The Role of Vitamin D Metabolites in Hypercalcemia of Zucker *fa/fa* Rats

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Summary In order to investigate mineral and vitamin D metabolism in obese rats with hyperinsulinemia, plasma calcium and vitamin D metabolites were measured in Zucker *fa/fa* rats. Body weight, plasma insulin, and calcium in *fa/fa* rats were significantly increased compared to their lean littermates ($p < 0.01$). However, no significant difference in plasma 25-hydroxyvitamin D (25(OH)D), 24,25-dihydroxyvitamin D, 1,25-dihydroxyvitamin D (1,25(OH)₂D) or the ratio of 1,25(OH)₂D to 25(OH)D was observed between *fa/fa* rats and their lean littermates. The hypercalcemia in the rats with hyperinsulinemia, therefore, might be caused by other calcium-regulating hormones or some factors other than 1,25-(OH)₂D. In addition, the hyperinsulinemia associated with obesity may not produce the accelerated conversion from 25(OH)D into 1,25(OH)₂D. **Key Words** calcium, 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D, 1,25-dihydroxyvitamin D, Zucker *fa/fa* rats, obesity, hyperinsulinemia

Zucker *fa/fa* rats with genetic obesity have been generally recognized to have symptoms such as hyperphagia, hyperlipemia, and hyperinsulinemia (1-3). In addition, there have been recent reports that Zucker *fa/fa* rats have C cell hyperplasia in the thyroid gland (4) and higher fasting plasma calcitonin and calcium levels (5). These observations suggest that there may be some alterations of mineral and vitamin D metabolism in hyperinsulinemic obese rats. In the present study, therefore, we have measured circulating levels of calcium and vitamin D metabolites in Zucker *fa/fa* rats.

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MATERIALS AND METHODS

Male Zucker *fa/fa* rats and their lean littermates (*fa/+*, *+/+*) were kindly supplied by Dr. H. Iwatsuka, Biological Research Laboratory, Central Research Division, Takeda Chemical Industry, Osaka, Japan. They were housed individually in a temperature- and humidity-controlled room and were given Oriental laboratory food (Oriental Yeast Co., Tokyo, Japan) containing 0.71 g calcium, 0.74 g phosphorus, and 200 IU vitamin D per 100 g (Table 1) and water *ad libitum*. All animals used for this experiment were aged 12–13 weeks. They were fasted overnight and blood samples were taken from the inferior vena cava with heparinized syringes after laparotomy under sodium pentobarbital anesthesia (40 mg/kg, i.p.). Blood samples were placed on ice and then centrifuged. Plasma was separated, frozen immediately, and stored at -20°C until assayed. Blood glucose was measured by the glucose oxidase method (6). Plasma calcium level was determined by Auto-Analyzer. Immunoreactive insulin was measured by radioimmunoassay using the polyethylene glycol method (7) with rat insulin as the standard. Plasma 25-hydroxyvitamin D (25(OH)D), 24,25-dihydroxyvitamin D (24,25(OH)₂D), and 1,25-dihydroxyvitamin D (1,25(OH)₂D) were determined using the methods previously reported (8).

Statistical analysis for the significance of the difference between groups was performed using unpaired “*t*” test.

RESULTS

Body weight, fasting blood glucose and plasma insulin (Table 2)

The rate of body weight increase was markedly higher in *fa/fa* rats than in their

Table 1. Composition of the diet.^a

Nutrients	%
Water	7.0
Ash	7.0
Protein	24.1
Fat	4.6
Carbohydrate	53.0
Dietary fiber	4.2
kcal/100 g	349.8

^a Provided the following vitamin and mineral mixture per 100 g diet: vitamin A, 1,000 IU; vitamin D, 200 IU; vitamin E, 1.6 mg; thiamin, 0.9 mg; riboflavin, 0.8 mg; vitamin B₆, 0.8 mg; vitamin B₁₂, 0.5 µg; ascorbic acid, 6 mg; niacin, 4 mg; pantothenic acid, 1.9 mg; biotin, 20 µg; folic acid, 30 µg; inositol, 60 mg; choline, 100 mg; Ca, 0.71 g; P, 0.74 g; Mg, 0.33 g; Na, 0.24 g; K, 0.69 g; Fe, 18 mg; Al, 5 mg; SiO₂, 0.48 g; Cu, 0.74 mg; Zn, 6.05 mg; Co, 0.21 mg; Mn, 8.17 mg.

Table 2. Body weight, blood glucose, and plasma insulin in Zucker *fa/fa* rats.^a

Rats	Body weight (g)	Blood glucose (mg/dl)	Plasma insulin (ng/ml)
<i>fa/fa</i> (6) ^b	422 ± 10**	230 ± 28*	4.2 ± 0.8**
Lean (6)	298 ± 8	150 ± 10	1.0 ± 0.3

^a Values are expressed as means ± SE. ^b Number of rats. ** $p < 0.01$ vs. lean littermates.* $p < 0.05$ vs. lean littermates.

