Calcium Metabolism of Pregnant Rats Fed a Vitamin D-Depleted Diet

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ABSTRACT. The effects of vitamin D (VD) deficiency on calcium (Ca) metabolism during pregnancy were evaluated in rats fed VD-repleted (VD-repleted rats) and VD-depleted (VD-depleted rats) diets. In the VD-depleted rats, the plasma concentrations of 1,25-dihydroxyvitamin D and Ca decreased severely, whereas the parathyroid hormone concentrations increased. The Ca contents of the feces of the VD-depleted rats were higher than those of the VD-repleted rats. The fetal Ca contents of the VD-repleted and VD-depleted rats increased continuously, but that of the VD-depleted rats was lower. These data reveal that VD deficiency during pregnancy induces severe hypocalemia due to reduced intestinal absorption of Ca and elevated fetal demand for Ca.

KEY WORDS: calcium metabolism, pregnancy, vitamin D deficiency.

Pregnancy induces dramatic changes in the homeostatic mechanisms for calcium (Ca) and other minerals; intestinal transport [1] and bone resorption [11] increase to meet fetal requirements. The alterations in Ca metabolism during pregnancy are accompanied by elevated circulating levels of 1,25-dihydroxyvitamin D [1,25(OH)₂D], a more active physiological form of vitamin D (VD) metabolites [5]. The mechanism that mediates this increase is still unclear but may involve stimulation of extra-renal 1-α-hydroxylase by placental synthesis of 1,25(OH)₂D [4] or an alteration in the balance between the production of 1,25(OH)₂D and 24,25-dihydroxyvitamin D [14].

Since 1,25(OH)₂D is synthesized from 25-hydroxyvitamin D (25(OH)D), which is converted from VD by the hepatic 25-hydroxylase enzymes [7], it is assumed that maternal VD status is one of the important factors in maintaining maternal and fetal Ca homeostasis during pregnancy. Although Ca is actively transported from the maternal to the fetal side of the placenta, 1,25(OH)₂D and other VD metabolites are not involved in placental transport [2, 4]. Intestinal Ca absorption increases by the middle of pregnancy [1], during which time the intestine upregulates the expression of Ca-transporting proteins in response to the rise in 1,25(OH)₂D [19]. However, in pregnant rats, intestinal Ca absorption doubles despite the absence of VD [1, 6]. Pregnancy induces VD-independent bone resorption and VD-dependent bone mineralization [11, 13]. These previous observations seem to indicate that VD is not always necessary to sustain maternal and fetal Ca homeostasis during pregnancy. To the best of our knowledge, the effects of maternal VD deficiency on maternal and fetal Ca metabolism have not been fully elucidated. The present study was designed to evaluate the effects of VD deficiency on Ca metabolism in pregnant rats.

Seventy-two, 3-week-old, female Sprague-Dawley rats were used in this study. The rats were born and bred at our laboratory under conditions of controlled temperature (23 ± 2°C), controlled relative humidity (55 ± 10%), and a 12-hr light/dark cycle (lights on from 6:00 to 18:00). The rats were divided into two groups: VD-repleted (n=36) and VD-depleted (n=36) rats. The VD-repleted rats were fed Diet 11–1, which contained 0.44% Ca, 0.20% phosphorus (P), 0.07% magnesium (Mg) and 46 IU of VD/100 g, whereas the VD-depleted rats were fed Diet 11–3, which contained 0.44% Ca, 0.20% P, and 0.07% Mg (without VD). Both of the diets were the custom-made ones made by CLEA Japan Inc. (Tokyo). The rats were allowed access to food and distilled water ad libitum and were individually housed in metabolic cages (250 × 145 × 210 mm; Tokiwa Co., Tokyo, Japan). At 9 weeks of age, each female rat in the VD-repleted and VD-depleted groups was mated to a male in a separate cage. The first day of pregnancy was confirmed by the presence of sperm in vaginal smears. On days 14, 16, and 20 of pregnancy, nine rats from each group were subjected to blood collection by cardiac puncture under light ether anesthesia, and were then autopsied following exsanguination of ether inhalation and were individually housed in metabolic cages (250 × 145 × 210 mm; Tokiwa Co., Tokyo, Japan). For each of the fetuses from each rat were collected. These experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals [8].

The plasma concentration of 25(OH)D was measured by a modification of the competitive protein-binding assay [15]. The plasma concentration of 1,25(OH)₂D was measured using a radioreceptor assay [17]. The plasma concentration of intact parathyroid hormone (PTH) was determined...
using a PTH radioimmunoassay kit (Incstar Corp, Stillwater, MN). The Ca concentrations in the plasma and urine were determined by atomic absorption spectrophotometry [16]. The fecal samples, femoral bones, and fetuses were ashed for 24 hr using a muffled furnace at 600°C in an electric furnace and then dissolved in 1 N nitric acid [12]. The Ca levels were determined by the same method as that used for the plasma and urine.

