Demonstration that the Vitamin D Metabolite 1,25(OH)$_2$-Vitamin D$_3$ and Not 24R,25(OH)$_2$-Vitamin D$_3$ Is Essential for Normal Insulin Secretion in the Perfused Rat Pancreas

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SUMMARY

It has previously been shown that vitamin D deficiency impairs arginine-induced insulin secretion from the isolated, perfused rat pancreas (Science 1980; 209:823–25). Since vitamin D is known to be metabolized to 1,25-dihydroxyvitamin D$_3$ (1,25[OH]$_2$D$_3$) and 24R,25-dihydroxyvitamin D$_3$ (24,25[OH]$_2$D$_3$), it is essential to clarify which vitamin D metabolite has the important role of enhancing insulin secretion. In this report, a comparison is made of the relative efficacy of 3-wk repletion with vitamin D$_3$ (980 pmol/day), 1,25(OH)$_2$D$_3$ (39 pmol/day or 195 pmol/day), and 24,25(OH)$_2$D$_3$ (650 pmol/day) on arginine-induced insulin secretion from the isolated, perfused rat pancreas; in this experiment, the daily caloric intake of the animals receiving vitamin D or its metabolites was controlled by pair feeding to the caloric intake of the vitamin D-deficient rats. 1,25(OH)$_2$D$_3$ repletion was found to completely restore insulin secretion to the levels seen in vitamin D$_3$-replete, pair-fed controls in both the first and second phases, while 24R,25(OH)$_2$D$_3$ only partially improved insulin secretion, and then only in the first phase.

Changes of both serum calcium levels and dietary caloric intake after vitamin D metabolite administration are concluded to play a lesser role on the enhancement of insulin secretion, since, in a separate experiment, vitamin D-deficient rats with normal serum calcium levels did not show recovery of insulin secretion equivalent to the vitamin D-replete animals under conditions of dietary pair feeding. These results suggest that 1,25(OH)$_2$D$_3$ but not 24,25(OH)$_2$D$_3$ plays an essential role in the normal insulin secretion irrespective of the dietary caloric intake and prevailing serum calcium levels. DIABETES 1985; 34:315–20.

In recent years, there have been several lines of evidence to suggest that the endocrine pancreas is also a target tissue for 1,25-dihydroxyvitamin D$_3$ (1,25[OH]$_2$D$_3$), along with the classical vitamin D target organs, the intestine, bone, and kidney. These include: (1) presence of a cytosol receptor protein for 1,25(OH)$_2$D$_3$ in the chick pancreas;[1–3] (2) presence of immunoreactive vitamin D-dependent calcium binding protein (CaBP) in the chick,[4] rat,[5] and porcine[6] pancreas and its induction by 1,25(OH)$_2$D$_3$,[7] as determined by radioimmunoassay; (3) immunohistochemical demonstration of CaBP in the endocrine B-cells of the chick[8] and rat[5] pancreas; and (4) demonstration of the localization of [^{3}H]-1,25(OH)$_2$D$_3$ within the rat pancreatic B-cells by autoradiography.[9] Collectively, these results strongly support the involvement of vitamin D metabolites in the calcium homeostasis of endocrine pancreatic B-cells and suggest that they may play an important role in regulating insulin secretion.

In this respect, we have recently demonstrated a direct interrelationship between pancreatic B-cell function and vitamin D;[10,11] vitamin D deficiency inhibited insulin, but not glucagon secretion from the isolated, perfused rat pancreas, and vitamin D repletion in vivo for 72 h restored insulin secretion. In results compatible with our own, Clark et al.[12] and Chertow et al.[13] have reported that administration in vivo of 1,25(OH)$_2$D$_3$ to vitamin D-deficient rats elevated the secretion of insulin by isolated pancreatic islets.

