Calcium is essential in normalizing intolerance to glucose that accompanies vitamin D depletion in vivo.

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Vitamin D is essential for normal insulin secretion in vivo and in vitro. The differential effect of calcium and of the vitamin D endocrine system in the insulin response to secretagogues is still a subject of debate. To study the roles of calcium and the vitamin D system in the in vivo insulin response, GTT and insulin sensitivity tests were conducted in rats presenting vitamin D depletion and hypocalcemia or vitamin D depletion supplemented with calcium alone for 3, 7, or 14 days, vitamin [D.sub.3] (6.5 nmol/day x 7 days), or [1,25(OH).sub.2][D.sub.3] (28 pmol/day x 7 days). Serum calcium was 1.28 [+ or -] 0.04 mM in hypocalcemic vitamin D--depleted rats, 1.47 [+ or -] 0.06 (NS), 1.8 [+ or -] 0.2 ($P < 0.0002$), and 2.04 [+ or -] 0.07 ($P < 0.0001$) mM after 3, 7, or 14 days, respectively, of calcium supplementation; vitamin [D.sub.3]- or [1,25(OH).sub.2][D.sub.3]- supplemented animals had serum calcium of 2.61 [+ or -] 0.04 or 2.48 [+ or -] 0.05 mM ($P < 0.0001$ vs. hypocalcemic vitamin D--depleted rats). Rats with hypocalcemia and vitamin D depletion had significantly higher glucose concentrations ($P < 0.0005$) and lower insulin response during GTT than all other groups ($P < 0.001$). Differences in insulin sensitivity could not account for differences in response because exogenous insulin administration led to a similar drop in glucose concentrations in all groups, with the nadir averaging 51.7 [+ or -] 2.6% of initial values. To distinguish between calcium and the vitamin D system in the GTT response, rats were treated with a nonhypercalcemic analogue of [1,25(OH).sub.2][D.sub.3], OCT (28 pmol/day x 4-7 days) with or without dietary calcium. Serum calcium was 1.23 [+ or -] 0.04 vs. 2.09 [+ or -] 0.02 mM in the absence or presence of dietary calcium ($P < 0.0001$), but normalization of GTT only happened in the presence of calcium. A time course of the calcium effect in vitamin D-depleted rats indicated that 7 days of hig hdietary calcium intake was needed to normalize GTT, with a significant correlation coefficient being observed between serum calcium and the maximum insulin response ($r = 0.5172$, $P < 0.004$). Our data indicate that vitamin D depletion with hypocalcemia is associated with normal insulin sensitivity but glucose intolerance caused by inadequate insulin secretion in response to glucose. Calcium alone contributes in normalizing GTT, whereas the [1,25(OH).sub.2][D.sub.3] analogue OCT is unable to normalize GTT at a dose equimolar to that of [1,25(OH).sub.2][D.sub.3] in the absence of calcium, suggesting that hypocalcemia predominates over vitamin D depletion in the glucose intolerance of vitamin D deficiency.

The action of [1,25(OH).sub.2][D.sub.3] is mediated through an intracellular nuclear receptor in a manner similar to that of other steroid hormones and of thyroid hormone [1]. Its most recognized action is to mediate active calcium transport in the intestine, which leads to increases in extracellular calcium that, in turn, may contribute in modulating the intracellular calcium pools. Besides its genomic action, [1,25(OH).sub.2][D.sub.3] is also known to promote rapid intracellular calcium mobilization in several normal cell types [2-5] and cell lines [6-8]. At the cellular level, it has been shown that [1,25(OH).sub.2][D.sub.3] can induce changes in membrane fluidity [9,10], cGMP [11], and several protein kinases [5, 12, 13]. [1,25(OH).sub.2[]D.sup.3] also has been shown to have effects on the immune system, the proliferation of normal and numerous cancer cells [14-19], and gene expression, such as PTH [20] and osteocalcin genes [21], the c-myc proto-oncogene [22,23], and the gene of its own receptor [24]. The endocrine pancreas is now known to express the [1,25(OH).sub.2][D.sup.3] nuclear receptor [25] and to possess [1,25(OH).sub.2][D.sup.3]-responsive elements, leading to the appearance of specific calcium-binding proteins [26,27].

