Calcium Intake Regulates 1,25-Dihydroxy-Vitamin D Formation in the Diabetic Rat

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SUMMARY

Duodenal calcium absorption and serum 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$] concentrations are decreased and body growth is arrested in the streptozotocin-diabetic rat taking commercial chow with high (1.2–2%) calcium content. Treatment with insulin restores 1,25-$(OH)_2D_3$, calcium absorption, and body growth to normal. We hypothesized that the depressed 1,25-$(OH)_2D_3$ in diabetics is due in part to the minimal requirement for vitamin-D-mediated intestinal calcium transport under conditions of arrested growth and high calcium intake. We tested this hypothesis by comparing the response of serum 1,25-$(OH)_2D_3$ concentration to low (0.02%) and normal (0.5%) calcium diets in control and streptozotocin-diabetic rats. To evaluate response to short-term insulin treatment, serum 1,25-$(OH)_2D_3$ was measured after 12 or 36 h of treatment. Serum 1,25-$(OH)_2D_3$ concentrations in the 0.5% calcium diet groups were 175, 25, and 120 pg/ml for control, diabetic, and insulin-treated 36-h groups, respectively. Low calcium diets increased concentrations to 625, 100, and 370 pg/ml for controls, diabetics, and insulin-treated 36-h groups, respectively. In conclusion, the diabetic retains the ability to respond to calcium deficiency, even in the insulin-deficient state. Low calcium intake, in addition to enhancing 1,25-$(OH)_2D_3$ formation in diabetics, also modulates the response to insulin treatment. These studies demonstrate that the regulatory factor(s) suppressing serum, 1,25-$(OH)_2D_3$ in diabetes is not simply insulin deficiency per se. Implications of these findings for diabetes are discussed. DIABETES 31:401–405, May 1982.

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Calcium has a central role in the coordination of insulin release,[1,2] and insulin release is impaired in vitamin D deficiency.[3] Insulin has a permissive role in the stimulation of 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$]* formation by parathyroid hormone in renal tubule cells in culture.[4] In this paper we describe studies relating to this interrelationship between diabetes, vitamin D, and calcium metabolism.

Duodenal calcium transport[5] and a specific vitamin-D-dependent calcium binding protein[6] are greatly decreased in the streptozotocin-diabetic rat. Decreased serum 1,25-$(OH)_2D_3$[7,8] appears to explain this response, since treatment with 1,25-$(OH)_2D_3$ but not 25-OHD_3 reverses the defects[9,10] and treatment with insulin for 10 days restores serum 1,25-$(OH)_2D_3$ to normal,[7,8] and corrects the decreased duodenal calcium absorption.[11] The defects in vitamin D metabolism and calcium transport in diabetes appear to be caused by decreased conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$, since (1) metabolic clearance of 1,25-$(OH)_2D_3$ is not increased in diabetes, (2) uptake of 1,25-$(OH)_2D_3$ by the intestinal mucosa is normal, and (3) conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ is decreased by 60% in diabetes.[12] The cause for decreased formation of 1,25-$(OH)_2D_3$ is unknown. Serum immunoreactive parathyroid hormone (iPTH) is increased in both fasted[13] and fed[14] diabetic rats, probably due to secondary hyperparathyroidism, since serum total and ionized calcium tend to be depressed in diabetic rats.[5,11,14] Insulin treatment restores the elevated iPTH[14] and depressed serum calcium to normal.[11] Serum inorganic phosphorus is normal in diabetes.[7,9,14] Thus, parathyroid insufficiency and elevated serum inorganic phosphorus, the two major factors depressing 1α-hydroxylation, are not responsible for the depression of 1,25-$(OH)_2D_3$. In addition, serum immunoreactive calcitonin is decreased in diabetes, and is restored to normal by insulin treatment.[14]

* The following abbreviations are used: 25-hydroxyvitamin D_3: 25-OHD_3; 1,25-dihydroxyvitamin D_3: 1,25-$(OH)_2D_3$; 25,26-dihydroxyvitamin D_3: 25,26-$(OH)_2D_3$; 24,25-dihydroxyvitamin D_3: 24,25-$(OH)_2D_3$; 25-hydroxyvitamin D_3-26,23-lactone: 25-OHD_3-26,23-lactone; serum immunoreactive parathyroid hormone: serum iPTH.