Since 25-hydroxyvitamin D$_3$ can be metabolized to either or both 1,25(OH)$_2$D$_3$ and 24R,25-dihydroxyvitamin D$_3$ (24,25[OH]$_2$D$_3$),[14] and since there is increasing evidence that 1,25(OH)$_2$D$_3$ cannot produce all the biologic responses attributable to the parent vitamin D,[15–17] it is the purpose of this report to compare vitamin D$_3$ and 24,25(OH)$_2$D$_3$ with 1,25(OH)$_2$D$_3$ with respect to the mediation of arginine-induced insulin secretion from the isolated, perfused pancreas of vitamin D-deficient rats.

MATERIALS AND METHODS

Male weanling rats, obtained from the Holzman Co. (Madison, Wisconsin), were fed ad libitum for 5 wk (while they became vitamin D depleted) a synthetic D-deficient diet[18]

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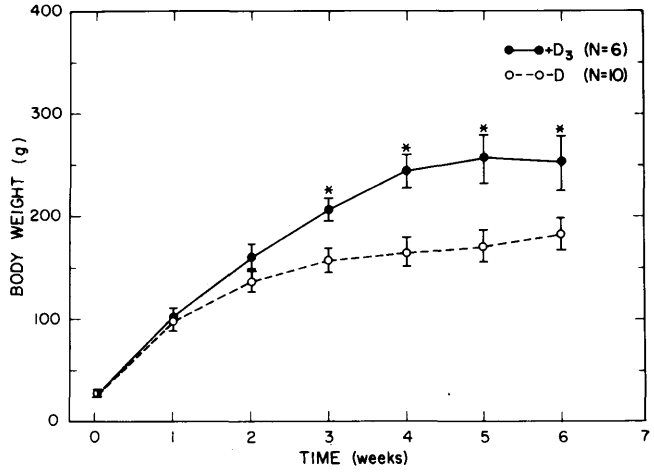

FIGURE 1. Growth curves of rats raised on either a vitamin D-replete or a vitamin D-depleted diet. The vitamin D-replete group (●——●) was given 4.5 nmol vitamin D_3 (three times per week) s.c., while the vitamin D-depleted group (○------○) was administered vehicle alone. Results are expressed as means ± SEM of the indicated numbers. *P < 0.05: significant difference from vitamin D-depleted group.

containing 0.4% Ca and 0.35% P. A group of the vitamin D-depleted animals was administered 4.5 nmol of vitamin D_3 orally three times per week from the weanling state as a normal control group; also the daily change of dietary intake and body weight in this group was studied in comparison with the vitamin D-depleted rats. All animals were housed individually in stainless steel cages with a 12-h light/12-h dark cycle free of UV light. After 5–6 wk, the following two types of experiments were carried out in animals that were allowed access to their available food up to the time of their receiving anesthesia.

Experiment 1. This study was designed to investigate the effect of dietary vitamin D_3 on arginine-induced insulin secretion under conditions designed to eliminate the usual reductions of serum calcium and dietary caloric intake present between vitamin D-depleted and vitamin D-replete animals. Rats that had been depleted of vitamin D for 5 wk (see above) were switched to a vitamin D-deficient diet containing 2% lactose, 1.7% calcium, and 0.35% P for an additional 2 wk. One-half of these rats were also administered vitamin D_3 (4.5 nmol, three times per week) for 2 wk. The vitamin D_3 was dissolved in 0.1 ml of 95% ethanol:1,2-propanediol (1:1, vol/vol), and injected subcutaneously (s.c.). In the control rats, vehicle alone was administered. It has already been reported that a vitamin D-deficient diet containing 20% lactose can support a normal serum calcium level despite an undetectable level of serum 25(OH)D_3.[19] Pancreatic perfusions were carried out using the vitamin D-replete and -deficient animals and their responses to arginine (10 mM)-induced insulin secretion were compared.

