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# Pancreatic secretion in man with subclinical vitamin D deficiency

B. L. Nyomba[1], J. Auwerx[1], V. Bormans[2], T. L. Peeters[2], W. Pelemans[3], J. Reynaert[4], R. Bouillon[1], G. Vantrappen[2] and P. De Moor[1]

[1]Laboratorium Experimentele Geneeskunde en Endocrinologie, [2]Gut Hormone Laboratory and [3]Geriatric Unit, Universitaire Ziekenhuizen, Katholieke Universiteit Leuven, and [4]Universitair Psychiatrisch Centrum St. Jozef, Kortenberg, Belgium

**Summary.** The effects of subclinical vitamin D deficiency and vitamin D supplementation on oral glucose tolerance and secretion of pancreatic hormones were studied in 10 diphenylhydantoin-treated epileptic patients and 15 geriatric patients. Their mean serum concentrations of 25-hydroxyvitamin $D_3$ and 1,25-dihydroxyvitamin $D_3$ decreased markedly, but returned to normal within 2 weeks of oral supplementation with 25-hydroxyvitamin $D_3$. The serum concentration of ionized calcium was within the normal range before treatment, and remained unchanged. Serum parathyroid hormone was increased during vitamin D deficiency, but decreased significantly ($p < 0.05$) afterwards. In vitamin D-deficient epileptic and geriatric patients, the 2- and 3-h insulin levels after glucose ingestion were increased when compared with control values, and glucagon secretion was not suppressed by glucose. Oral glucose tolerance of both groups of patients did not change after 25-hydroxyvitamin $D_3$ supplementation. Insulin secretion remained unchanged in geriatric patients, but was reduced to normal values in epileptic patients. Glucagon suppressibility by glucose was partly restored after vitamin D supplementation in epileptic patients but not in geriatric patients. In contrast to that observed in severely vitamin D-deficient rats or rabbits, correction of subclinical vitamin D deficiency failed to enhance insulin secretion or to improve glucose tolerance in man.

**Key words:** Vitamin D, calcium, insulin, glucagon, glucose, anticonvulsants, geriatrics.

Several hormones influence the production of the active vitamin D hormone 1,25-dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$). Recent data also suggest a reciprocal influence of the vitamin D endocrine system on the secretion of these hormones. Indeed, parathyroid hormone, growth hormone, prolactin, oestrogens, thyroid hormones, and insulin modulate the renal biosynthesis of 1,25-$(OH)_2D_3$ [1]. Conversely, specific receptors for 1,25-$(OH)_2D_3$ have been found in parathyroid gland, pituitary and ovary. The vitamin D hormone also affects the secretion of various hormones and the growth of ovarian cells in culture [2].

Pancreatic B cells are also possible targets for vitamin D, since they contain specific receptors for 1,25-$(OH)_2D_3$ [3] and a specific vitamin D-dependent calcium binding protein [4]. In fact, few studies have shown that insulin secretion is reduced in vitamin D-deficient rats [5, 6] or rabbits [7], and that this defect can be corrected by vitamin D repletion but not by calcium alone [7, 8]. Pharmacological doses of 1,25-$(OH)_2D_3$ could further increase insulin secretion in vitamin D-replete rats [9].

Little is known about the effects of vitamin D deficiency on the activity of the endocrine pancreas in man. Moreover, since the activity of the pancreatic B cells also depends on the effect of other hormones [10], we examined the glucose-induced insulin response and the concomitant response of glucagon in vitamin D-deficient man before and after vitamin D repletion.

## Subjects and methods

### *Subjects*

The investigation was carried out in 10 epileptic patients (one male, nine females) with a mean age of 56 years and confined to a psychiatric clinic, and in 15 non-epileptic elderly patients (three males, 12 females) aged 78 years and restricted to a geriatric home. The epileptic patients had been treated with a combination of barbiturates (mean dose: 200 mg/day) and diphenylhydantoin (mean dose: 200 mg/day) for a mean duration of 28 years (range 12–54 years). All the medications were kept constant during the course of the study. The geriatric patients were taking no medication known to interfere with calcium or glucose metabolism. The exposure to sunshine of both groups of patients was negligible, their vitamin D intake was estimated to average 250 IU daily, and they ate about 2100 calories daily. Control values were obtained in 34 healthy adult subjects with a mean age of 50 years and in 61 non-institutionalized elderly subjects with a mean age of 77 years. Six subjects from either control group and all the patients volunteered for an oral glucose tolerance test.

