Impaired intestinal active calcium absorption and reduction of serum 1α, 25(OH)₂D₃ in streptozotocin-induced diabetic pregnant rats with hypocalcemia in their fetuses

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Summary
Purpose: The effects of maternal diabetes mellitus on fetal hypocalcemia were studied in streptozotocin-induced diabetic rats. Materials and Methods: Experiments were performed using Wistar rats rendered diabetic with streptozotocin. Treatment with insulin was started in six diabetic pregnant rats after diabetes induction. On day 21 of gestation, cesarean section was performed and serum ionized calcium, parathyroid hormone and 1α, 25-dihydroxyvitamin D₃ were measured and the active intestinal calcium absorption was measured in mother rats using the everted gut sac technique.

Results: In untreated diabetic pregnant rats, serum 1α, 25-dihydroxyvitamin D₃ and the active intestinal calcium absorption were significantly decreased, and the placental calcium transfer was disturbed compared with the control and insulin-treated groups. Furthermore, serum ionized calcium levels were markedly reduced in fetuses from untreated diabetic pregnant rats. However, these abnormalities of calcium metabolism in untreated diabetic rats could be corrected by treatment with insulin.

Conclusions: These data indicate that diabetes mellitus in pregnant rats a contribute to negative calcium homeostasis which is probably related to the development of fetal hypocalcemia.

Key words: Calcium; Diabetes mellitus; Duodenum; Everted gut sac technique; Intestine; 1α, 25-dihydroxyvitamin D₃; Parathyroid hormone; pregnancy; Rat; Streptozotocin.

Introduction
Infants of diabetic mothers have an increased incidence of neonatal hypocalcemia [1]. In humans, serum ionized calcium and parathyroid hormone (PTH) levels were found to be lower in the umbilical arterial blood of infants from diabetic mothers than in controls [2]. However, the precise pathogenesis of neonatal hypocalcemia remains uncertain at present. Multifactors are likely to be involved including altered maternal calcium metabolism, abnormalities of fetal calcitropic hormones, disturbance of placental calcium transport and fetal delayed bone maturation [3]. The aim of the present study was to determine whether some alterations in calcium metabolism may influence the development of fetal hypocalcemia in utero in streptozotocin-induced diabetic pregnant rats.

Materials and Methods
Experiments were performed using female Wistar rats weighing 150-160 grams. Rats were fed a high calcium-phosphorus diet containing 1.10% calcium, 0.83% phosphate and vitamin D₃, 80IU/100 g and deionized water throughout the study. Rats were mated with male Wistar rats and the day a vaginal copulation plug was found was designated day 0 of gestation (Wistar rats usually deliver on days 20/21). On day 0, rats were rendered diabetic with streptozotocin (50 mg/kg, iv) dissolved in 200 μl of citrate buffer and diabetes was confirmed by the development of hyperglycemia. Six diabetic rats were injected with six international units of NPH porcine insulin dissolved in 200 μl of citrate buffer subcutaneously every day from day 3 and other diabetic rats received citrate buffer alone. Blood sugar levels were measured on days 7, 14 and 20, respectively, and pregnant rats were divided into three groups; [1] a group of nine nondiabetic control pregnant rats, [2] a group of five untreated diabetic pregnant rats with mean blood sugar levels > 300 mg/dl and [3] a group of six insulin-treated diabetic pregnant rats.

On day 21, cesarean section was performed under pentobarbital anesthesia and fetuses were weighed and blood was collected by decapitation. Maternal blood was sampled from the abdominal aorta and serum was stored at −20 °C. At the same time, the duodenum was removed and active calcium transport was measured using the everted gut sac technique [4]. One end of a resected 6 cm long proximal duodenum was ligated and 30 mM Tris HCl (pH = 7.4) containing 125 mM NaCl, 10 mM fructose, 0.25 mM CaCl₂, 20,000 cpm/ml was introduced into the everted sac. This end was then ligated and the sac was placed in a flask. The flask was placed in an incubator, shaken, and the content of the flask was aerated continuously with 95% O₂ and 5% CO₂ at 37 °C for 90 minutes. Following incubation, 50 μl of both mucosal and serosal fluid was recovered and the ratio of ⁶⁷Ca radioactivity was measured using liquid scintillation counter (Packard Corp., Tri-Carb). Active calcium transport (S/M ratio) was expressed as the ratio of ⁶⁷Ca radioactivity inside the everted sac (serosal) to the ⁶⁷Ca radioactivity outside the sac (mucosal).