Experiment 2. Five-week-old, vitamin D-depleted animals (fed the 0.4% Ca, 0.35% P diet) were divided into five groups of 7–11 animals; they were administered daily doses of vitamin D or metabolites according to the following schedule: group 1, vehicle (D-deficient control); group 2, vitamin D_3, 980 pmol/day (D-replete control); group 3, 1,25$(OH)_2D_3$ 39 pmol/day; group 4, 1,25$(OH)_2D_3$, 195 pmol/day; and group 5, 24R,25$(OH)_2D_3$, 650 pmol/day. All animals were injected s.c. daily for 2 wk with their appropriate doses of vitamin D metabolite dissolved in 0.1 ml of 95% ethanol:1,2-propanediol (1:1, vol/vol). The rats of groups 2–5 were pair fed with the rats of group 1, which were given free access to their diet. These doses were selected based on this laboratory's understanding of the relative biologic activities of the two dihydroxylated metabolites when they are administered chronically; thus, 1,25$(OH)_2D_3$ is some 4–5 times more potent than the parent vitamin D_3,[18] while 24R,25$(OH)_2D_3$ has been shown to be 1.5 times more active than vitamin D_3.[27]

Method of pair feeding. The daily dietary intake of the vitamin D-deficient control rats for both experiments 1 and 2 was measured gravimetrically every day using a commercial rat food device (Wahnann Co., Maryland) and the mean dietary intake of these animals was given to the vitamin D-replete rats (scheduled to be pair fed) the next day.

Pancreatic perfusion. The pancreas was isolated and perfused by the procedure described by Grodsky et al.[20] with minor modifications.[21] The pancreas and duodenum were perfused with a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.5% BSA and 4.6% dextran T70 (Sigma, St. Louis, Missouri) equilibrated with 95% O_2-5% CO_2 at 37°C. After 15 min equilibration time with this medium containing 5.5 mM glucose, L-arginine hydrochloride solution was infused from a side-arm syringe at 0.5 ml/min for 20 min to maintain a final concentration of 10 mM. Successive 2-ml fractions of portal effluent were collected for 30–40 min and frozen until assayed for insulin. For all rats undergoing pancreatic perfusion, blood samples (3 ml) were obtained from the jugular vein just before removal of the pancreas and collected in heparinized tubes. Plasma was stored at −20°C until it was assayed for glucose and serum calcium.

Immunoreactive insulin (IRI) was measured by RIA using antibody raised against porcine insulin, and rat insulin (Novo, Denmark) was used as a standard.[22] Serum Ca was determined by atomic absorption analysis.[23] Serum phosphorus was measured by the method of Chen et al.[28] Statistical assessment of data was made using Student's *t*-test of means.

RESULTS

Change of body weight and dietary intake in vitamin D-treated and vitamin D-deficient rats (Figures 1 and 2). Figures 1 and 2 show the change of body weight and daily

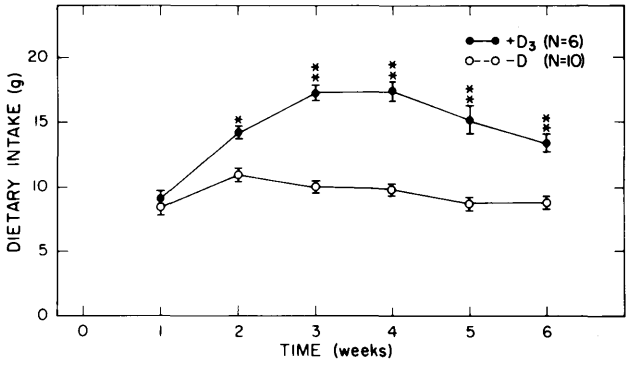

FIGURE 2. Change of daily dietary intake between vitamin D-replete and vitamin D-depleted rats for 6 wk after weaning. The vitamin D-replete group (●——●) was given 4.5 nmol vitamin D_3 (three times per week) s.c., while the vitamin D-depleted group (○------○) was administered vehicle alone. Results are expressed as means ± SEM of the indicated numbers. *P < 0.05, **P < 0.001: significant difference from vitamin D-depleted group.

