Diabetologia (1986) 29: 142-145

Diabetologia

Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man

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Summary. We have studied the effects of vitamin D deficiency on pancreatic A- and B-cell function. Four subjects with vitamin D deficiency and 10 healthy subjects were studied. Pancreatic B-cell function was assessed by the insulin response to an oral glucose tolerance test. An insulin tolerance test was used to evaluate pancreatic A-cell function. The patients were then treated with 2000 U/day of vitamin D_3 for 6 months, after which the clinical, metabolic, biochemical and radiological features of vitamin D deficiency resolved, and pancreatic A- and B-cell function was repeated. In the vitamin D-deficient subjects pre-treatment and post-treatment serum calcium levels (mean ± SEM) were 2.22 ± 0.01 mmol/l and 2.24 ± 0.01 mmol/l respectively, and 2.27 ± 0.02 mmol/l in healthy subjects (NS). The pre-treatment level of 1,25-dihydroxy vitamin D (1,25-$(OH)_2$D) of 29.7 ± 3.3 pg/ml in the vitamin D deficient subjects rose to 70.3 ± 10.3 pg/ml after treatment ($p < 0.05$). The 1,25-$(OH)_2$D level in the healthy subjects was 50.0 ± 13.7 pg/ml ($p < 0.05$ versus pre- and post-treatment values in the patients). Insulin secretion, calculated by the area under the insulin curve, was significantly lower before vitamin D_3 treatment in the patients (9.09 ± 0.7 mU × min, $p < 0.05$) compared with the healthy subjects (11.9 ± 0.5 mU × min) and post-treatment values of the patients with vitamin D deficiency (13.7 ± 0.5 mU × min). Similar changes were seen in the insulogenic indices ($\Delta I/\Delta G$). While $\Delta I/\Delta G$ was 1.71 ± 0.4 (mean ± SEM) during vitamin D deficiency, it increased to 2.48 ± 0.3 with vitamin D repletion. The insulogenic index in the healthy subjects was 2.68 ± 0.3. The glucose areas were not significantly different. Insulin-induced glucagon secretion was similar in all instances. The results of this study suggest that vitamin D deficiency reduces pancreatic insulin secretion but it does not affect pancreatic A-cell function.

Key words: Vitamin D, insulin, glucagon secretion.

Vitamin D is essential in higher animals and man for the maintenance of calcium and phosphorus homeostasis [1]. This is accomplished by the sequential metabolism of vitamin D by the liver and kidney into its principal biologically active metabolite, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) [2, 3]. The effect of 1,25-$(OH)_2$D has been associated with calcium and phosphate handling in intestine, bone and kidney. It has been established that 1,25-$(OH)_2$D acts on these three tissues, as receptors for 1,25-$(OH)_2$D have been found in these tissues [4-6]. Moreover, a large number of previously unrecognized target organs have been identified. Among them are stomach, skin, pituitary, brain and the endocrine pancreas [7-8]. Recent autoradiographic studies have provided evidence that 1,25-$(OH)_2$D is selectively concentrated in and retained by pancreatic islet cells, suggesting a genomic action on B-cell function [8].

Because of the known effects of vitamin D on membrane and calcium transport [9, 10], abnormalities in vitamin D metabolism could alter endocrine cell membrane function and calcium transport and, thereby, hormone release. In fact, it has been demonstrated that vitamin D deficiency is associated with marked impairment of insulin release from the rat pancreas [11-14].

The purpose of this study was to investigate any relationship between 1,25-$(OH)_2$D levels and pancreatic A- and B-cell function in man.

Subjects and methods

We studied two groups of subjects; group 1 consisted of 4 female patients, aged 17-54 years, and group 2 consisted of 10 healthy volunteers (5 men, 5 women), aged 24-46, who underwent the same study protocol. Written informed consent was obtained from all subjects.

Case histories of the vitamin D-deficient subjects

Case 1. A 54-year-old woman complained of difficulty in walking and extensive bone pains. She had 10 pregnancies with prolonged lactation. She had no relevant medical history. Bone survey revealed the pseudofractures of both femurs with generalized demineralization.

Case 2. A 27-year-old woman complained of generalized bone pain for one year. She had two pregnancies with normal lactation periods, although she had minimal exposure to sunshine because of traditional clothing. There was no relevant medical history. Radiographs showed diffusely decreased bone density and pseudofractures of both scapulae.

Case 3. A 17-year-old girl complained of difficulty in walking and bone pains, and she had minimal exposure to sunlight. She had not been pregnant or had no history of relevant conditions. Radiographs showed pseudofractures of the ishio-pubic rami and extreme decalcification.

Case 4. A 33-year-old woman complained of extensive bone pains. She had six pregnancies and three abortions. Her past medical history was unremarkable. X-rays showed generalized decreased bone density and a pseudofracture of the left fibula.