Early reports have shown that calcium was implicated in the release of insulin by the pancreas [28-30]. Subsequent reports have shown that vitamin D depletion was accompanied by a decreased insulin secretion in vivo [31] and in vitro as judged by the glucose, L-arginine, and tolbutamide stimulation of insulin release in isolated, perfused pancreas preparations [32-34] and isolated pancreatic islets [35,36]. Moreover, observations that vitamin D depletion decreased insulin secretion has been documented in laboratory animals and humans [37,38]. Among the vitamin [D.sub.3] metabolites, the hormone [1,25(OH).sub.2][D.sub.3] is considered the essential metabolite for normal insulin secretion [39].

The respective roles of calcium and vitamin D status on the insulin response to secretagogues is still a subject of debate. In some studies, the effect of the vitamin D status on insulin secretion appeared to be linked to the systemic effect of the vitamin, including its beneficial effect on food intake, weight gain recovery, and the rise in extracellular calcium concentrations [40,41]. Others have observed that the effect seemed independent of nutritional factors and the prevailing circulating calcium and phosphate, suggesting that the action of vitamin [D.sub.3] on glucose-stimulated insulin secretion may be through a mechanism that involves more than its regulating action on extracellular calcium homeostasis and nutrient availability [32,33,36,42,43]. Tanaka et al. [44], however, have reported, using isolated, perfused pancreas preparations, that in vivo calcium pretreatment was essential for normal glucose-mediated insulin secretion. At the cellular level, vitamin D deficiency is accompanied by impaired islet calcium handling [41], which seems to be restored along with a marked improvement in glucose-induced insulin release by vitamin [D.sub.3] or [1,25(OH).sub.2][D.sub.3] treatment [35,45]. These data strongly implicate calcium in the islet insulin response and suggest that the vitamin D endocrine system could maximize glucose-induced insulin release through its participation in the handling of calcium.

The purpose of this study was to evaluate the respective roles of calcium and the vitamin D endocrine system in the in vivo insulin response to glucose challenge and in insulin sensitivity. We now report that insulin sensitivity is not perturbed by vitamin D depletion, and that calcium repletion alone is sufficient to normalize glucose tolerance and insulin secretion in vitamin D--depleted rats. The need for calcium was further confirmed by the use of a nonhypercalcemic analogue of [1,25(OH).sub.2][D.sub.3] (possessing several of the genomic attributes of the hormone [[46-48]]), which failed to normalize GTTs in the absence of calcium feeding.

RESEARCH DESIGN AND METHODS

To investigate the influence of the vitamin D endocrine system and/or of calcium on glucose metabolism, two sets of studies were undertaken. First, GTTs were conducted in three experimental conditions to study the effect of vitamin D status, calcium status using a time course of calcium repletion alone in hypocalcemic vitamin D--depleted rats, and calcium versus the vitamin D endocrine system using the nonhypercalcemic [1,25(OH).sub.2][D.sub.3] analogue OCT (Chugai Pharmaceutical, Tokyo Japan) as a testing agent.

Second, to study the responsiveness to insulin, experiments were conducted on insulin sensitivity in animals presenting hypo- or normocalcemic vitamin D depletion or in animals repleted with vitamin [D.sub.3] or [1,25(OH).sub.2][D.sub.3].

All animals were treated according to the standard of ethics for animal experimentation of the Canadian Council on Animal Care. All protocols were approved by the local animal ethics committee.

To evaluate the influence of vitamin D and/or calcium status on glucose metabolism, animals presenting normal blood chemistry (except for the variables under study) and representative of the following vitamin D endocrine status were sought: 1) vitamin D depletion and hypocalcemia; 2) full vitamin [D.sub.3] repletion with normal circulating vitamin [D.sub.3] metabolites and calcium concentrations; 3) hormonal ([1,25(OH).sub.2][D.sub.3] repletion only with normal circulating calcium and [1,25(OH).sub.2][D.sub.3] concentrations; 4) vitamin D depletion followed by a time course of calcium repletion; and 5) treatment with the [1,25(OH).sub.2][D.sub.3] angalogue OCT in a state of hypo- or normocalcemia. To unify experimental conditions, all animals were randomly chosen from a common pool of vitamin D--depleted animals, as described previously [31,49]. An a posteriori verification of food intake in animals similarly treated revealed no significant between-group difference [19-21 g/24 h] in food consumption.