TABLE 1
Comparison of the effect of dietary vitamin D_3 treatment on the 10 mM arginine-induced first and second phases of insulin secretion from the isolated, perfused pancreas of the vitamin D-deficient rat with normal serum calcium level (experiment 1)

Group	Treatment	N	Body wt (g)	Serum Ca (mg/dl)	Serum P (mg/dl)	Insulin release (ng) First phase (t_{0-6})	Second phase (t_{7-20})	Total (t_{0-20})
1	Vehicle	6	175 ± 8	9.5 ± 0.4	2.7 ± 0.4	147 ± 19	194 ± 27	340 ± 40
2	Vitamin D_3	6	168 ± 4	10 ± 0.2	5.1 ± 0.3*	240 ± 15†	448 ± 18*	687 ± 31*

*$P < 0.001$, †$P < 0.05$: significant difference from group 1.
Results are expressed as mean ± SEM of the indicated numbers. Vitamin D_3 (4.5 nmol) was administered to the rats of group 2 s.c. three times per week for 2–3 wk; vehicle alone was administered to the rats of group 1. Also, the daily caloric intake of the vitamin D-replete rats was restricted to the daily dietary consumption of the vitamin D-deficient rats.

dietary intake between the vitamin D-replete and -depleted groups for weeks 1–6. The daily dietary intake was measured in each rat, and the mean daily dietary intake was calculated at the end of every week. The body weight was measured every week. The mean daily dietary intake was not significantly different in the first week after weaning, while this value was significantly higher in the vitamin D-replete group thereafter.

These results suggested that pair feeding is required between vitamin D-replete and vitamin D-depleted rats to eliminate nutritional factors that may affect insulin secretion.

Effect of vitamin D_3 on arginine (10 mM)-induced insulin secretion in the isolated, perfused pancreas of the vitamin D-deficient rat subjected to normalization of serum calcium level (Table 1, Figure 3). In this study, as shown in Table 1, we successfully prepared normocalcemic, vitamin D-deficient animals (experiment 1); serum calcium = 9.5 ± 0.4 mg/dl. The vitamin D repletion in these animals resulted in a serum calcium of 10.0 ± 0.2 mg/dl serum, which is not significantly different from the control animals. Also, as noted in Table 2, the serum P levels in these two groups were significantly different. Moreover, since pair feeding was carried out between these two groups during the treatment of vitamin D_3 or vehicle, similar body weights were also obtained. The blood glucose level was also not significantly different between the two groups (112 ± 4 versus 108 ± 5 mg/dl). As shown in Figure 3, arginine-induced insulin secretion showed a typical biphasic pattern in both groups. The peak value of insulin secretion in the first phase appeared 3 min after the introduction of arginine, and showed a higher value in the vitamin D-replete group as compared with the vitamin D-deficient group (32 ± 5 versus 20 ± 4 ng/ml, $P < 0.05$). The second phase of insulin release also showed significantly higher values at all points measured during the arginine infusion. The data on the cumulative insulin secretion (Table 1) in the first (t_{0-6}) and second phase (t_{7-20}) showed a significant enhancement in the vitamin D-replete group, which was more markedly different in the second phase. These results demonstrate that vitamin D_3 or its metabolites stimulate insulin secretion through some mechanism irrespective of the dietary intake and prevailing serum calcium level.

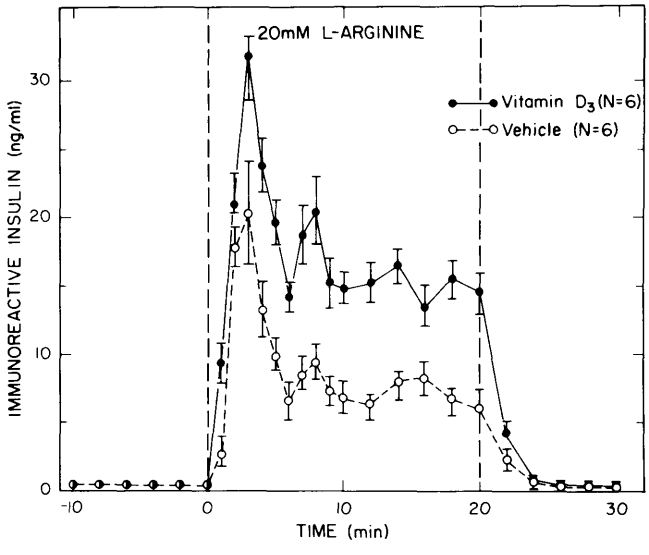

FIGURE 3. Effect of vitamin D_3 treatment on the 10 mM arginine-induced insulin release from the isolated, perfused pancreas of the vitamin D-deficient rat with normocalcemia. The daily dietary caloric intake of the vitamin D-replete rats was also restricted to the daily dietary consumption of the vitamin D-deficient rats. The means ± SEM of the indicated number of experiments are recorded.

