning water and food intakes of FAS rats in the present study were increased. FAS offspring also showed higher food intake per 100 g body weight. Gestational consumption of high folate increased body weight and food intake in the offspring (Cho et al., 2013). This enhanced food intake would lead to increased caloric intake and ultimately promote weight gain. Alternatively, the increased food intake and body weight may be associated with improved appetite. Namdari et al. (2014) reported a positive association between folate level and increased appetite. Therefore, it is not surprising that we recorded a noticeable increase in the body weight of offspring that were exposed to perinatal FAS. Given that the body weight was notably higher than in control rats even after the animals had been switched to the control diet for 10 weeks suggests that gestational FAS modifies feeding behavior in adult life and engenders weight gain. Our data are consistent with previous work in which the capacity of a folate-rich diet to promote weight gain was reported in young rats fed 5 mg/kg folic acid (Burdge et al., 2009). This finding, therefore, suggests that a high folic acid diet programs the offspring to consume increased amounts of food and stimulates weight gain. Whether the alteration of feeding behavior and weight gain by high FAS diet can precipitate obesity is unknown and deserves further attention.
Next, we investigated whether FAS modulated glucose metabolism by measuring glucose response to glucose and/or insulin load. Our data showed that although FAS impaired insulin sensitivity in the offspring, glucose tolerance remained unaffected. Since insulin is critically required for maintaining glucose homeostasis, we measured plasma insulin level in the fasting state. We recorded an elevated insulin level in FAS offspring. Insulin as a key regulator of carbohydrate metabolism stimulates the uptake of glucose into insulin-sensitive tissues such as fat, liver, and skeletal muscles (Wei et al., 2013) and thus serves as the primary regulator of glucose concentration (Saltiel and Kahn, 2001). Therefore, concomitant hyperinsulinemia and deficient glucose clearance after insulin challenge in FAS rats is indicative of poor insulin sensitivity and glucose regulation. Insulin resistance exists when insulin is unable to promote glucose uptake and inhibit hepatic glucose production.

Several pathways contribute to the etiology of insulin resistance at the cellular level, including defective insulin signal transduction, impaired effector molecules within insulin-dependent pathways, and impaired insulin-sensitizing pathways (Boucher et al., 2014). Considering that adiponectin is a widely recognized insulin sensitizer with several identified approaches and insulin-sensitizing activities (Berg et al., 2001; Tomas et al., 2002; Yamauchi et al., 2002), we decided to investigate the availability of this antidiabetic adipokine in insulin-stimulated tissues of FAS offspring. Supplementation with FAS was found to cause decreased expression of adiponectin in adipose tissue and gastrocnemius muscle; this result is consistent with a previous report (Huang et al., 2014) in mice. Adiponectin is known to increase glucose uptake in the skeletal muscles of rats (Ceddia et al., 2005) and a low level of this insulin-sensitizing adipokine has been shown to exacerbate insulin resistance in mice fed a high-fat diet (Kubota et al., 2002; Nawrocki et al., 2006). Thus, reduced expression of adiponectin may contribute to the impaired insulin sensitivity and deranged glucose metabolism in FAS offspring.

Dyslipidemia is a major component of metabolic disorder and a strong risk factor for insulin resistance. Lipid profile analysis in this study showed that TRIG level was remarkably higher in FAS offspring compared to controls. Elevated TRIG level is linked to insulin resistance; similarly, reduction of adiponectin has been associated with insulin resistance (Yamauchi et al., 2002; Yoon et al., 2006). Previous studies have shown that adiponectin increases fatty acid oxidation and therefore improves insulin sensitivity. Thus, it is plausible to suggest that the mechanism linking FAS to insulin resistance may involve adiponectin-mediated dyslipidemia.

In conclusion, our findings suggest that a perinatal high-dose FAS diet promotes insulin resistance and dyslipidemia and disrupts glucose metabolism through the depression of adiponectin. Although this is an animal model and the effects of the diets cannot be directly transposed to humans, this study provides indications of the possible adverse effects of a high FAS maternal diet on glucose metabolism in offspring during adult life. Caution should be taken to avoid excessive intake of folic acid in the maternal diet during gestation and lactation as folate intake above the approved limit may occur from unregulated availability of multivitamin supplements and increasing doses of vitamins, particularly folate, being recommended for pregnant women.

Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.
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