Animal conditioning was achieved by procedures similar to those described previously [49]. Nursing female Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) were fed ad libitum a vitamin D--deficient diet. At weaning, male rats were housed in polycarbonate cages and fed a vitamin D--deficient diet for a 6-wk period before being randomly assigned to placebo, vitamin [D.sub.3], [1,25(OH).sub.2][D.sub.3], oral calcium, or OCT supplementation regimens. Oral calcium supplementation was achieved by supplying a 3% (wt/vol) solution of calcium gluconate as drinking water for a period of 3, 7, or 14 days. Vitamin and hormonal supplementation were achieved by intraperitoneal implantation of osmotic minipumps (Alza, Palo Alto, CA), which delivered a constant infusion of vitamin [D.sub.3] at a dose of 6.5 nmol/24 h for 7 days; OCT was administered at a dose of 28 pmol/24 h for 4-7 days with a diet containing either 0.01% calcium to maintain hypocalcemia or 0.9% dietary calcium and 3% calcium gluconate as drinking water to rapidly normalize circulating calcium concentrations. At minipump implantation, loading doses of 13 nmol of vitamin [D.sub.2] or 14 pmol of [1,25(OH).sub.2][D.sub.3] or OCT were administered intraperitoneally, to rapidly raise serum concentrations of vitamin [D.sub.3], [1,25(OH).sub.2][D.sub.3], or OCT and hence accelerate the establishment of steady-state conditions. Vitamin D--depleted and calcium-supplemented animals were implanted with minipumps containing the vehicle only (ethanol:1,2-dihydroxy-propane:saline,3:13:4 [vol/vol/vol]).

Serum parameters of the vitamin D endocrine system. Serum vitamin D metabolites were measured after extraction and chromatography [50]. 25(OH)D was analyzed by a competitive-binding assay with sheep serum in a dilution of 1:20,000. [1,25(OH).sub.2]D was measured by competitive protein binding with cytosolic receptor from chick intestine. The lower limits of sensitivity of the methods were 0.2 nM and 7 pM for 25(OH)D and 1,25[(OH).sub.2]D, respectively. The CVs were 7% for 25(OH)D and 11% for [1,25(OH).sub.2]D. Plasma calcium was measured colorimetrically [51].

Insulin response to glucose load. The GTT was used to investigate the insulin status of the animals. Serum insulin and glucose concentrations were measured before and 30, 60, and 120 min after a single intraperitoneal injection of glucose (2 g/kg). Glucose was measured with the glucose oxidase reaction on an Astra 8 analyzer (Beckman Instruments, Palo Alto, CA). Immunoreactive insulin was measured as described by Bleicher and Herbert [52] with porcine insulin (Lilly, Indianapolis, IN) as standard and [[.sup.125]I]insulin (specific activity: 225 [mu]Ci/[mu]g insulin) labeled by the chloramine T method [53]. The CV of the assay was 5%. The displacement curve produced by rat insulin (Lilly, Indianapolis, IN) in the RIA was parallel to that of the porcine insulin standard, as illustrated in Fig. 1.

Insulin sensitivity. Insulin sensitivity was assessed by measurements of the circulating glucose concentrations before and 10, 20, 30, and 60 min after the intravenous administration of 0.2 U of porcine insulin.

Statistical analyses. Statistically significant differences between group means were evaluated by multifactorial ANOVAs with individual contrasts being evaluated according to the method of Tukey [54]. Differences between hypo- and normocalcemic OCT-treated rats were evaluated by Student's t tests.

RESULTS

Table 1 presents the circulating 25(OH)D and 1,25 [(OH).sub.2]D concentrations obtained in relation to the vitamin D or calcium status. As expected, serum 25(OH)D concentrations were only increased after vitamin [D.sub.3] supplementation ($P < 0.0001$), whereas serum [1,25(OH).sub.2]D concentrations were significantly increased after vitamin [D.sub.3] or [1,25(OH).sub.2][D.sub.3] supplementation compared with the hypo- or normocalcemic vitamin D--depleted state ($P < 0.0001$); no significant difference in [1,25(OH).sub.2]D concentrations was observed between vitamin [D.sub.3]--and 1,25 [(OH).sub.2][D.sub.3]-repleted animals.