Serum samples were analysed for ionized calcium and calcitropic hormones. Ionized calcium was measured using a calcium flow-through electrode (Cera 250, Horiba Corp.) and parathyroid hormone was determined by a radioimmunoassay
Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic control pregnant rats (n = 9)</th>
<th>Untreated diabetic pregnant rats (n = 5)</th>
<th>Insulin-treated diabetic pregnant rats (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionized calcium (mmol/l)</td>
<td>1.29±0.05</td>
<td>1.20±0.11</td>
<td>1.28±0.04</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/l)</td>
<td>66.7±28.0</td>
<td>87.9±18.2</td>
<td>60.0±19.7</td>
</tr>
<tr>
<td>1, 25 (OH)₂D₃ (pg/ml)</td>
<td>66.7±11.7</td>
<td>27.5±5.0</td>
<td>63.3±7.5</td>
</tr>
<tr>
<td>S/M ratio</td>
<td>1.60±0.43</td>
<td>1.17±0.28</td>
<td>1.53±0.50</td>
</tr>
</tbody>
</table>

Material mean blood sugar, serum ionized calcium, parathyroid hormone, 1, 25 (OH)₂D₃, concentrations are significantly lower in fetuses from diabetic mothers than in those from the other two groups (Table 2). However, serum PTH levels were not significantly different among all groups (Table 2).

On the other hand, insulin treatment reversed decreased serum ionized calcium and 1, 25 (OH)₂D₃, levels and impaired active intestinal calcium absorption seen in diabetic pregnant rats toward the levels of the control rats. Moreover, insulin treatment recovered the fetal birth weight and serum ionized calcium levels in fetuses from diabetic mothers.

Discussion

We have previously reported that the increase in serum 1, 25 (OH)₂D₃, levels and the enhancement of the intestinal calcium absorption during pregnancy are adaptive changes on the maternal side in order to maintain maternal calcium homeostasis and supply calcium for the fetus in humans and rats [5, 6].

In this study, we investigated the influence of the altered calcium metabolism in streptozotocin-induced diabetic pregnant rats on their fetal calcium balance.

From experimental series in the present study, calcium metabolism in diabetic pregnant rats was characterized by the marked reduction of serum 1, 25 (OH)₂D₃, concentrations, the reduced active intestinal calcium absorption and the fetal hypocalcemia. In addition, insulin therapy further increased maternal and fetal levels of serum ionized calcium, maternal serum 1, 25 (OH)₂D₃, concentrations, active intestinal calcium absorption and fetal body weight toward the levels of the controls.

1, 25 (OH)₂D₃, is an active metabolite of vitamin D₃, which promotes active calcium absorption from the intestines.

Low concentrations of serum 1, 25 (OH)₂D₃, have been reported in nonpregnant diabetic rats [7-9] as an effect of diabetes on vitamin D₃ metabolism. The main hypothesis for the reduction of serum 1, 25 (OH)₂D₃, in untreated diabetic pregnant rats involves an inhibitory effect of insulin deficiency on renal 25-hydroxyvitamin D₃, 1α-hydroxylase. Indeed, insulin permits PTH stimulation of 1, 25 (OH)₂D₃ production in cultured kidney cells [10].

Furthermore, 1α-hydroxylase has been shown in the placental tissue of the rat during pregnancy [11]. An alternative hypothesis is a disturbance in the placental synthesis of this metabolite due to the functional immaturity of the placenta in diabetic rats. However, a recent study demonstrated that low concentrations of 1, 25 (OH)₂D₃ in diabetic rats were associated with reduced vitamin D binding protein and that 1, 25 (OH)₂D₃, levels were not decreased [8].

In addition, Verheghe et al. [12] also showed that maternal free 1, 25 (OH)₂D₃, levels in diabetic rats were

Kit that measured rat PTH (44-68) (INC Corp.). Serum 1, 25-dihydroxyvitamin D₃, (1, 25 (OH)₂D₃) was measured by a radioreceptor assay using a Yamasa 1, 25 (OH)₂D₃, receptor after HPLC purification.

Data were presented as mean ± SD and the student’s t-test was used to quantify the levels of significance between the control group, the untreated diabetic group and the insulin-treated diabetic group.

Results

The untreated diabetic pregnant rats were hyperglycemic compared with the nondiabetic control pregnant rats (98.2±14.5 mg/dl) (Table 1). Mean blood glucose levels in the insulin-treated diabetic pregnant rats (172.2±26.0 mg/dl) were lower than in diabetic pregnant rats (369.1±9.0 mg/dl).