**Table 1.** Parameters of calcium metabolism in epileptic and geriatric patients during vitamin D treatment

| | Epileptic patients | | | | Geriatric patients | | | |
|---|---|---|---|---|---|---|---|---|
| | Before treatment | 1 week after treatment | 2 weeks after treatment | Control values | Before treatment | 1 week after treatment | 2 weeks after treatment | Control values |
| Total calcium (mmol/l) | 2.17 ± 0.03[a] | 2.15 ± 0.03[a] | 2.20 ± 0.02[b] | 2.35 ± 0.04 | 2.28 ± 0.02 | 2.30 ± 0.02 | 2.30 ± 0.20 | 2.27 ± 0.04 |
| Ionized calcium (mmol/l) | 1.13 ± 0.01 | 1.14 ± 0.04 | 1.13 ± 0.01 | 1.14 ± 0.03 | 1.15 ± 0.004 | 1.17 ± 0.005 | 1.16 ± 0.005 | 1.13 ± 0.02 |
| Phosphorus (mmol/l) | 0.94 ± 0.06[a] | 1.00 ± 0.07[d] | 1.00 ± 0.08[d] | 1.07 ± 0.07 | 0.90 ± 0.02[a] | 1.00 ± 0.02[b] | 1.13 ± 0.14[b,d] | 1.37 ± 0.10 |
| Alkaline phosphatase (U/l) | 165 ± 28[b] | 159 ± 21[a] | 164 ± 26[a] | 78 ± 6 | 112 ± 18 | 112 ± 18 | 110 ± 18 | 121 ± 10 |
| Parathyroid hormone (mU/l) | 174 ± 82[b] | 128 ± 49[b] | 118 ± 50[b] | 39 ± 10 | 129 ± 26 | 103 ± 19 | 110 ± 20 | 133 ± 14 |
| 25-$OHD_3$ (nmol/l) | 17 ± 3[c] | 44 ± 5[e] | 36 ± 6 | 38 ± 4 | 19 ± 3[c] | 37 ± 3[e] | 35 ± 3[e] | 42 ± 3 |
| 1,25-$(OH)_2D_3$ (pmol/l) | 59 ± 6[c] | 104 ± 12[f] | 101 ± 14[f] | 122 ± 10 | 75 ± 7[c] | 111 ± 10[e] | 99 ± 7[e] | 105 ± 6 |
| Vitamin D-binding protein (μmol/l) | 7.2 ± 0.5 | 6.2 ± 0.7 | 7.0 ± 0.4 | 6.7 ± 0.1 | 6.5 ± 0.2 | 6.8 ± 0.3 | 6.7 ± 0.3 | 6.6 ± 0.2 |
| Free 1,25-$(OH)_2D_3$ index | 0.82 ± 0.04[a] | 1.54 ± 0.20[f] | 1.42 ± 0.22[f] | 1.83 ± 0.39 | 1.12 ± 0.11[a] | 1.76 ± 0.18[f] | 1.54 ± 0.13[f] | 1.64 ± 0.10 |

Data are expressed as mean ± SE. 25-$OHD_3$ = 25-hydroxyvitamin $D_3$; 1,25-$(OH)_2D_3$ = 1,25-dihydroxyvitamin $D_3$. [a] $p < 0.01$ vs. control values; [b] $p < 0.05$ vs. control values; [c] $p < 0.001$ vs. control values; [d] $p < 0.05$ vs. values before vitamin D administration; [e] $p < 0.001$ vs. values before vitamin D administration; [f] $p < 0.01$ vs. values before vitamin D administration