Glucose tolerance in relation to vitamin D and calcium status. A preliminary experiment conducted between vitamin [D.sub.3]--supplemented animals and normal rats (always fed a normal rat chow diet) established (as already reported by others [43]) that no significant differences between normal rats and vitamin [D.sub.3]--supplemented rats were present in both serum glucose and insulin concentrations after glucose admininstration (Table 2). Because normal (rat chow-fed) animals cannot be matched for age and weight or, most likely, for body composition with vitamin D--depleted rats, all further comparisons were conducted by using [D.sub.3]--supplemented animals as surrogate controls; the latter were, indeed, considered the most appropriate controls because they had been subjected to experimental variables identical to those of all other groups except for the last week of supplementation with vitamin [D.sub.3] to normalize their vitamin D endocrine status, as illustrated in Table 1 and Fig. 3.

Figure 2 presents the glucose and insulin concentrations obtained in hypocalcemic vitamin D--depleted rats and in rats pretreated with vitamin [D.sub.3] or [1,25(OH).sub.2][D.sub.3]. The circulating calcium concentrations obtained in each of the experimental groups are presented in Fig. 3. As expected, serum calcium concentrations were below normal in vitamin D--depleted animals but significantly increased after vitamin [D.sub.3] ($P < 0.0001$) or [1,25(OH).sub.2][D.sub.3] ($P < 0.0001$) supplementation. Body weight was 155 [+ or -] 4, 177 [+ or -] 4, and 184 [+ or -] 4 g in vitamin D--depleted, vitamin [D.sub.3]-supplemented, and [1,25(OH).sub.2][D.sub.3]-supplemented rats, respectively (vitamin D-depleted significantly different from vitamin [D.sub.3]- or [1,25(OH).sub.2][D.sub.3]-treated rats: $P < 0.05$; difference between vitamin [D.sub.3]-and [1,25(OH).sub.2][D.sub.3]-supplemented rats: NS). As illustrated in Fig. 2A, circulating glucose was significantly influenced by the vitamin D status, with higher glucose concentrations before glucose challenge in hypocalcemic vitamin D--depleted animals ($P < 0.0001$) and significantly higher increases 30 ($P < 0.0006$), 60 ($P < 0.0001$), and 120 min ($P < 0.0002$) after glucose challenge than in the vitamin [D.sub.3]-- or [1,5(OH).sub.2][D.sub.3]-supplemented groups. The overall insulin response to glucose (Fig. 2B) was lower in hypocalcemic vitamin D--depleted rats than in the two other groups ($P < 0.02$), with a highly significant difference in maximum insulin concentration 30 min after glucose stimulation ($P < 0.0001$). The response to glucose challenge is well illustrated by the insulin-glucose concentration ratio (Fig. 2C), which was significantly lower throughout all the GTTs in vitamin D--depleted hypocalcemic rats than after vitamin [D.sub.3] or [1,25(OH).sub.2][D.sub.3] repletion ($P < 0.0002$).

Glucose tolerance in relation to calcium repletion in vitamin D--depleted rats. To investigate whether calcium supplementation could improve the response to glucose challenge in vitamin D--depleted animals, GTTs were conducted 3, 7, and 14 days after high calcium feeding in hypocalcemic vitamin D--depleted rats and compared with the data obtained in hypocalcemic vitamin D--depleted rats (Fig. 4). The effect of calcium feeding on serum calcium concentrations is presented in Fig. 3B. As illustrated, high calcium feeding progressively increased circulating calcium with mean increases of 14.8 (NS), 40.6 ($P < 0.0002$), and 59.4% ($P < 0.0001$) above basal values after 3, 7, or 14 days of calcium supplementation. As illustrated in Fig. 4A, glucose concentrations significantly increased in all groups after glucose administration ($P < 0.0001$), but calcium supplementation for 3, 7, and 14 days contributed to significantly lower glucose concentration compared with the values obtained in vitamin D--depleted hypocalcemic rats ($P < 0.0002$). The insulin response to glucose (Fig. 4B) was not significantly increased by 3 days of calcium feeding compared with that observed in the vitamin D--depleted hypocalcemic group but was significantly increased after 14 days of calcium supplementation ($P < 0.02$) with serum insulin levels not significantly different from those observed in vitamin [D.sub.3]-- or [1,25(OH).sub.2][D.sub.3]-treated [D.sub.3]-treated rats (Fig. 2). The insulin/glucose concentration ratio (Fig. 4C) illustrates the insulin hyporesponsiveness to glucose in the absence of or after 3 days of calcium supplementation compared with the response obtained after 7 or 14 days of supplementation ($P < 0.003$). Moreover, a significant correlation coefficient was observed between the prevailing circulating calcium and the maximum

serum insulin concentrations obtained (30 min after glucose challenge)-r = 0.5172 (P < 0.004)-whereas that observed between serum calcium and maximum glucose concentrations was r = -0.3419 (P < 0.009).