Effect of vitamin D metabolite administration on arginine (10 mM)-induced insulin secretion in the isolated, perfused rat pancreas. The serum level of calcium (Table 2) in the vitamin D-deficient control group (group 1) was 5.6 ± 0.1 mg/dl, which was significantly lower than in any of the vitamin D metabolite-replete groups (groups 2–5); no significant difference was observed in the serum Ca levels among groups 2–5. As expected, the serum P-values for the rats of group 1, which were hypocalcemic, were significantly higher than for the normal calcemic rats of groups 2–5. Also vitamin D deficiency and repletion under the conditions of pair feeding resulted in no differences in the body weights and serum glucose levels (Table 2).

As shown in Figure 4, arginine-induced insulin secretion showed a typical biphasic pattern in vitamin D-deficient and in all vitamin D-replete groups. However, peak values and cumulative insulin secretion of the first and second phases were significantly higher in vitamin D_3-replete controls compared with vitamin D-deficient controls (peak of first phase: 25 ± 2.7 ng/ml versus 8.8 ± 1.8 ng/ml, $P < 0.01$; integrated first phase 150 ± 12 ng versus 53 ± 7 ng, $P < 0.001$; peak of second phase 7.6 ± 1.8 ng/ml versus 4.1 ± 0.6 ng/ml, $P < 0.005$; integrated second phase 181 ± 14 ng versus 91 ± 12 ng). For clarity, only the results from one group (group 4) treated with $1,25(OH)_2D_3$ are presented in Figure 4; however, essentially identical results were obtained with the rats in groups 3 and 4, which are the rats that received the low and high physiologic doses of $1,25(OH)_2D_3$ alone.

TABLE 2
Parameters of vitamin D-deficient and vitamin D-replete rats (experiment 2)

Group	Treatment	N	Body wt (g)	Serum Ca (mg/dl)	Serum P (mg/dl)	Serum glucose (mg/dl)
1	Vehicle	11	182 ± 8	5.6 ± 0.1	7.2 ± 0.3	110 ± 5
2	D_3	7	177 ± 8	10 ± 0.2*	5.4 ± 0.2*	107 ± 5
3	$1,25(OH)_2D_3$	8	177 ± 4	9.7 ± 0.5*	6.7 ± 0.3	109 ± 6
4	$1,25(OH)_2D_3$	6	178 ± 4	9.8 ± 0.8*	6.5 ± 0.2	106 ± 9
5	$24,25(OH)_2D_3$	7	177 ± 12	9.5 ± 0.7*	5.3 ± 0.3*	115 ± 9

*$P < 0.001$: significant difference from group 1.
Results are expressed as mean ± SEM of the indicated numbers. As described in the text, the rats of groups 2–5 were pair fed with the vitamin D-deficient rats of group 1 for 2 wk while they received the indicated vitamin D or D-metabolites.

The $1,25(OH)_2D_3$-treated rats (group 3) also showed a statistically higher insulin secretion than vitamin D-deficient control rats in both first and second phases; the extent of first- and second-phase secretion of insulin (for both groups 3 and 4) was also indistinguishable from that achieved by the parent vitamin D_3 (group 2).

In contrast, while $24,25(OH)_2D_3$ treatment enhanced first-phase insulin release to a value significantly higher than in the vitamin D-deficient controls (peak first-phase value: 16 ± 3 versus 8.8 ± 1.8 ng/ml, $P < 0.01$), the level achieved was still significantly less than that achieved by the vitamin D-replete group (peak first phase: 25 ± 2.7 ng/ml, $P < 0.05$).

