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Impaired glucose tolerance in vitamin D deficiency can be corrected by calcium

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Vitamin D_3, via its active metabolite 1α,25-dihydroxyvitamin D_3, helps maintain normal calcium levels in the body. Apart from the maintenance of calcium homeostasis, the active form of vitamin D_3 is now known to be involved in a number of other functions including that of pancreatic β cells. Low serum insulin levels and impaired glucose tolerance in a vitamin D-deficient state have been reported in experimental animals. Hypocalcemia is a major consequence of vitamin D deficiency. Whether the impairment observed is due to vitamin D deficiency per se or is secondary to low calcium is still a matter of controversy. The present study was conducted to delineate the roles of vitamin D and calcium in glucose intolerance associated with vitamin D deficiency in vivo. It was found that supplementation with either vitamin D_3 or high calcium alone to vitamin D-deficient rats could correct the defects. In addition, insulin sensitivity was found to be enhanced in the vitamin D-deficient group compared with vitamin D control or calcium-supplemented groups. Hence the present study demonstrates that calcium per se in the absence of vitamin D increases insulin secretion and normalizes intolerance to glucose seen in vitamin D deficiency. (J. Nutr. Biochem. 11:170–175, 2000)

Keywords: vitamin D_3; 25-hydroxyvitamin D_3 (25-OH-D_3); glucose tolerance; insulin tolerance; area under the curve (AUC)

Introduction

Vitamin D_3 is well known to function in calcium homeostasis.[1] One of the important developments in the field of vitamin D endocrinology is the strong evidence for the existence of specific receptors for 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] and the consequent biological effects in many nonclassical target tissues.[2] Several lines of evidences suggest that the β cells of endocrine pancreas are among the nonclassical target tissues for the action of 1α,25$(OH)_2D_3$, which include (1) the presence of receptor protein for 1α,25$(OH)_2D_3$ in chick pancreas[3,4] and (2) the presence of immunoreactive vitamin D-dependent calcium binding protein (CaBP) in pancreas.[5] All these findings suggest a probable role for vitamin D_3/metabolites in pancreatic β-cell function.

A role for vitamin D in β-cell activity was first suggested by Boquist et al.[6] Later studies showed that vitamin D deficiency leads to lowered insulin secretion and impaired glucose tolerance.[7–9] Most of the studies have attributed these defects to the lack of vitamin D/1α,25$(OH)_2D_3$. Whether vitamin D is the actual factor that is essential for insulin secretion is complicated by other changes that occur due to vitamin D deficiency. Some of these changes include a decrease in serum calcium and islet CaBP. Calcium is known to play a crucial role in hormone release.[10] The role of calcium per se in the absence of vitamin D in improving glucose tolerance and insulin secretion is still inconclusive.

Therefore, the present study was conducted to evaluate the role of calcium and vitamin D in insulin secretion in response to glucose challenge, and also to test the in vivo insulin sensitivity.

Materials and methods

Experimental protocol

Fifty male weanling Wistar NIN rats weighing 30 to 40 g were randomly divided into three groups. The first group of 10 rats (group I) served as the ad libitum control and the second group, which also contained 10 rats (group II), was pair-fed to the vitamin D-deficient group (group III). Group III contained 30 rats. All the rats were housed in individual cages in a dark room maintained at

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24°C with only red light being provided. Rats were fed on a basal vitamin D-deficient diet[11] containing normal calcium (0.4%) and phosphorus (0.35%) and had free access to distilled water. Rats from groups I and II were administered vitamin D_3 orally at a dose of 40 IU/day/rat and group III received only vehicle (arachis oil).