Differential effect of calcium and vitamin D status. Figure 5 illustrates the response to glucose in animals pretreated with the [1,25(OH).sub.2][D.sub.3] analogue OCT in conditions of hypo- or normocalcemia. OCT administration at a dose equimolar to that of [1,25(OH).sub.2][D.sub.3] to hypocalcemic vitamin D--depleted rats did not affect serum calcium when animals were fed a low-calcium diet with values of 1.23 [+ or -] 0.04 mM, but high-calcium feeding contributed to a significant increase in serum calcium to 2.09 [+ or -] 0.02 mM (P < 0.0001; Fig.3C). Furthermore, body weight was not significantly influenced by calcium feeding with weights of 155 [+ or -] 9 and 163 [+ or -] 10 g in OCT-treated hypo-and normocalcemic animals, respectively (NS). As illustrated in Fig. 5A, glucose concentrations after glucose administration were significantly higher in hypocalcemic OCT-treated animals than in their normocalcemic counterparts (P < 0.03). Figure 5B illustrates the lower insulin response observed in the hypocalcemic compared with the normocalcemic group (P < 0.0002). This observation is further illustrated by the insulin/glucose concentration ratio showing a significantly decreased ratio in the hypothan in the normocalcemic group receiving OCT at a dose equimolar to that given to animals treated with [1,25(OH).sub.2[]D.sub.3] (P < 0.003).

Figure 6 presents the insulin-mediated decrease in serum glucose in animals with various calcium and/or vitamin D statuses. As illustrated, after intravenous insulin administration, serum glucose concentrations decreased significantly in all groups. No significant between-group differences were observed in the nadir of glucose concentrations (20 - 30) min after insulin administration) nor in the recovery in glucose concentrations 60 min after insulin administration, indicating that no major defect in the response to insulin seemed associated with hypocalcemic vitamin D deficiency.

DISSCUSSION

Early studies clearly demonstrated the need for the vitamin D endocrine system for optimum insulin secretion in response to secretagogues (32 - 34). Many of these studies established a link between the overall systemic effect of vitamin D administration and the insulin response, which suggested that the vitamin D system itself overrode its effect on calcium metabolism. Subsequently, it became more and more evident that calcium status played a major role in the insulin response, although the attribution of the effects to calcium or some other genomic effect of the vitamin D system remained largely unresolved.

The data obtained during this study clearly establish that hypocalcemic vitamin D depletion leads to insulinopenia, as suggested by the low circulating insulin concentrations before glucose loading and leads also to glucose intolerance, as evidenced by significantly higher glucose and lower insulin concentrations after glucose challenge with resulting significantly lower insulin/glucose concentration ratio than in any other groups. Insulin insensitivity attributable to vitamin D depletion cannot be evoked as a significant parameter in the response observed in these animals because inappropriate insulin secretion was clearly observed relative to the circulating glucose concentrations attained after glucose administration, and insulin sensitivity tests suggest that the sensitivity to insulin was well maintained in hypocalcemic vitamin D--depleted rats compared with all other groups. Repletion of these animals with vitamin [D.sub.3] or the hormone [1,25(OH).sub.2][D.sub.3] fully restored glucose tolerance. Furthermore, repletion with calcium alone greatly improved glucose tolerance, with a time course indicating improvement in glucose concentrations after glucose administration as early as 7 days after calcium feeding. Significant improvement in the insulin response, however, seemed to be delayed because significant changes in insulin concentrations were only noted 14 days after calcium feeding but, nevertheless, at circulating calcium concentrations still significantly lower than those observed in vitamin [D.sub.3]-- or [1,25(OH).sub.2][D.sub.3]-treated animals. These observations suggest that full normalization of insulin secretion may need a more adequate calcium repletion than that needed for the glucose response. The data also may indicate that in these severely hypocalcemic and insulinopenic animals, insulin sensitivity may have been enhanced by calcium feeding, a hypothesis that would support the data of Williams et al. (55), which indicate that calcium increases insulin binding and insulin sensitivity. Our data would also be well in agreement with those of Kergoat et al. [56] showing that in mild insulinopenia such as in the n0-streptozocin model, insulin sensitivity as measured by glucose utilization tends to be increased rather than decreased. Collectively, the calcium-supplementation studies strongly indicate that calcium is a major determinant in the response to glucose challenge, because the animals studied were all vitamin D depleted and strongly hypocalcemic before calcium feeding to ascertain the predominant contribution of calcium over that of the vitamin D system in the response observed.