Prior studies of calcium and vitamin D metabolism in diabetes were carried out using commercial rat chows, which have a high calcium content (1–2%). Since low calcium intake increases formation of 1,25-$(OH)_2D_3$ in normal animals,[15,16] we tested this stimulus in diabetes. We also evaluated the effects of short-term insulin treatment.

MATERIALS AND METHODS

Animals and diets. Male albino rats (Sprague-Dawley strain, King) weighing 120–140 g were housed in the animal care unit with temperature and humidity controlled and a dark cycle from 6 p.m. to 6 a.m. After 1 wk, during which weight was monitored to establish normal growth, the shipment was divided randomly into two groups and each animal was housed in a stainless steel metabolic cage. One group received a semisynthetic diet of 0.5% calcium content ("normal calcium diet") and the other group received a diet containing no added calcium (0.02% calcium by analysis, "low calcium diet"). Both diets contained 0.3% phosphorus, 18% protein (vitamin-free casein, Teklad Test Diets, Madison Wisconsin), 10% fat (cottonseed oil, Teklad), 0.2% L-cystine (Fisher), 2.9% calcium-free mineral mix, and 1% vitamin-D-deficient vitamin mix (ICN Nutritional Biochemicals, Cleveland, Ohio). Dextrose monohydrate 63.1% was present in the low calcium diet, and dextrose content was reduced by 1.1% in the normal calcium diet to compensate for 1.1% $CaCO_3$ added to give 0.5% calcium content. Each rat was given orally 222 IU vitamin D_3 (Sigma Co., St. Louis, Missouri) dissolved in corn oil daily.

Food intakes and body weights were measured daily. After 7 days, each diet group was subdivided. One-fourth of each diet group was injected intraperitoneally with citrate buffer (controls) and the remainder were injected with streptozotocin (Upjohn, Kalamazoo, Michigan), 80 mg/kg body weight, freshly dissolved in citrate buffer pH 4.5 (diabetics). Onset of diabetes was established by glucosuria, polyuria, and weight loss. Rats continued on their respective diets, the controls being restricted to the intake of diabetics until diabetics became hyperphagic (day 4). Subsequently, diabetics were limited to intake of the controls.

Insulin treatment. At 9:00 p.m. on the 12th or 13th day, one-third of the diabetics in each diet group received insulin (Lilly, Indianapolis, Indiana), 1 U per 50 g body weight subcutaneously. Insulin injection of this group was repeated at 9:00 p.m. on the following day and this group is referred to as the Diabetic + Insulin—36-h group. A second group of diabetics was treated with insulin at 9:00 p.m. on the 13th or 14th day and killed 12 h later and is referred to as the Diabetic + Insulin—12-h group. The remainder of the diabetics from each diet were untreated. The rats were killed between 8:00 and 10:00 a.m. on the 14th and 15th days following the start of dietary regimen. Animals from each of the four subgroups of each diet group were killed each day. Animals were killed, after anesthetization with intraperitoneal sodium pentobarbital (Nembutal) and opening of the abdomen at the midline, by exsanguination from the bifurcation of the aorta. Blood was clotted on ice and centrifuged, and the serum was separated. An aliquot was removed for analysis of calcium (atomic absorption spectrometry), phosphorus,[17] and glucose,[18] and the remainder was stored at −60° until assay for metabolites of vitamin D.

Vitamin D metabolite assay. Vitamin D and its metabolites were extracted from serum and the extract resolved by three purification steps involving one Sephadex LH-20 and two high-pressure liquid chromatographic (HPLC) columns.[19] 1,25-$(OH)_2D_3$ and 25,26-$(OH)_2D_3$ were separated by HPLC on Zorbax Sil (11:89 isopropanol:hexane). The 24,25-$(OH)_2D_3$ complex from this column was resolved using HPLC on Zorbax Sil with 2:98 isopropanol:methylene chloride. Individual compounds were quantitated by competitive protein binding assay.

The study plan was replicated three times, and data from the three studies were pooled and analyzed by one-way analysis of variance and Tukey's multiple comparison test for comparisons within diet groups, or unpaired Student's *t* test, for comparing effects of low calcium diet.

RESULTS

Body weights of all groups were similar at the start of the study and 7 days later when groups were injected with buffer or streptozotocin (Figure 1A and B). Diabetic rats lost weight progressively, whereas controls gained approximately 8 g/day, except for the several days postinjection when they were restricted to intake of diabetics. Insulin reversed the decline in weight in the 12- and 36-h treatment groups.