Serum calcium levels were monitored in all groups of rats from the third week of feeding. At the end of 4 weeks, rats in group III were hypocalcemic; the serum calcium levels of rats were found to be in the range of 5 to 6 mg/dL (indicating vitamin D deficiency) compared with control rats, which had serum calcium levels in the range of 10 to 11 mg/dL. The rats in group III were further randomly divided into three groups (groups III, IV, V) of 10 rats each. Group III continued to receive the same diet without any supplementation, group IV was supplemented with vitamin D_3, and group V received a high calcium diet. (The composition of the high calcium diet was similar to that of the basal diet except that the calcium content was 4%.) All the rats were fed their respective dietary regimens for a period of 2 weeks. Daily food intake and weekly body weight were monitored throughout the experimental period.

At the end of the 6 weeks of experimental period, blood was drawn from rats in all five groups rats for the analysis of serum vitamin D-dependent parameters such as calcium, 25-hydroxyvitamin D_3(25-OH-D_3), phosphorus, and alkaline phosphatase.

Methods

Serum parameters. The serum parameters such as calcium, phosphorus, alkaline phosphatase, and 25-OH-D_3 were estimated using standard methods.[12–15] Serum insulin was assessed by radioimmunoassay[16] and plasma glucose was done by glucose oxidase kit method (Stangen Immunodiaganostics, Hyderabad, India).

Glucose tolerance test. After an overnight fast, an initial (0 minutes) blood sample was collected from the orbito-sinus plexus. Glucose (200 mg/100 g body weight) was administered intraperitoneally. Blood samples were collected at 30, 60, and 120 minutes after the glucose load in tubes containing sodium fluoride. Blood was also collected in a separate set of tubes for the estimation of serum insulin.

Insulin tolerance test. An initial (0 minutes) blood sample was collected. The rats were then given a single intraperitoneal injection of porcine insulin (50 mU/100 g body weight). This was immediately followed by a intraperitoneal glucose load (200 mg/100 g body weight). Blood was drawn at 30, 60, and 120 minutes following the glucose load for estimation of plasma glucose.

Calculation of area under the curve

The area under the glucose/insulin curves (AUC) was calculated using their concentrations at 0 minutes, 30 minutes, 60 minutes, and 120 minutes as per the following formula[17]:

$$AUC = \frac{[(C_0 + C_1)(T_1 - T_0)]}{2} + \frac{[(C_1 + C_2)(T_2 - T_1)]}{2} + \frac{[(C_2 + C_3)(T_3 - T_2)]}{2}$$

where C_0, C_1, C_2, and C_3 are the concentrations of glucose/insulin at time points T_0, T_1, T_2, and T_3, respectively (i.e., $T_0 = 0$ minutes; $T_1 = 30$ minutes; $T_2 = 60$ minutes; $T_3 = 120$ minutes).

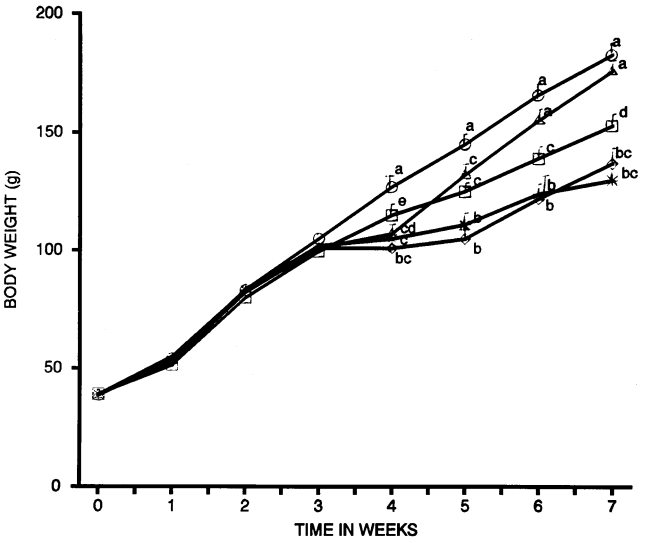


Figure 1 Body weights of different groups of rats. Values are mean ± SEM ($n = 8$). Values bearing different superscripts are significantly different by analysis of variance ($P < 0.001$). ○, + vitamin D_3 control (group I); □, + D_3 pair-fed control (group II); *, vitamin D_3 deficient (group III); △, vitamin D_3 supplemented with vitamin D_3 (group IV); ◇, vitamin D_3 supplemented with high calcium (4%) (group V).