Fig. 1. Plasma calcium, 25(OH)D, 24,25(OH)₂D, 1,25(OH)₂D, and the ratio of 1,25(OH)₂D to 25(OH)D (1,25(OH)₂D/25(OH)D) in Zucker *fa/fa* rats (O) and their lean littermates (L). Circulating calcium was significantly higher in *fa/fa* rats ($p < 0.01$) than in their lean littermates. Plasma vitamin D metabolites and the ratio of 1,25(OH)₂D to 25(OH)D were not significantly different between them.

lean littermates. Immediately before the rats were killed, the mean body weight of the *fa/fa* rats was 422 ± 10 g ($\pm$ SE), significantly higher than that of the lean littermates ($p < 0.01$). The blood glucose levels in the obese rats were significantly higher than in the lean controls ($p < 0.05$). The fasting plasma insulin levels were also significantly higher in the *fa/fa* rats than in their lean littermates ($p < 0.01$).

Calcium and vitamin D metabolites (Fig. 1)

Plasma calcium levels in the *fa/fa* rats were significantly higher than in their lean littermates ($p < 0.01$). No significant difference in circulating 25(OH)D, 24,25(OH)₂D, or 1,25(OH)₂D levels was observed between the *fa/fa* rats and the

lean controls. In addition, the ratio of $1,25(\text{OH})_2\text{D}$ to $25(\text{OH})\text{D}$ in *fa/fa* obese rats was not statistically different from controls.

DISCUSSION

Flynn *et al.* (9) have recently observed that both plasma calcium and calcitonin levels are high in young (10 weeks of age) *fa/fa* obese rats. In the present study, we also found elevated calcium concentrations in rats with hyperinsulinemia aged 12–13 weeks. To the contrary, in experimental hypoinsulinemic diabetic rats, hypocalcemia has been reported (8) which may be caused by a negative calcium balance resulting from intestinal calcium malabsorption, hypercalciuria, and increased fecal calcium excretion (10–13). Since renal $25(\text{OH})\text{D}$ - 1α -hydroxylase activity has been reported to be reduced in diabetic rats and reversed by insulin (14), the activity of 1α -hydroxylase seems to be increased in the hyperinsulinemic state. Accordingly, the observed hypercalcemia in *fa/fa* rats might be thought to be due to the increased intestinal calcium absorption and calcium mobilization from bone found with high plasma $1,25(\text{OH})_2\text{D}$ levels. However, in the present study, circulating $1,25(\text{OH})_2\text{D}$ was not elevated and the ratio of $1,25(\text{OH})_2\text{D}$ to $25(\text{OH})\text{D}$ was not increased in *fa/fa* rats compared to their lean littermates. Hyperinsulinemia with obesity, therefore, may not produce the accelerated conversion into $1,25(\text{OH})_2\text{D}$ from $25(\text{OH})\text{D}$ found in the present study. Calcium-regulating hormones other than this active vitamin D metabolite, such as parathyroid hormone or calcitonin, could be involved in the hypercalcemia in these rats. Recent reports (4,5,9) have shown, however, that there are high plasma calcitonin levels and thyroid C cell hyperplasia under the condition of hypercalcemia in *fa/fa* rats. It seems likely, therefore, that calcitonin is not responsible for the hypercalcemia, and that C cell hyperplasia might be a secondary phenomenon, possibly to compensate for the elevated plasma calcium levels in these rats. Furthermore, *fa/fa* rats and lean littermates have been observed to show similar levels of plasma parathyroid hormone as measured by radioimmunoassay in which the antiserum used recognized primarily the C-terminal region (5). Therefore, at present, there seems to be no direct evidence that parathyroid hormone is related to the high plasma calcium levels in *fa/fa* rats. However, measurement by antiserum against the N-terminal region of parathyroid hormone should be considered. It is possible, accordingly, that the increased calcium intake stemming from hyperphagia might be important in the positive calcium balance. Another possibility regarding the unchanged ratio of $1,25(\text{OH})_2\text{D}$ to $25(\text{OH})\text{D}$ is that the conversion from $1,25(\text{OH})_2\text{D}$ into further metabolites or lactone bodies might be accelerated, resulting in normal plasma $1,25(\text{OH})_2\text{D}$ levels even if the production of $1,25(\text{OH})_2\text{D}$ is increased in the hyperinsulinemic state. Further studies are needed to clarify the details of mineral and vitamin D metabolism in genetic obesity.

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