Serum glucose (Table 1) is similar in controls for the 0.5% and 0.02% calcium diet groups. Serum glucose is also similar for diabetics taking 0.5% and 0.02% calcium diets, but greatly elevated compared with controls. Insulin-treatment lowered serum glucose to near control values except in the 0.5% calcium diet Diabetic + Insulin—12-h group. Serum calcium (Table 1) was lower in all groups fed low calcium diet as compared with the 0.5% calcium diet. When the 0.5% calcium diet was fed, diabetes lowered serum calcium compared with controls, but insulin restored serum calcium to the control level. However, insulin treatment for 12 or 36 h did not change the serum calcium depression in the low calcium diet groups. Phosphorus in serum did not differ among the groups taking a given diet. Diabetes depressed serum phosphorus in the 0.5% as compared with the low calcium diet groups (Table 1, Diabetics, untreated, and Diabetics + Insulin—12-h).

Concentration of 1,25-$(OH)_2D_3$ in serum is shown in Figure 2. Serum 1,25-$(OH)_2D_3$ concentration was 3.5- to 4-fold greater in all groups taking the low calcium diet compared with groups taking 0.5% calcium: controls, 625 vs. 175 pg/ml; diabetics, 100 vs. 25 pg/ml; 36-h insulin treatment, 370 vs. 120 pg/ml. Diabetes decreased 1,25-$(OH)_2D_3$ concentration below control levels in both 0.5% calcium and low calcium diet groups.

Insulin treatment for 12 h increased mean serum 1,25-$(OH)_2D_3$ concentration above that of the untreated diabetics in both 0.5% and 0.02% calcium diet groups, but not significantly. In the 0.5% calcium diet group insulin treatment for 36 h increased 1,25-$(OH)_2D_3$ concentration from 25 pg/ml in the untreated diabetic group to 120 pg/ml, which did not differ from the control level (175 pg/ml). Serum 1,25-$(OH)_2D_3$ in the low calcium diet group treated with insulin for 36 h was 370 pg/ml, which was below the control level of 625 pg/ml.

Serum concentrations of four other metabolites of vitamin D_3 are shown in Table 2: 25-OH-D_3, 25,26-$(OH)_2D_3$, 24,25-$(OH)_2D_3$, and 25-OH-D_3-26,23-lactone. Concentrations of