Statistical analysis

The data was analyzed by the appropriate use of one-way analysis of variance using SPSS computer package with a test of critical difference.[18] A probability level of 5% was considered to be significant.

Results

Body weights

Figure 1 depicts the body weights of the different experimental groups of rats. Growth of rats in the ad libitum control (group I), pair-fed control (group II), and vitamin D-deficient (group III) groups was similar up to the end of 3 weeks of feeding. The vitamin D-deficient rats had significantly ($P < 0.001$) lower body weights starting from fourth week compared with groups I, II, and IV, and it continued to be so until the end of the experiment. Body weight gains in the vitamin D-supplemented group (group IV) were similar to those in group I at the sixth and seventh weeks of feeding, whereas rats in the high calcium-supplemented group had significantly ($P < 0.001$) lower body weights compared with groups I, II, and IV, and similar to those in the vitamin D-deficient group (group III).

Serum vitamin D-dependent parameters

Table 1 shows the vitamin D-dependent parameters in the different experimental groups of rats. The vitamin D-deficient group had significantly ($P < 0.001$) lower serum calcium than did the vitamin D replete controls. Supplementation with either vitamin D or high calcium normalized the serum calcium, which was significantly ($P < 0.001$) higher than that in the deficient group. No significant differences in serum calcium levels were observed between groups I, II, IV, and V.

Table 1 Serum vitamin D-dependent parameters in different groups at the end of 6 weeks

Groups		Serum parameters			
		Calcium (mg/dL)	25-OH-D_3 (ng/mL)	Phosphorus (mg/dL)	Alkaline phosphatase U/lt.
I	Control	10.3 ± 0.11[a]	22.4 ± 5.42[b]	7.42 ± 0.39[a]	104 ± 5.48[a]
II	Control (pair-fed)	9.8 ± 0.17[a]	21.0 ± 5.52[b]	7.05 ± 0.29[a]	110 ± 8.90[a]
III	Vitamin D deficient	5.4 ± 0.15[b]	1.02 ± 0.82[a]	9.77 ± 0.34[c]	194 ± 11.5[b]
IV	Vitamin D deficient (rehab with vitamin D)	9.6 ± 0.22[a]	18.4 ± 6.22[b]	7.48 ± 0.27[a]	116 ± 17.1[a]
V	Vitamin D deficient (Supp with Ca 4%)	9.8 ± 0.13[a]	0.92 ± 0.85[a]	4.30 ± 0.21[b]	183 ± 18.8[b]

Values are mean ± SEM ($n = 8$).
Values in a column bearing different superscripts are significantly different ($P < 0.001$) by analysis of variance.
25-OH-D_3–25-dihydroxyvitamin D_3.

The serum 25-OH-D_3 levels in vitamin D-deficient and calcium-supplemented rats were found to be very low and were significantly ($P < 0.001$) lower than those in group I. When vitamin D-deficient rats were supplemented with vitamin D, as expected, the serum 25-OH-D_3 levels increased and were similar to those of control group (group I). The pair-fed control had serum 25-OH-D_3 levels similar to those in the ad libitum control.

The serum phosphorus levels in the vitamin D-deficient group were significantly higher ($P < 0.001$) compared with controls. The serum alkaline phosphatase levels also showed a similar trend. The calcium-supplemented group had significantly ($P < 0.001$) lower serum phosphorus compared with rest of the groups; however, the serum alkaline phosphatase levels in this group of rats were similar to those of the vitamin D-deficient group and significantly ($P < 0.001$) higher than those of the other three groups.