To further strengthen the contribution of the calcium status over that of the vitamin D endocrine system in glucose tolerance, a protocol was initiated where animals were treated with the [1,25(OH).sub.2][D.sub.3] analogue OCT along with a normalization of the calcium status or in the presence of an extracellular calcium concentration similar to that of hypocalcemic vitamin D--depleted rats. It is interesting to note that at a dose equimolar to that of [1,25(OH).sub.2][D.sub.3], OCT alone did not contribute to improve insulin secretion and glucose concentrations. Normalization of extracellular calcium, however, contributed to fully normalize glucose tolerance, indicating that when amputated of its calcium branch, the vitamin D system was unable to sustain the insulin secretion needed to match the glucose challenge. Although the comparative effect of [1,25(OH).sub.2][D.sub.3] and OCT at the [beta]-cell level has not yet been studied, OCT in the presence of low extracellular calcium concentrations is suspected to exhibit several of the genomic actions of [1,25(OH).sub.2][D.sub.3] as illustrated by the repression of the PTH gene in vivo [46] and the proliferation inhibition of psoriatic fibroblasts [47] and breast cancer cells [48] in vitro. Moreover, in this study, OCT treatment seemed to have elicited the normalization of the vitamin D system, as evidenced by the expected increase in serum calcium concentrations observed when animals were fed a calcium-containing diet. These observations further strengthen the contention that calcium predominates over the vitamin D system in the in vivo insulin secretion secondary to acute glucose administration in the rat and illustrate that the positive direct effects the vitamin D system might have on [beta]-cell function cannot overcome, in themselves, the effect associated with a long-lasting pronounced calcium deficiency.

This study strongly indicates that the secretion of insulin after glucose challenge is mainly calcium rather than vitamin D dependent. It has now been established that glucose elicits intracellular calcium movements in pancreatic [beta]-cells [57]. The pattern of intracellular calcium indicates an initial decline in cytosolic calcium, possibly caused by calcium sequestration by intracellular pools [58], which is, however, followed by subsequent increases often associated with intracellular calcium oscillations necessitating the presence of extracellular calcium [59]. A causal relationship between the intensity of these calcium movements and insulin secretion is not unequivocally established, but a link is probable as proposed previously [60] not only for both phases of insulin secretion but also for the secretion of other hormones such as that of aldosterone after angiotension II or ACTH stimulation [61]. It is, therefore, possible that the vitamin D system exerts its major effect on insulin secretion by raising extracellular calcium, ensuring, then, that sufficient calcium is available to fill the intracellular pools for subsequent agonist-mediated calcium mobilization. It is postulated that high calcium feeding alone during a sufficiently long period of time succeeds, by normalizing extracellular calcium, in bypassing the necessity for [1,25(OH).sub.2][D.sub.3] (normally needed for the absorption of physiological quantity of calcium) and in mimicking, therefore, the action normally vested in the vitamin D endocrine system. The exact cellular and molecular mechanisms involved in the respective action of vitamin D and calcium in pancreatic [beta]-cell physiology still, however, remain to be elucidated.

The data do not preclude, however, a participation of the vitamin D endocrine system in long-term glucose metabolism and insulin homeostasis. Indeed, it is not excluded that the vitamin D system could affect the synthesis rather than the acute secretion of insulin as suggested by the studies of Ozono et al. [62], in which vitamin D deficiency was shown to be accompanied by decreased pancreatic preproinsulin mRNA, a phenomenon found to be reversible by [1,25(OH).sub.2][D.sub.3] treatment, providing, however, rats were fed. Collectively, data indicate that the effect of [1,25(OH).sub.2][D.sup.3] and/or of calcium on insulin-gene expression and insulin secretion follows the demand for insulin and should not lead to overexpression and/or secretion of the hormone.

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