Table 1. Pre-treatment and post-treatment clinical data from the vitamin D deficient subjects

Case number	Sex	Age (years)	Pre-treatment							Post-treatment						
			Body mass index	Serum Ca^{++} mmol/l	Serum Mg^{++} mmol/l	Serum PO_4^- mmol/l	Serum total prot. g/dl	Alk. phos IU/l	PTH mIU/ml	Body mass index	Serum Ca^{++} mmol/l	Serum Mg^{++} mmol/l	Serum PO_4^- mmol/l	Serum total prot. g/dl	Alk. phos IU/l	PTH MIU/ml
1	F	54	23.5	2.22	0.90	0.90	6.8	244	2.27	23.7	2.29	0.90	0.96	7.0	111	2.51
2	F	27	21.0	2.24	1.15	1.0	7.3	255	2.33	21.7	2.22	0.98	1.06	7.0	100	2.37
3	F	17	23.1	2.19	0.74	1.06	7.0	200	2.60	22.5	2.24	0.78	1.13	6.9	111	2.73
4	F	33	23.9	2.22	1.02	0.96	7.0	222	2.41	23.9	2.27	1.19	1.13	6.9	120	2.38
Mean		32.7	22.8	2.22	0.95	1.00	7.0	230	2.40	22.9	2.25	0.96	1.07	6.9	110	2.49
SEM		15.6	1.3	0.01	0.09	0.03	0.2	6.1	0.12	1.0	0.01	0.07	0.04	0.16	5.0	0.15

Table 2. Serum calcium and 1.25-$(OH)_2$ vitamin D levels

	Group 1 Vitamin D deficient subjects			Group 2 Healthy subjects	
	Pre-treatment	Post-treatment	p^a		p^b
Serum calcium mmol/l	2.22 ± 0.01	2.25 ± 0.01	> 0.05	2.25 ± 0.02	> 0.05
Serum 1.25-$(OH)_2$D pg/ml	29.7 ± 3.3	70.3 ± 10.3	< 0.05	50.00 ± 13.7	< 0.05

Results are given as mean ± SEM. [a] Significance of differences between pre-treatment and post-treatment vitamin D deficient subjects. [b] Significance of differences between pre-treatment healthy subjects and post-treatment healthy subjects

Table 1 shows the pre-treatment and post-treatment laboratory values in the vitamin D deficient patients.

All patients were admitted to a metabolic ward and given a diet of 50–55% carbohydrate, 30–35% fat, and 15–20% protein, supplemented with 15 g of calcium gluconate. After serum calcium and parathyroid hormone levels were normalized, pancreatic A- and B-cell function was evaluated by dynamic tests.

Following an overnight fast, blood was drawn from an antecubital vein at 08.00 hours for blood glucose, serum calcium, magnesium, phosphate, PTH, 1,25-$(OH)_2$D and insulin levels. They were then given 1.75 g glucose/kg orally, and venous samples were collected at 30-min intervals for glucose and insulin levels for 3 h.

To investigate glucagon release, 0.1 U crystalline insulin/kg was given as an intravenous bolus, and blood samples were drawn for blood glucose and plasma glucagon levels at 15-min intervals for 2 h.

The patients were then treated with 2000 IU of vitamin D_3 per day. X-rays were taken at 3-month intervals. After 6 months, the pseudofractures had disappeared. Alkaline phosphate, PTH and 1.25-$(OH)_2$D levels became normal. The body mass indices of the patients were unchanged. The same study protocol was then repeated.

Blood glucose was measured by the Somogyi method [15]. Serum phosphate and alkaline phosphatase were measured by a standard Technicon Autoanalyzer. Normal values for phosphate and alkaline phosphatase are 0.8–1.6 mmol/l and 45–130 IU/l respectively. Calcium and magnesium levels were measured by an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 403, Norwalk, CT, USA). Normal ranges for calcium and magnesium are 2.25–2.74 mmol/l and 0.77–1.18 mmol/l respectively. Serum PTH was measured by RIA using an antibody recognising the C-terminal region of PTH. A PTH kit was purchased from Institut National des Radioelements, Fleurus, Belgium. The coefficients of variation of the kits used were 9.4% at low values and 8.6% at high values. Normal levels for PTH are 2.7 ± 0.8 mIU/ml. For plasma pancreatic glucagon determinations, plasma samples were prepared by adding 1000 IU of Trasylol + 2 mg of EDTA Na per ml of blood. The plasma was kept frozen until the day of the assay. Plasma glucagon was measured with a commercially available glucagon assay kit (Glucagon PEG Kit, Biodata, Milan, Italy). The antiserum (rabbit) of the kit has a 100% cross-reaction with pancreatic glucagon. The coefficients of variation were 12.1% at low and 9.3% at high values. The basal values of pancreatic glucagon fasting subjects at rest range were between 50–250 pg/ml. Serum insulin levels were measured by RIA using commercially available kits (Sorin Biomedica Gruppo Radiochemica, Verecelli, Italy). The coefficients of variation were 8.1% at low and 6.6% at high values and normal fasting levels were between 3 and 15 μU/ml. Serum 1.25-$(OH)_2$D levels were determined by Endocrine and Metabolic Center Laboratories, Oakland, CA, USA according to the cytoreceptor assay method as previously described [16]. Using this assay, normal values of 1,25-$(OH)_2$D in our healthy subjects were 50.0 ± 13.7 pg/ml.