Intraperitoneal glucose tolerance test

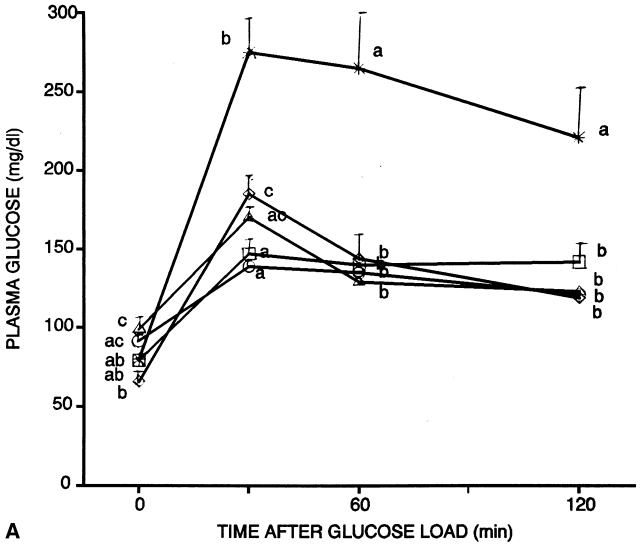
Plasma glucose levels. The glucose response curves after an intraperitoneal load of glucose in different experimental groups are depicted in *Figure 2A*. As illustrated in the figure, the fasting plasma glucose levels in the different groups ranged between 66 and 99 mg/dL. The peak plasma glucose concentration was observed 30 minutes after the glucose load in all the groups. The rise in glucose levels at this time point was significantly ($P < 0.001$) higher in the vitamin D-deficient group (275 ± 21.5 mg/dL) compared with the rest of the groups (group I, 139 ± 4.29; group II, 147 ± 4.33; group IV, 170 ± 9.42; group V, 185 ± 11.0 mg/dL). Sixty minutes following the load, the glucose levels showed a trend similar to that seen at 30 minutes. The plasma glucose values at 120 minutes returned to near fasting levels in groups I (121 ± 8.76 mg/dL), II (142 ± 9.18 mg/dL), IV (123 ± 5.1 mg/dL), and V (119 ± 5.86 mg/dL) whereas the vitamin D-deficient (i.e., group III) still had elevated glucose levels (221 ± 26.2 mg/dL) that were significantly ($P < 0.001$) higher than the rest. In addition, the vitamin D-deficient group had significantly ($P < 0.001$) higher AUC values compared with groups I, II, IV, and V (467 ± 42.5 vs. 255 ± 7.18, 270 ± 7.54, 268 ± 8.41, and 277 ± 18.7 mg/dL/hr).

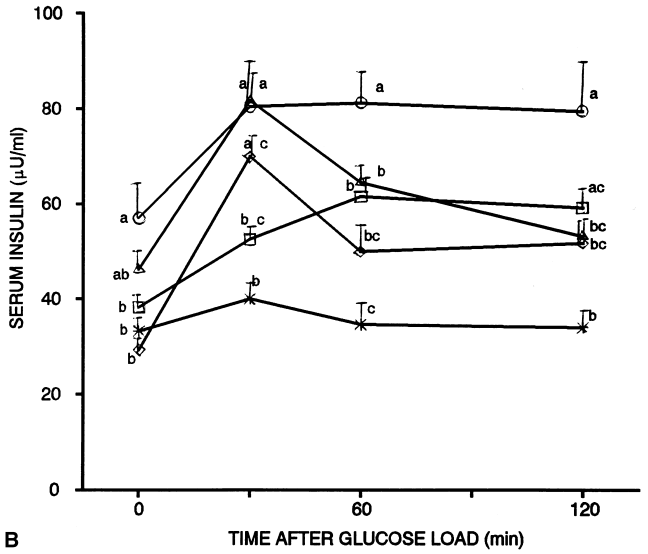
Serum insulin levels. The insulin levels during intraperitoneal glucose tolerance testing are shown in *Figure 2B*. The fasting (0 minutes) insulin levels in the vitamin D-deficient group were significantly ($P < 0.001$) lower than those in the ad libitum control but were similar to the remaining groups.