The data from each group on each day of pregnancy are expressed as means and standard deviation (SD). Student’s t-test was used to compare each timed value between the groups, and significance was set at P<0.05.

The plasma 25(OH)D levels of the VD-depleted rats were <0.1 pg/ml for each day of pregnancy, i.e., significantly lower than those of VD-repleted rats (P<0.01) (Table 1). The plasma 1,25(OH)2D levels of the VD-depleted rats were lower than those of the VD-repleted rats throughout pregnancy (P<0.01), with the levels decreasing to 32.0 pg/ml on day 18 and to 19.1 pg/ml on day 20 of pregnancy. The plasma PTH levels of the VD-depleted rats increased on days 16 to 20 of pregnancy, and were higher than the corresponding levels of the VD-repleted rats (P<0.01) on day 20 of pregnancy.

The plasma Ca levels of the VD-depleted rats were lower than those of the VD-repleted rats (P<0.05 and P<0.01) for all the days of pregnancy (Table 2), and markedly decreased on days 18 and 20 of pregnancy. The fecal Ca contents on days 14, 18, and 20 of pregnancy were higher for the VD-depleted rats than for the VD-repleted rats (P<0.01). The urinary Ca concentrations in the VD-depleted rats decreased on days 16 to 20 of pregnancy, and were lower than those in the VD-repleted rats (P<0.01). Femoral bone Ca content was lower in the VD-depleted rats than in the VD-repleted rats (P<0.01).

The fetal Ca content per rat in both the VD-repleted and VD-depleted groups increased continuously between days 14 and 20 of pregnancy (Table 3). VD-depleted rats had significantly lower fetal Ca contents than VD-repleted rats on days 16 and 20 of pregnancy (P<0.01), whereas they showed higher Ca contents on day 18 of pregnancy (P<0.05). The fetal weights were lower in the VD-depleted group than in the VD-repleted group (P<0.01) on days 14, 18, and 20 of pregnancy.

In general, Ca absorption and urinary Ca excretion are higher during pregnancy than before conception or after delivery [14]. This increase is evident in early-to-mid pregnancy and precedes the increased demand for Ca for skeletal growth by the fetus. In bone metabolism, resorption and

### Table 1. Plasma concentrations of Ca-regulating hormones in VD-repleted and -depleted rats during pregnancy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 18</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VD-repleted (SD)</td>
<td>VD-depleted (SD)</td>
<td>VD-repleted (SD)</td>
<td>VD-depleted (SD)</td>
</tr>
<tr>
<td>25-OHD</td>
<td>pg/ml</td>
<td>22.9 (2.5)</td>
<td>&lt; 0.1**</td>
<td>22.8 (2.5)</td>
<td>&lt; 0.1**</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>pg/ml</td>
<td>77.8 (4.0)</td>
<td>44.1 (2.5)**</td>
<td>72.8 (4.1)</td>
<td>42.1 (3.7)**</td>
</tr>
<tr>
<td>PTH</td>
<td>pmol/l</td>
<td>88.8 (5.2)</td>
<td>84.2 (4.0)</td>
<td>85.9 (5.9)</td>
<td>147.7 (18.1)**</td>
</tr>
</tbody>
</table>

* P<0.05 compared to VD-repleted rats at the same day of pregnancy, ** P<0.01 compared to VD-repleted rats at the same day of pregnancy.

### Table 2. Plasma Ca concentration and Ca contents of feces, urine and femoral bones in VD-repleted and -depleted rats during pregnancy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 18</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VD-repleted (SD)</td>
<td>VD-depleted (SD)</td>
<td>VD-repleted (SD)</td>
<td>VD-depleted (SD)</td>
</tr>
<tr>
<td>Plasma Ca</td>
<td>mg/d</td>
<td>9.6 (0.2)</td>
<td>8.7 (0.2)*</td>
<td>9.9 (0.2)</td>
<td>8.6 (0.3)**</td>
</tr>
<tr>
<td>Fecal Ca</td>
<td>mg/kg/day</td>
<td>175.2 (11.8)</td>
<td>215.7 (5.7)**</td>
<td>223.2 (17.3)</td>
<td>223.7 (10.4)</td>
</tr>
<tr>
<td>Urinary Ca</td>
<td>mg/kg/day</td>
<td>5.9 (0.7)</td>
<td>5.5 (1.1)</td>
<td>6.3 (1.6)</td>
<td>3.6 (0.6)**</td>
</tr>
<tr>
<td>Femoral bone Ca</td>
<td>mg/g</td>
<td>184.2 (6.5)</td>
<td>186.2 (4.8)</td>
<td>188.4 (3.0)</td>
<td>188.6 (4.3)</td>
</tr>
</tbody>
</table>

* P<0.05 compared to VD-repleted rats at the same day of pregnancy, ** P<0.01 compared to VD-repleted rats at the same day of pregnancy.