Data on the cumulative insulin secretion (Table 3) in the first ($t_{0\ 6}$) and second phase ($t_{7\ 20}$) showed a significant enhancement of insulin secretion in both $1,25(OH)_2D_3$-treated groups, which was comparable to that achieved by the vitamin D-replete controls. The $24,25(OH)_2D_3$ treatment was approximately equivalently effective in supporting both first- and second-phase insulin release. Thus, as shown in Table 4, the ratio of insulin secreted for ($24,25[OH]_2D_3$, group 4)/(−D, group 1) was 1.70 and 1.31, respectively, for first and second phase; this was 40% and 34%, respectively, of the first- and second-phase response achieved by the vitamin D_3 (group 2) or $1,25(OH)_2D_3$-treated (group 4) animals (Table 4).

DISCUSSION

The present study clearly demonstrates that vitamin D_3 repletion improves the impaired insulin secretion associated with vitamin D deficiency through mechanisms that are not dependent on the prevailing serum calcium level or dietary caloric intake; this confirms our previous observation.[10] In addition, the results strongly suggest that it is the $1,25(OH)_2D_3$ metabolite rather than the $24,25(OH)_2D_3$ metabolite of the parent vitamin D_3 that is capable of generating this biologic response. This result is not unexpected, since it is already known[4] that $1,25(OH)_2D_3$ is much more effective than $24,25(OH)_2D_3$ in inducing vitamin D-dependent calcium binding protein (CaBP). Thus, the facts that CaBP is present in the pancreatic B-cell and that CaBP is normally induced by $1,25(OH)_2D_3$ were suggestive that $1,25(OH)_2D_3$ was the responsible metabolite of vitamin D_3 with regard to insulin secretion.

It is possible to identify several possible physiologic mechanisms that might explain the involvement of $1,25(OH)_2D_3$ in insulin secretion. These include: (1) an enhanced dietary intake; (2) enhanced levels of serum or ionized calcium; and (3) a direct interaction of the biologically active forms of vitamin D metabolites, $1,25(OH)_2D_3$ and/or $24,25(OH)_2D_3$, with pancreatic islet B-cells to effect secretion of insulin.

It is amply documented that differences in dietary caloric intake affect the secretion of insulin.[24,25] Our study, however, was carried out under conditions of pair feeding between vitamin D-deficient and all groups of animals receiving vitamin D or metabolite repletion. Therefore, we conclude that the putative effects of vitamin D or its metabolites on caloric intake, which are reported in Figure 2, are not involved in the present study. However, it was not possible to control the rate of food delivery and, hence, caloric intake to the pair-fed animals of groups 2–5. Thus, it is possible that these animals, which tended to consume their dietary aliquot more

TABLE 3
Comparison of the effect of vitamin D_3, $1,25(OH)_2D_3$, and $24,25(OH)_2D_3$ treatment on the 10 mM arginine-induced first and second phases of insulin secretion from the isolated, perfused pancreas of the rat (experiment 2)

Group	Treatment	N	First phase (ng) (t_{0-6})	Second phase (ng) (t_{7-20})	Total (ng) (t_{0-20})
1	Vehicle	11	53 ± 7	91 ± 12	145 ± 16
2	Vitamin D_3	7	150 ± 12†	181 ± 14†,‡	331 ± 19†,‡
3	$1,25(OH)_2D_3$	8	138 ± 13†	178 ± 11†,‡	316 ± 42†,‡
4	$1,25(OH)_2D_3$	6	158 ± 19†,‡	177 ± 21†,‡	336 ± 22†,§
5	$24,25(OH)_2D_3$	7	90 ± 12*	119 ± 10	209 ± 21*

*$P < 0.05$, †$P < 0.001$: significant difference from group 1.
‡$P < 0.05$, §$P < 0.001$: significant difference from group 5.
Results are expressed as mean ± SEM of the indicated numbers.