Calculations of glucose and insulin areas were made according to the methods of Chiles and Tzagournis [17]. The results of the calculations were expressed as mol × min for glucose and milliunit × min for insulin.

The Mann-Whitney test was used for statistical analysis [18].

Results

Serum calcium levels before and after vitamin D_3 treatment in the group 1 patients were 2.22 ± 0.01 mmol/l and 2.24 ± 0.01 mmol/l respectively. In group 2, serum calcium levels were 2.27 ± 0.02 mmol/l (NS, Table 2).

Serum 1,25-$(OH)_2$D levels before and after treatment with vitamin D_3 in the group 1 patients were 29.7 ± 3.3 pg/ml and 70.3 ± 10.3 pg/ml respectively, and 50.0 ± 13.7 pg/ml in group 2 ($p < 0.05$ group 1 pre-treatment versus group 1 post-treatment and versus group 2, Table 2).

Figure 1 shows the serum insulin response to oral glucose. During vitamin D deficiency the insulin area (9.09 ± 0.7 mU × min) was significantly lower than after vitamin D repletion (13.6 ± 0.5 mU × min) in the healthy subjects (11.9 ± 0.5 mU × min) ($p < 0.05$). The insulogenic indicies ($\Delta I/\Delta G$) were calculated for all groups. In the vitamin D deficient state, $\Delta I/\Delta G$ was 1.71 ± 0.4. It increased to 2.48 ± 0.3 after vitamin D treatment. The healthy subjects had a $\Delta I/\Delta G$ of 2.68 ± 0.3. Pre-treatment $\Delta I/\Delta G$ values were significantly lower compared with post-treatment values and the values of the healthy subjects ($p < 0.05$) (Table 3).

Mean blood glucose levels did not alter significantly between the groups over six months. Similarly, the glucose areas were unchanged (pre-treatment 1.15 mol ×

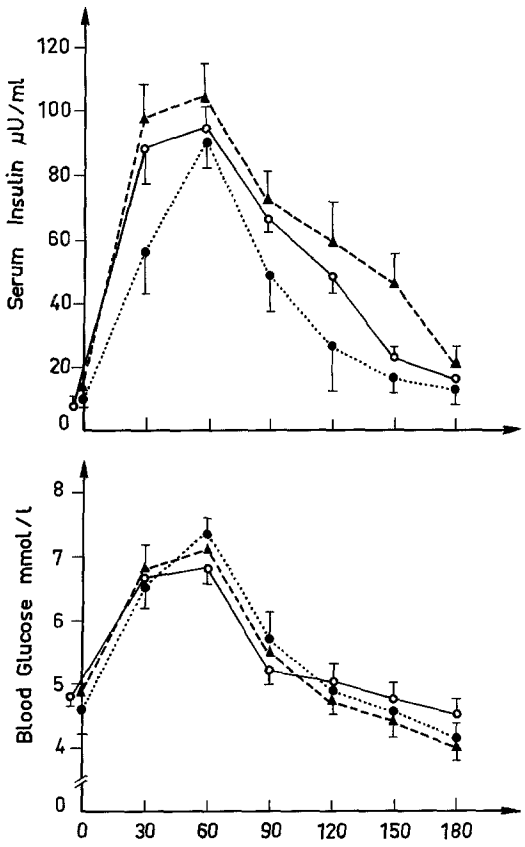

Fig. 1. Blood glucose and serum insulin levels (mean ± SEM) during oral glucose testing in healthy subjects (○—○) and in vitamin D deficient subjects before (● · · · · ●) and after (▲ ----- ▲) treatment

min, post-treatment 1.13 mol × min and healthy subjects 1.16 mol × min) (Table 3).

Mean basal plasma glucagon levels before and after vitamin D treatment in the group 1 patients were 87.0 ± 4.2 pg/ml and 89.0 ± 5.4 pg/ml respectively. In the healthy subjects the mean plasma glucagon level was 85.1 ± 4.9 pg/ml (NS). Similarly, during insulin tolerance testing mean plasma glucagon levels were not significantly different between all groups at any time interval.