Thirty minutes after the glucose load, there was a rise in the serum insulin levels in all the groups. The vitamin D-deficient group (group III; 40.5 ± 5.16 μU/mL) exhibited a minimal rise, which was significantly lower ($P < 0.001$) compared with that of the ad libitum control (group I; 80.6 ± 9.01 μU/mL), vitamin D-supplemented (group IV; 81.8 ± 5.08 μU/mL), and calcium-supplemented (group V; 70.0 ± 3.82 μU/mL) groups. Although the pair-fed control group had higher insulin levels compared with the deficient group, it was not significant (40.5 ± 5.16 vs. 52.6 ± 1.28 μU/mL). At 60 and 120 minutes the deficient group continued to exhibit lower insulin levels than the rest of the groups. The insulin levels in both the vitamin D and calcium-supplemented groups were similar, with no significant difference between them. The AUC values of the vitamin D-deficient group were significantly ($P < 0.001$) lower than those in groups I, II, IV, and V (70 ± 6.88 vs. 155 ± 14.9, 111 ± 5.48, 127 ± 6.45, and 106 ± 5.36 μU/mL/hr).

Insulin/glucose ratio. The insulin response to glucose challenge in different experimental groups is well illustrated by the insulin-glucose concentration ratio in *Figure 2C*. As seen from the figure, this ratio was found to be significantly lower ($P < 0.001$) in the vitamin D-deficient group at 30, 60, and 120 minutes compared with controls and supplemented groups.

Insulin tolerance test. The effect of intraperitoneal insulin administration on plasma glucose levels is depicted in *Figure 3*. Thirty minutes following the insulin load, the plasma glucose levels were significantly ($P < 0.001$) lower in deficient group (64.0 ± 4.76 mg/dL) than in the vitamin D control (105 ± 5.89 mg/dL) and calcium-supplemented (130 ± 14.6 mg/dL) groups. At 60 minutes, the deficient group (54.0 ± 5.28 mg/dL) continued to exhibit significantly ($P < 0.001$) lower plasma glucose compared with the control (132 ± 2.97 mg/dL) and calcium-supplemented (105 ± 6.56 mg/dL) groups. A similar trend was seen at 120 minutes also. The AUC values of the vitamin D-deficient group were significantly ($P < 0.001$) lower than those in