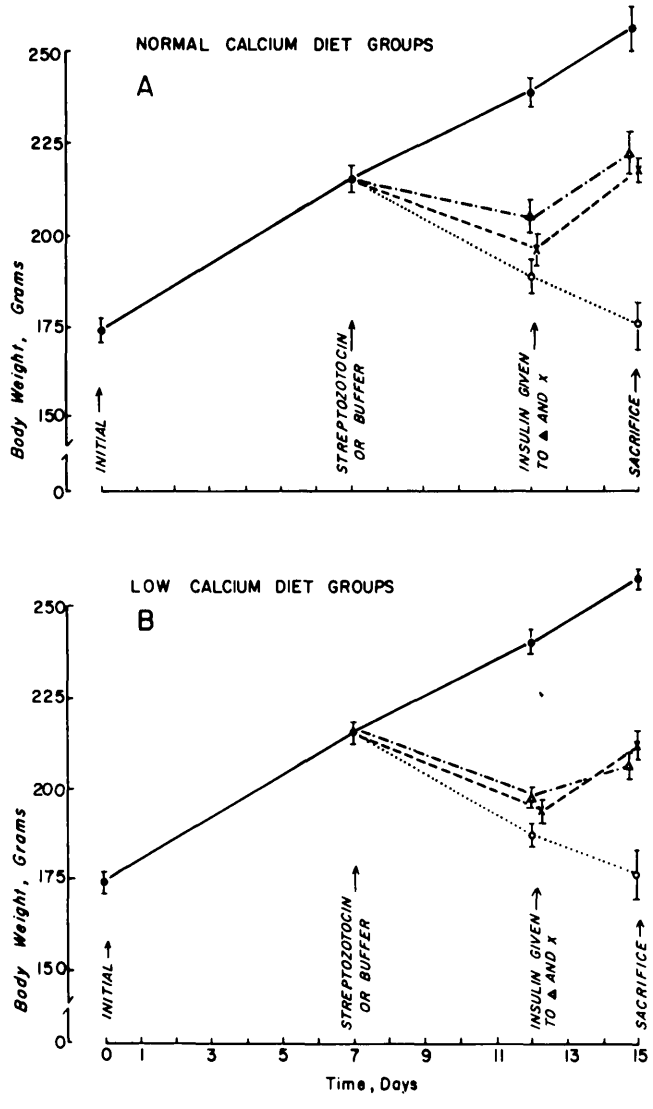

FIGURE 1. Mean body weights of Controls (●), Diabetics (○), Diabetics + Insulin—12-h (△), and Diabetics + Insulin—36-h (x) for normal calcium diet groups are shown in (A). The same groups taking low calcium diet are shown in (B). Vehicle buffer or streptozotocin in buffer was injected after rats had been on diets 7 days. Insulin was given at 9:00 p.m., 36 and 12 h before the rats were killed, to the Diabetic + Insulin—36-h groups, and at 9:00 p.m. to the Diabetic + Insulin—12-h groups. Start of insulin treatment was adjusted so that animals from all groups were killed on either the 14th or 15th day. Sacrifice weights are the mean of animals killed on the 14th and 15th day.

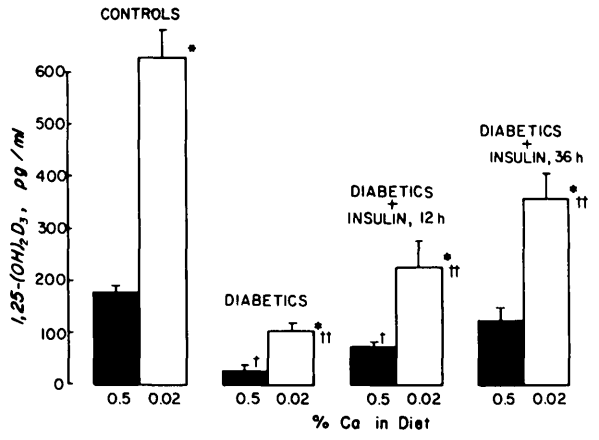

FIGURE 2. Concentrations of 1,25-$(OH)_2D_3$ in serum (mean ± SE) are shown for the Controls, Diabetics, Diabetics + Insulin—12-h, and Diabetics + Insulin—36-h groups taking 0.5% calcium diet by solid bars. Open bars indicate the same groups taking 0.02% calcium diet. *0.02% calcium diet groups > 0.05% calcium diet group, $P < 0.01$; †less than control group within 0.5% calcium diet group, $P < 0.05$; ††less than control group within 0.02% calcium diet group, $P < 0.05$. Data were analyzed by one-way analysis of variance for comparing groups within a diet set. The unpaired Students' *t* test was used to compare 0.5% calcium diet groups with 0.02% calcium diet groups.

the precursor of 1,25-$(OH)_2D_3$, 25-OH-D_3, are the same in all groups, and there is no effect of diet or diabetes. Serum concentrations of 25,26-$(OH)_2D_3$, 24,25-$(OH)_2D_3$, and 25-OH-D_3-26,23-lactone are elevated in the Diabetic + Insulin—12-h group, comparing normal calcium groups with the low calcium groups. 24,25-$(OH)_2D_3$ in untreated diabetic and the Diabetic + Insulin—36-h groups is lower than in the control and Diabetics + Insulin—12-h groups, when 0.5% calcium diet was fed.

DISCUSSION

This study demonstrates that the concentration of 1,25-$(OH)_2D_3$ in serum is greatly depressed in the streptozotocin-diabetic rat fed either 0.5% or low calcium diets as compared with corresponding controls (Figure 2). Low calcium diet increased 1,25-$(OH)_2D_3$ concentration fourfold in diabetics and threefold in controls, compared with corresponding groups fed the 0.5% calcium diet. Thus, the response of serum 1,25-$(OH)_2D_3$ to low calcium diet in diabetics is appropriate.

Animals received a daily dose of vitamin D_3, which produced similar serum 25-OH-D_3 levels in all groups (Table 2).