Discussion

Several investigators have suggested the presence of a cytosol receptor for 1,25-$(OH)_2$D in the endocrine pancreas [6-8]. How these receptors affect pancreatic function is unknown. To investigate a possible effect of vitamin D on pancreatic islet cell function, Norman et al. [11] used the perfused pancreas of vitamin D-deficient rats and showed that they secreted less insulin than in vitamin-D-repleted rats. Clark et al. [12] demonstrated that basal serum insulin in vitamin D-deficient rats increased in response to treatment with 1,25-$(OH)_2$D. More recently, Chertow et al. [13] suggested that vitamin D-deficiency is associated with marked impairment of biphasic insulin release by rat pancreas. Tanaka et al.

Table 3. Glucose areas, insulin areas, insulogenic indices

	Glucose area mol × min	Insulin area mU × min	Insulogenic index $\Delta I/\Delta G$
Pre-treatment with vitamin D	1.15 ± 0.01	9.09 ± 0.7	1.71 ± 0.4
Post-treatment with vitamin D	1.13 ± 0.01	13.6 ± 0.5	2.48 ± 0.3
Healthy subjects	1.16 ± 0.02	11.9 ± 0.9	2.68 ± 0.3
p[a]	> 0.05	< 0.05	< 0.05

Results are given as mean ± SEM. [a] Significance of differences between pre-treatment, post-treatment and healthy subjects

[14] studied insulin and somatostation secretion in vitamin D-deficient and -repleted rats and found that there was a marked inhibition of insulin release in the former group; after treatment with vitamin D, insulin secretion was restored to normal.

To our knowledge, the effects of vitamin D deficiency and repletion on insulin and glucagon release have not been investigated in humans. Our study demonstrated that, during vitamin D deficiency, pancreatic insulin response to glucose was impaired. Our results also showed that, during vitamin D deficiency, insulin areas were significantly smaller. After treatment with vitamin D_3, glucose-stimulated peak insulin levels, as well as insulin areas, were greater than those found in the healthy subjects ($p < 0.05$). In vitamin D-deficient and -repleted states, glucose areas were identical with those of the healthy subjects.

These findings indicate that vitamin D influences pancreatic B-cell function. Vitamin D can affect insulin release directly and indirectly. This steroid plays a significant role in the regulation of serum calcium [19, 20]. Serum calcium, in turn, is an important regulator of insulin release [21, 22]. Studies in man have shown an impairment in insulin release during hypocalcaemia [23, 24]. It is possible that vitamin D affects insulin release indirectly by altering serum calcium levels. In order to eliminate the effect of low extracellular calcium levels on insulin release, we gave the patients supplemental calcium in their diets. After calcium supplementation, serum calcium levels were not different from the calcium levels of the healthy subjects, yet insulin release was significantly impaired. Nevertheless we cannot argue that extracellular and intracellular calcium deficiency has been overcome entirely by short-term calcium replacement. Thus, one can accept an indirect effect of vitamin D, yet normal PTH values of vitamin D-deficient patients indicate that intracellular calcium has reached, at least, near normal values. As a result of calcium replacement, sufficient amounts of ionized calcium must be entering the cell and lowering high PTH values to the normal range [25]. On the other hand, due to 1,25-$(OH)_2$D deficiency, pancreatic B cells probably cannot respond to the increasing ionized calcium by

augmenting insulin release. Therefore, it can be suggested that vitamin D, through its metabolite 1,25-$(OH)_2$D, plays a direct role in insulin secretion. As shown by Rasmussen et al. [26], and later by Drücke et al. [27], 1,25-$(OH)_2$D alters membrane phospholipid composition; this change modifies the function of membrane proteins, leading to an increase in calcium and phosphorus uptake velocity. This liponomic regulation of protein function can be considered as a direct action of vitamin D on hormone release. In fact, more recently a study in rats demonstrated that vitamin D, through its metabolite 1,25-$(OH)_2$D, plays a direct role in insulin secretion independent of the prevailing level of serum calcium [28].

The presumed action of vitamin D on food intake may provide another indirect mechanism. It has been shown in animal experiments that impaired insulin release during vitamin D deficiency may be the result of malnutrition and weight loss [13]. This, however, cannot be applied to the present study, since the patients maintained their body mass indices and there was no change in the composition of their diets or their caloric intake.

In the present study, as in previous studies [11], we have not demonstrated a significant alteration in glucagon secretion during vitamin D deficiency. This tends to support the possibility that pancreatic A cells are less sensitive than B cells to the effects of vitamin D.

In summary, the results of this study suggest that 1,25-$(OH)_2$D increases the secretion of insulin from the B cell but does not influence A cell function.

Acknowledgement. The authors gratefully acknowledge the help of Dr. R. E. Reitz for the assay of 1.25-$(OH)_2$ vitamin D.

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Received: 21 June 1985
and in revised form: 31 December 1985

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