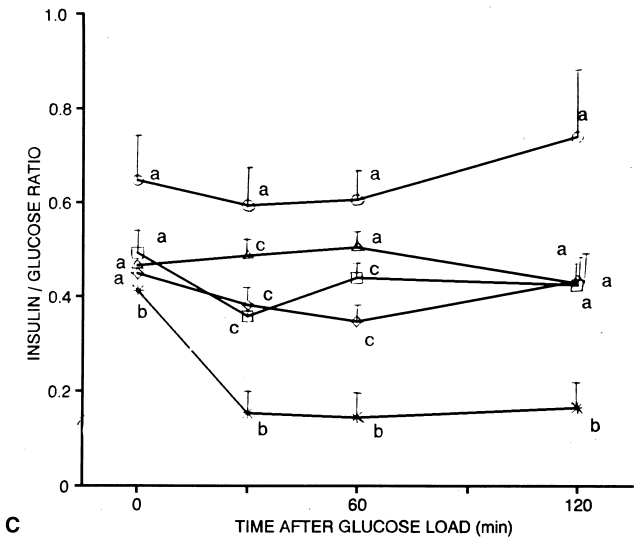


Figure 2 (A) Effect of vitamin D_3 deficiency and supplementation with vitamin D_3/high calcium on intraperitoneal glucose tolerance in rats. Values are mean ± SEM ($n = 6$). Values bearing different superscripts are significantly different by analysis of variance (ANOVA; $P < 0.001$). (B) Effect of vitamin D_3 deficiency and supplementation with vitamin D_3/high calcium on serum insulin levels during intraperitoneal glucose tolerance in rats. Values are mean ± SEM ($n = 6$). Values bearing different superscripts are significantly different by ANOVA ($P < 0.001$). (C) Insulin to glucose ratios during intraperitoneal glucose tolerance in vitamin D_3 deficiency and supplementation with vitamin D_3/high calcium in rats. Values are mean ± SEM ($n = 6$). Values bearing different superscripts are significantly different by ANOVA ($P < 0.001$). ○, + vitamin D_3 control (group I); □, + D_3 pair-fed control (group II); *, vitamin D_3 deficient (group III); △, vitamin D_3 supplemented with vitamin D_3 (group IV); ◇, vitamin D_3 supplemented with high calcium (4%) (group V).

groups II and V (136 ± 5.63 vs. 235 ± 4.94 and 230 ± 11.8 mg/dL/hr).

Discussion

Earlier studies have demonstrated that vitamin D or its metabolites are essential for normal insulin secretion.[8,9] The evidence from the literature does not necessarily corroborate proposals of a direct action of vitamin D on the pancreatic β cell. Vitamin D deficiency is a complex metabolic state wherein the diet intake of animals is poor, and they are hypocalcemic and consequently have impaired growth. Calorie restriction has been reported to result in inhibition of insulin secretion.[19] Therefore, the present study was conducted to clarify the role of nutritional factors in the reduced insulin levels in vitamin D deficiency, to delineate the role of vitamin D and calcium in mediating insulin secretion, and to check insulin sensitivity under these conditions.

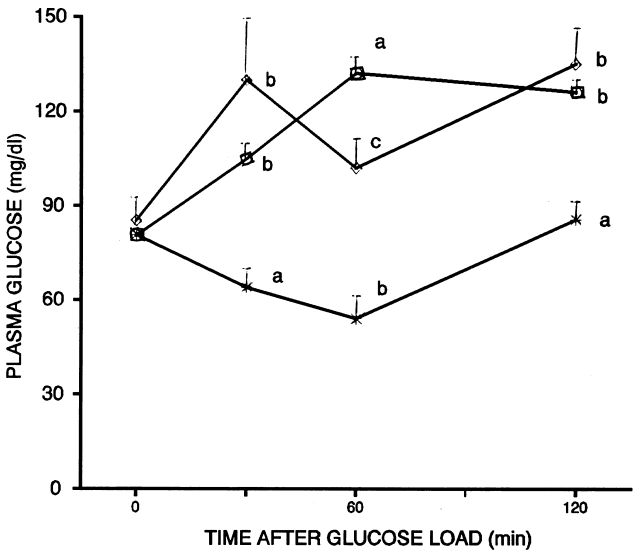


Figure 3 Effect of exogenously administered insulin on intraperitoneal glucose tolerance in rats. Values are mean ± SEM ($n = 6$). Values bearing different superscripts are significantly different by analysis of variance ($P < 0.001$). □, + D_3 pair-fed control (group II); *, vitamin D_3 deficient (group III); ◇, vitamin D_3 supplemented with high calcium (4%) (group V).

A vitamin D-deficient rat model was employed. The decreased levels of serum 25-OH-D_3 correlated well with the decreased serum calcium levels in them. Our results are in line with earlier studies that demonstrated that frank hypocalcemia in vitamin D deficiency is associated with a concomitant decrease in circulating levels of vitamin D metabolites.[20,21] Apart from hypocalcemia, the other vitamin D-dependent biochemical changes observed in vitamin D deficiency include increases in serum phosphorus levels and alkaline phosphatase activity. Serum phosphorus levels have been reported to be either elevated or unaltered in this state.[22] In addition, the high levels of serum alkaline phosphatase are in line with earlier findings[23,24] that suggested leakage of the enzyme from bone.