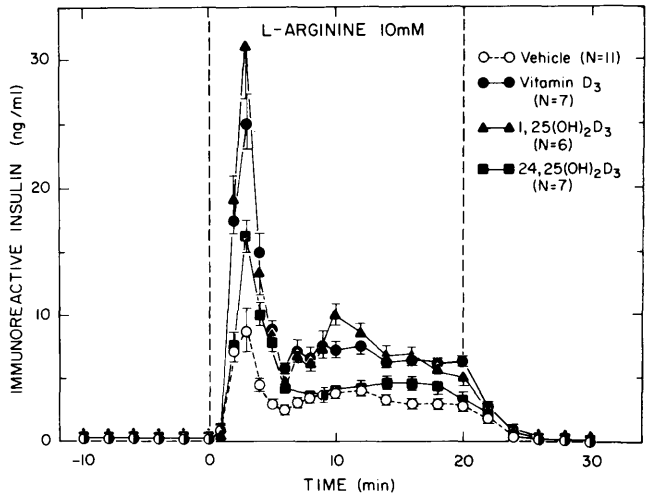

FIGURE 4. Effect of vitamin D_3, $1,25(OH)_2D_3$, and $24R,25(OH)_2D_3$ treatment on the 10 mM arginine-induced insulin release from the isolated, perfused pancreata of rats pair fed in the same manner as the vitamin D-deficient controls and the vitamin D-replete rats. The means ± SEM of the indicated number of experiments are recorded.

rapidly than the D-deficient rats of group 1, which were fed ad libitum, were relatively more fasted at the time of perfusion of their pancreata.

Second, normalization of serum or ionized calcium levels after vitamin D repletion might have affected insulin release, since calcium is known to play a crucial role in the stimulus-secretion coupling process of insulin secretion.[26] However, calcium levels in our perfusion media were maintained at 2.5 mM throughout the entire perfusion in all experiments, and group 5, which received $24,25(OH)_2D_3$ treatment and achieved a normal level of serum Ca, failed to increase insulin release to the normal value. Moreover, in experiment 1 (Table 1), the serum Ca level of the vitamin D-deficient rats was elevated to the normal range by increasing the dietary level of calcium. In spite of this, the vitamin D-deficient normocalcemic rats had a significantly impaired first- and second-phase insulin secretion in response to arginine. A similar result was also obtained when glucose or tolbutamide was used as an insulin secretagogue.[11] Thus, we suggest that vitamin D, through its metabolite $1,25(OH)_2D_3$, plays a direct role in insulin secretion independent of the prevailing level of serum calcium.

Alternatively, it may be possible that the hypophosphatemia of vitamin D deficiency plays a contributory role to the blunted secretion of insulin. The role of phosphate in the insulin-secretory mechanism has been investigated in several laboratories. In these studies, it has been shown that hypophosphatemia enhances,[29,30] inhibits,[31] and has no effect.[32] In the latter study, the perfusate contained the same concentration of glucose (5.5 mM) and secretagogue, arginine (10 mM), as employed in the present studies. These authors show that the presence (1.2 mM) or the absence of phosphate had no effect on either phase of insulin secretion. We, therefore, suggest that it is unlikely that the hypophosphatemia seen in our animals was a factor in the diminished insulin secretion. We conclude that there is a direct interaction between $1,25(OH)_2D_3$ and the islet B-cell. Based on the results of the presence of CaBP in the endocrine B-cells

TABLE 4
Calculated ratio of insulin release

Ratios	First phase (peak 1)	Second phase (peak 2)	Integrated total
$(24,25[OH]_2D_3/-D)$	1.70	1.31	1.44
$(1,25[OH]_2D_3$-high/ $-D)$	2.98	1.95	2.32
$(+D_3/-D)$	2.83	1.99	2.28

The ratio of insulin release (for the indicated groups) was calculated by using the integrated values for insulin release from each group (reported in Table 3) for "first phase," "second phase," or "integrated total," respectively. Thus, for the first phase, the ratio of $(-D/24,25[OH]_2D_3) = 90/53 = 1.70$.

of the chick[7] and rat[5] together with morphologic and biochemical evidence for the presence of a 1,25-dihydroxyvitamin D_3 receptor in chick pancreas,[2,3] we suggest a direct action of vitamin D, especially $1,25(OH)_2D_3$, on pancreatic B-cell function. It is not yet known what role, if any, CaBP may play in the sequence of steps associated with insulin secretion.

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