Serum ionized calcium and PTH concentrations were not significantly different among all groups (Table 1). However, the diabetic pregnant rats tended to have lower ionized calcium levels and higher PTH levels than the other two groups (Table 1). Serum 1, 25 (OH)₂D₃, concentrations were significantly lower in diabetic pregnant rats than in the control pregnant rats and the insulin-treated diabetic pregnant rats (Table 1).

Active intestinal calcium absorption expressed as S/M ratio was significantly lower in the diabetic pregnant rats than in the control pregnant rats (Table 1).

Fetuses from diabetic mothers weighed significantly less than those from the other two groups (Table 2). Serum ionized calcium levels in fetuses from diabetic mothers were lower than maternal levels but those in fetuses from the control mothers and the insulin-treated diabetic mothers were higher than maternal levels. Furthermore, serum ionized calcium concentrations were significantly lower in fetuses from the diabetic mothers than in those from the other two groups (Table 2). However, serum PTH levels were not significantly different among all groups (Table 2).

On the other hand, insulin treatment reversed decreased serum ionized calcium and 1, 25 (OH)₂D₃, levels and impaired active intestinal calcium absorption seen in diabetic pregnant rats toward the levels of the control rats. Moreover, insulin treatment recovered the fetal birth weight and serum ionized calcium levels in fetuses from diabetic mothers.

Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Fetuses from nondiabetic control mothers (n = 15)</th>
<th>Fetuses from untreated diabetic mothers (n = 9)</th>
<th>Fetuses from insulin-treated diabetic mothers (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>4.45±0.30</td>
<td>3.15±0.83b</td>
<td>4.40±0.48</td>
</tr>
<tr>
<td>Ionized calcium (mmol/l)</td>
<td>1.62±0.12</td>
<td>1.10±0.36a</td>
<td>1.54±0.21</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/l)</td>
<td>123.8±40.0</td>
<td>120±22.5</td>
<td>127.5±12.5</td>
</tr>
</tbody>
</table>

Fetal weight, serum ionized calcium and parathyroid hormone concentrations in fetuses from nondiabetic control mothers, untreated diabetic mothers and insulin-treated diabetic mothers.

a: p<0.001 vs nondiabetic control pregnant rats
b: p<0.01 vs insulin-treated diabetic pregnant rats
c: p<0.05 vs nondiabetic control pregnant rats.
not different from those in controls when fed a high calcium-phosphorus diet. These results suggest an impairment of the intracellular calcium transport system in the intestine of diabetic pregnant rats. At the cellular level, 1 α, 25 (OH)₂D₃ modulates the synthesis of vitamin D-dependent proteins by binding to a specific vitamin D receptor within the target cell. Calcium binding protein 9K (CaBP₂₉) is concentrated in rat duodenal mucosa and may act to facilitate calcium diffusion [13]. Although the intestinal CaBP mRNA increases 2- to 3-fold during rat pregnancy [14], diabetic pregnant rats have lower duodenal CaBP mRNA than controls [12]. In addition, genetically diabetic db/db mice have a decreased number of 1 α, 25 (OH)₂D₃ receptors in the intestine [15]. These data are compatible with the disturbed intestinal calcium transfer seen in our study.

Impairment of duodenal calcium absorption places diabetic pregnant rats in a state of negative calcium balance. Maternal negative calcium homeostasis may, in turn, decrease the transplacental calcium transfer to fetuses, resulting in fetal hypocalcemia. In fact, the present study has shown that the fetuses from the untreated diabetic mothers had lower serum ionized calcium levels in contrast to their mothers. The rat placenta contains a CaBP identical to the intestinal CaBP₉ [16] and the increase in placental CaBP mRNA suggests a role for this CaBP in maternal-fetal calcium transfer. However, Husain et al. [17] demonstrated reduced placental CaBP mRNA in the placenta of the untreated diabetic rats compared with the control and insulin-treated groups. A decrease in placental CaBP may reduce fetal calcium accretion by preventing the placentatal transport of calcium in diabetic rats.

We conclude that abnormalities of calcium metabolism in the untreated diabetic pregnant rats may have contributed to fetal hypocalcemia and that insulin therapy is effective in correcting the altered calcium homeostasis in diabetic mothers, possibly by recovering the synthesis of 1 α, 25 (OH)₂D₃, the intestinal calcium absorption and the placental calcium transfer.

References


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