TABLE 1
Glucose, calcium, and phosphorous in serum (mg/dl, mean ± SE)

Parameter	Diet calcium content (%)	Controls	Diabetics, untreated	Diabetics + Insulin	
				12 h before killing	36 and 12 h before killing
Glucose	0.5	195 ± 7	527 ± 20	436 ± 92*	220 ± 94
	0.02	204 ± 6	492 ± 21	87 ± 10	204 ± 68
Calcium	0.5	10.34 ± 0.06*	9.90 ± 0.12*‡	10.21 ± 0.13*	10.34 ± 0.22*
	0.02	9.55 ± 0.08	9.51 ± 0.11	9.52 ± 0.10	9.54 ± 0.10
Phosphorous	0.5	8.46 ± 0.32	7.61 ± 0.18†	7.71 ± 0.38†	7.28 ± 0.58
	0.02	8.96 ± 0.27	8.53 ± 0.32	9.04 ± 0.48	7.17 ± 0.34

* 0.5% Ca diet group > 0.02% diet group (unpaired *t* test, $P < 0.05$).
† 0.5% Ca diet group < 0.02% Ca diet group (unpaired *t* test, $P < 0.05$).
‡ Diabetics < controls (ANOVA and Tukey's test, $P < 0.05$).

TABLE 2
Responses in serum concentrations of other vitamin D_3* metabolites to diabetes and calcium content of diet (ng/ml, mean ± SE)

Metabolite	Calcium content of diet (%)	Controls	Diabetics, untreated	Diabetics + Insulin	
				12 h before killing	12 and 36 h before killing
25-OH-D_3	0.5	38 ± 4	37 ± 3	37 ± 3	44 ± 4
	0.02	28 ± 3	38 ± 2	37 ± 2	38 ± 2
25,26-$(OH)_2D_3$	0.5	4.9 ± 0.4	3.7 ± 0.6	4.6 ± 0.3	2.8 ± 0.3
	0.02	3.8 ± 0.3	2.6 ± 0.3	2.6 ± 0.3†	2.5 ± 0.2
24,25-$(OH)_2D_3$	0.5	13.0 ± 1.8	8.5 ± 0.3‡	15.0 ± 0.7	7.3 ± 0.3‡
	0.02	9.5 ± 1.4	9.2 ± 1.2	8.7 ± 1.1†	6.5 ± 0.3
25-OH-D_3-26,23 Lactone	0.5	2.6 ± 0.2	2.9 ± 0.5	4.6 ± 0.3	2.2 ± 0.1
	0.02	2.4 ± 0.2	1.8 ± 0.3	1.8 ± 0.1†	1.6 ± 0.2

* Serum vitamin D_3 concentrations did not differ among groups.
† 0.5% Ca diet group differs from 0.02% group, $P < 0.001$.
‡ Differs from Control and Diabetic + Insulin–12-h within 0.5% Ca diet group, $P < 0.05$.

Hence, precursor 25-OHD_3 was not limiting for sudden changes in vitamin D metabolism produced by insulin treatment. To obtain a more definitive picture of vitamin D metabolism in diabetes and insulin treatment under conditions of normal and low calcium diet, we measured several other metabolites. In comparison with effects of diabetes and insulin treatment on 1α-hydroxylation (Figure 2), effects on other metabolites were minimal.

Although the mechanisms determining response of 1,25-$(OH)_2D_3$ to low calcium diet are intact in the diabetic rat, they determine much lower concentrations in diabetics than in controls. Metabolic factors in diabetics probably determine the regulation of 1,25-$(OH)_2D_3$ at low levels. In contrast to controls, which are growing rapidly and have a large requirement for exogenous calcium, growth is arrested in diabetics and most animals are losing weight (Figure 1). Hence, diabetics would be overloaded with calcium and at risk for calcium intoxication if they continued to absorb calcium at the high rate set in the growing animals. This is prevented in diabetics taking diets of normal calcium content by the low rate of the vitamin-D-dependent component of calcium absorption[5] determined by decreased 1,25-$(OH)_2D_3$. Feeding a low calcium diet lowers serum calcium, eliciting a requirement for bone calcium mobilization and exogenous calcium in diabetics, and they responded normally by increasing 1,25-$(OH)_2D_3$ (Figure 2). These animals with untreated diabetes would not be expected to achieve the levels of 1,25-$(OH)_2D_3$ of the controls, which are growing rapidly and have a greater requirement for calcium.