The intraperitoneally administered glucose led to clear impairment in glucose tolerance in vitamin D-deficient rats and a corresponding 50 to 60% decrease in insulin levels. This was further confirmed by the lowered insulin/glucose ratio in vitamin D-deficient animals, indicating a poor response to stimulate insulin on glucose challenge in vitamin D deficiency. These results are consistent with earlier studies that reported glucose intolerance and impaired insulin response in a vitamin D-deficient state.[7,9] To delineate the roles of vitamin D and calcium, the vitamin D-deficient rats were supplemented either with vitamin D or calcium alone. Normalization of serum calcium levels was accomplished by both the supplementations. Earlier studies[25] have suggested that calcium absorption occurs primarily by passive diffusion when the luminal calcium concentration is high.

Both the vitamin D and calcium-supplemented groups showed a substantial rise in insulin levels in response to glucose load, resulting in normal glucose tolerance curves. The role of calcium per se in the absence of vitamin D in enhancing insulin secretion is still a debatable question with studies both in favor[26] and against[27] the hypothesis. The present study suggests that calcium alone corrects glucose intolerance observed in vitamin D deficiency. Thus, it can be postulated that high calcium feeding alone succeeds in normalizing extracellular calcium concentrations needed for insulin release. It was observed that rats in the high calcium-supplemented group were hypophosphatemic. Frankel and Sjunghall[28] reported that moderate changes in phosphate concentrations (0.3–2 mmol/L) had no effect on glucose stimulated insulin release from mouse islets in vitro. Therefore, the elevated insulin levels observed in high calcium-supplemented rats was not secondary to decreased phosphorus levels; it may be due instead to direct effect of calcium on β cells.

Our findings are in line with the findings of Cade and Norman[8] who observed insulin deficiency in vitamin D deficiency. The chronic insulin deficiency in a vitamin D-deficient state might alter insulin sensitivity of the peripheral tissues, thereby affecting glucose utilization. The simplest method to estimate in vivo insulin sensitivity is to measure plasma glucose levels after intravenous or intraperitoneal insulin administration [i.e., insulin tolerance test (ITT)]. This test has been used in both humans[29,30] and animals[31] to estimate in vivo insulin action. The ITT conducted in different experimental groups has revealed that vitamin D-deficient rats were hyperresponsive to insulin, suggesting that insulin sensitivity is enhanced in these animals. Our results are comparable to the study in the n0-streptozotocin model wherein rats exhibited chronic moderate insulin deficiency and increased insulin action has been reported in these rats.[32] From our data, it may be postulated that the insulin-dependent glucose utilization by tissues is clearly enhanced as a consequence of mild chronic hypoinsulinism. Earlier studies indicate that there is an inverse relationship between circulating insulin levels and insulin binding.[33,34] In addition, the circulating insulin concentration has been shown to modulate the number of insulin receptors.[34,35] Similar to our results in a vitamin D-deficient rat model, the diabetic Chinese hamsters have low plasma insulin, a low insulin response to glucose load, and consequently low glucose tolerance. In this model, the liver has been reported to adapt to decreased hormone concentration by raising the number of receptors in plasma membrane; similar results were observed in experimentally induced diabetes.[36] Hence it could be presumed that insulin receptor concentration may be enhanced in a vitamin D-deficient insulinopenic rat model. Interestingly, the high calcium-supplemented group that had high circulating insulin had a response similar to vitamin D given controls, suggesting no alteration in insulin sensitivity.

In conclusion, the present study demonstrates that impaired glucose tolerance and reduced serum insulin levels encountered in vitamin D deficiency are reversible not only by vitamin D administration but also by calcium supplementation alone, and insulin sensitivity appears to be enhanced in a vitamin D-deficient state.

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