### Table 3. Ca contents and weights of the fetuses in each VD-repleted and -depleted rat during pregnancy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 18</th>
<th>Day 20</th>
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<tr>
<td></td>
<td></td>
<td>VD-repleted (SD)</td>
<td>VD-depleted (SD)</td>
<td>VD-repleted (SD)</td>
<td>VD-depleted (SD)</td>
</tr>
<tr>
<td>Fetal Ca</td>
<td>mg</td>
<td>0.009 (0.001)</td>
<td>0.009 (0.001)</td>
<td>0.047 (0.005)</td>
<td>0.025 (0.003)**</td>
</tr>
<tr>
<td>Fetal weight</td>
<td>g</td>
<td>1.47 (0.17)</td>
<td>1.21 (0.08)**</td>
<td>3.89 (0.41)</td>
<td>4.01 (0.16)</td>
</tr>
</tbody>
</table>

* P<0.05 compared to VD-repleted rats at the same day of pregnancy, ** P<0.01 compared to VD-repleted rats at the same day of pregnancy.
formation are elevated in a similar manner [14]. The alterations in Ca and bone metabolism during pregnancy are mainly accompanied by increases in 1,25(OH)2D, with little discernible alteration in either PTH or calcitonin concentration. These observations well agree with our present results obtained from VD-repleted rats; increased plasma 1,25(OH)2D concentrations, negligible changes in plasma PTH levels, and increases in fetal Ca contents and fetal weights in the latter stages of pregnancy.

During pregnancy, the maternal blood concentrations of 25(OH)D correlated with dietary VD intake [18]. In the present study, the undetectable levels of plasma 25(OH)D in VD-depleted rats were considered as evidence of successful induction of VD deficiency during pregnancy. Maternal general nutrition has a major impact on fetal growth and weight, as well as on skeletal mass, and poor nutrition during pregnancy may reduce neonatal bone density and size [10]. The developing fetus in the pregnant rat requires approximately 20 mg of Ca for skeletal formation during the last 4–5 days of gestation [1]. Brommage and DeLuca [2] have indicated that 1,25(OH)2D and other VD metabolites are not involved in the active transport of Ca across the placenta in the rat, which corroborates previous data showing normal Ca levels in VD-depleted fetuses. The homeostasis of fetal plasma Ca levels is clearly controlled independently of the maternal values; the fetus is capable of maintaining normal Ca levels in VD-depleted fetuses. The increase in 1,25(OH)2D concentrations, negligible changes in plasma PTH concentrations on days 16 to 20 of pregnancy may reduce neonatal bone density and size [10]. The developing fetus in the pregnant rat requires approximately 20 mg of Ca for skeletal formation during the last 4–5 days of gestation [1]. Brommage and DeLuca [2] have indicated that 1,25(OH)2D and other VD metabolites are not involved in the active transport of Ca across the placenta in the rat, which corroborates previous data showing normal Ca levels in VD-depleted fetuses. The homeostasis of fetal plasma Ca levels is clearly controlled independently of the maternal values; the fetus is capable of maintaining adequate supplies of Ca for its own needs even under conditions of dietary deprivation and VD deficiency, during which maternal plasma Ca concentrations are low [2]. In the present study, there was some concern that the significantly lower weight and Ca content of the fetuses in the VD-depleted rats resulted from decreased placental Ca transport due to severe maternal hypocalcemia.

Severe hypocalcemia was observed in the VD-depleted rats in this study, which possibly resulted from the decreased plasma levels of maternal 1,25(OH)2D and the elevated trans-placental supply of Ca to the fetuses. Low concentrations of circulating 1,25(OH)2D fail to stimulate active Ca absorption in the intestine. Generally, the pregnant rat responds by increasing food intake by 60–70% and increasing small intestinal villous height and colon length [3]. These changes increase total dietary Ca absorption, even when rats are fed a VD-depleted diet [1]. Therefore, for the VD-depleted rats described here, it appears that intestinal Ca absorption depended on 1,25(OH)2D-independent Ca transport rather than active transport. The higher fecal Ca contents in VD-depleted rats appear to reflect reduced levels of active Ca transport in the intestine. Hypocalcemia in VD-depleted rats also induced increases in the plasma PTH concentrations on days 16 to 20 of pregnancy. Therefore, it seems likely that the elevated levels of plasma PTH reduced urinary Ca excretion due to stimulation of renal Ca resorption. Bone metabolism was considered to reflect the increase in PTH-induced bone resorption and the decrease in 1,25(OH)2D-dependent bone formation [11, 13].

In conclusion, VD deficiency in pregnant rats induces severe hypocalcemia due to reduced intestinal Ca absorption and elevated fetal Ca demand. Therefore, serious suppression of increases in fetal Ca content and weight occur despite PTH-stimulated elevation of renal and bone Ca resorption. Our results suggest that adequate dietary Ca content should be maintained to meet fetal Ca demand in VD-depleted pregnant rats, as blood Ca levels enter the normal range in VD-depleted rats that are fed a high Ca (2%) diet [9].

REFERENCES