Seven days of insulin treatment of diabetic rats taking a normal calcium diet restores body growth rate and serum 1,25-$(OH)_2D_3$ to normal.[7] Thus, insulin treatment is required to reset 1,25-$(OH)_2D_3$ regulation in the diabetic to the normal response range. In the present study, 36 h of insulin treatment restored 1,25-$(OH)_2D_3$ concentration to the corresponding control level in diabetics taking the 0.5% calcium diet (Figure 2). The response of serum 1,25-$(OH)_2D_3$ in diabetics to insulin treatment was much greater in the group taking the low calcium diet than in the 0.5% calcium diet group. The finding that the diabetic requires insulin to restore vitamin D metabolism to normal is consistent with prior work. Insulin is permissive for the PTH stimulation of 1,25-$(OH)_2D_3$ production in cultured kidney cells.[4]

Taken together, the responses of serum 1,25-$(OH)_2D_3$ of diabetics to low calcium diet and to insulin treatment are consistent with the following conclusions: (1) the response to low calcium diet demonstrates that the low 1,25-$(OH)_2D_3$ in diabetics is not determined solely by insulin deficiency; (2) the fact that the level of response to insulin treatment depends in part on calcium content of the diet is also consistent with the conclusion that insulin deficiency per se is not the sole factor determining low 1,25-$(OH)_2D_3$; and (3) insulin treatment and restoration of body growth resets the regulation of 1,25-$(OH)_2D_3$ concentration at the control level.

The increased 1,25-$(OH)_2D_3$ in response to low calcium diet is determined by parathyroid hormone (PTH) activity and inorganic phosphorus concentrations, which are usually measured in serum. The increased conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ in response to low calcium diet does not occur in the thyroparathyroidectomized rat.[16] The increased PTH secondary to low calcium diet may increase 25-OHD-1α-hydroxylase directly[20] or indirectly through effects on inorganic phosphorus concentration. PTH blocks reabsorption of phosphate in the renal tubule. In the animal with low 1,25-$(OH)_2D_3$ (and probably decreased phosphate absorption by the gut such as the diabetic), elevated PTH is expected to depress serum inorganic phosphorus by promoting renal phosphate excretion. Decreased inorganic phosphorus concentration increases 1α-hydroxylase activity independently of PTH,[21] and activity of the renal 25-OHD_3-1α-hydroxylase in the chick is inversely proportional to serum phosphorus concentration.[22] In prior studies, we found elevated serum PTH in diabetic rats fed normal to high calcium diets. Yet we found no relationship of serum phosphate to 1,25-$(OH)_2D_3$ in our studies (Table 1). In fact, serum inorganic phosphorus is not depressed by low calcium diet in comparison with the 0.5% calcium diet. Untreated diabetic and diabetic rats treated with insulin 12 h before they are killed have significantly higher serum phosphorus when fed low calcium diet than when fed 0.5% calcium. Thus, by serum data, inorganic phosphate cannot be identified as a factor determining the low concentration of 1,25-$(OH)_2D_3$ in diabetes (i.e., high phosphate) or the stimulation by low calcium diet (low phosphate).

The elevated PTH in diabetics even when taking the high calcium intake (1–2% calcium by weight) of commercial rat

chows[13,14] rules out parathyroid insufficiency as a cause for low 1,25-$(OH)_2D_3$ in diabetics. This elevated PTH in diabetics presumed to be subjected to calcium overload is paradoxical, but may be the consequence of the nature of the regulatory processes for maintaining serum calcium in the narrow physiologic concentration range. It is clear that the gross regulation of serum calcium through control of calcium absorption rate by 1,25-$(OH)_2D_3$ requires PTH. Under conditions of suppression of calcium absorption, as in diabetics taking a normal calcium diet, the fine regulation of serum calcium may require high PTH concentrations.

Several studies have established relationships between diabetes, vitamin D metabolism, and insulin secretion. Vitamin-D-deficiency depresses insulin secretion.[3] The physiologic significance of this observation is supported by the presence of a cytosol receptor for 1,25-$(OH)_2D_3$[23] and a vitamin-D-dependent calcium binding protein[24] in the pancreas. In addition, insulin is permissive for increased production of 1,25-$(OH)_2D_3$ in response to PTH in kidney cells.[4] Our finding that 1α-hydroxylation is greatly depressed in the diabetic rat is consistent with these interrelationships. The present paper demonstrates that insulin is not an absolute requirement for responses in vitamin D metabolism to improve calcium homeostasis in diabetes.

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