**Table 2.** Fasting glucose and hormonal levels in epileptic and geriatric patients during vitamin D administration

| | Epileptic patients | | | | Geriatric patients | | | |
|---|---|---|---|---|---|---|---|---|
| | Before treatment | 1 week after treatment | 2 weeks after treatment | Control values | Before treatment | 1 week after treatment | 2 weeks after treatment | Control values |
| Glucose (mmol/l) | 3.8 ± 0.1 | 4.2 ± 0.1 | 4.0 ± 0.1 | 4.1 ± 0.2 | 4.5 ± 0.3 | 4.6-0.3 | 4.3 ± 0.2 | 4.9 ± 0.2 |
| Insulin (mU/l) | 14.8 ± 2.3 | 8.8 ± 1.5[a] | 5.4 ± 0.9[a,b] | 11.5 ± 0.6 | 10.4 ± 1.7 | 12.5 ± 2.5 | 10.1 ± 1.5 | 9.8 ± 3.1 |
| Glucagon (pmol/l) | 12.5 ± 3.0[c] | 22.6 ± 4.7[a,d] | 21.3 ± 3.1[a,d] | 38.5 ± 5.3 | 23.3 ± 2.3 | 23.6 ± 2.6 | 23.4 ± 2.2 | 18.2 ± 4.9 |

Data are expressed as mean ± SE. [a] $p < 0.01$ vs. values before vitamin D administration; [b] $p < 0.001$ vs. control values; [c] $p < 0.01$ vs. control values; [d] $p < 0.05$ vs. control values

### *Study design*

Following an overnight fast, venous blood was collected before and after the ingestion of 75 g glucose for the measurement of glucose, insulin and glucagon. The blood samples were also used to measure biochemical parameters of calcium metabolism. The patients then received a loading dose of 200 μg 25-$OHD_3$ (Dedrogyl, Roussel, Paris, France) followed by 10 μg daily for 2 weeks. The evaluation of calcium metabolism and fasting hormonal values were repeated 1 week after vitamin D treatment. These parameters and the glucose tolerance test were evaluated again after 2 weeks of vitamin D supplementation.

### *Biochemical methods*

Glucose, calcium, phosphorus, alkaline phosphatase and creatinine were measured on a SMAC continuous flow analyzer (Technicon Instruments, Tarrytown, New York, USA). Plasma ionized calcium was measured with an ion selective electrode (Nova 2 Ionized Analyzer, Nova Biomedical, Newton, Massachusetts, USA). The concentrations of serum 25-$OHD_3$ and vitamin D-binding protein were measured by competitive protein binding assay [11] and radial immunodiffusion [12] respectively. Serum parathyroid hormone and 1,25-$(OH)_2D_3$ were measured by radioimmunoassay as previously described [13, 14]. The free 1,25-$(OH)_2D_3$ index was calculated as the molar ratio between the concentrations of this vitamin D metabolite and vitamin D-binding protein [15]. Serum insulin was determined using the charcoal-dextran radioimmunoassay technique [16], and plasma glucagon was determined by radioimmunoassay using blood samples collected in pre-chilled heparinized tubes containing aprotinin. The glucagon radioimmunoassay was carried out according to standard procedures using iodinated glucagon (New England Nuclear, Brussels, Belgium) and standard and antibody (UCB Bioproducts, Brussels, Belgium).

### *Statistical analysis*

In the analysis of the effect of vitamin D treatment on the parameters of calcium metabolism, analysis of variance with repeated measures was used. The Newman-Keuls method for testing a posteriori contrasts was used for comparisons among means [17]. The effect of vitamin D on the secretion of pancreatic hormones was evaluated using the Student's t-test and the Wilcoxon test for paired observations. The Student's t-test for unpaired data and the Mann-Whitney test were used to compare means of independent groups. Data are expressed as mean ± SE.

## Results

### *Evidence for subclinical vitamin D deficiency*

Deficiency of vitamin D was demonstrated by low circulating concentrations of 25-$OHD_3$ and 1,25-$(OH)_2D_3$ in both groups of patients studied (Table 1). The mean level of 25-$OHD_3$ was clearly below the mean levels of

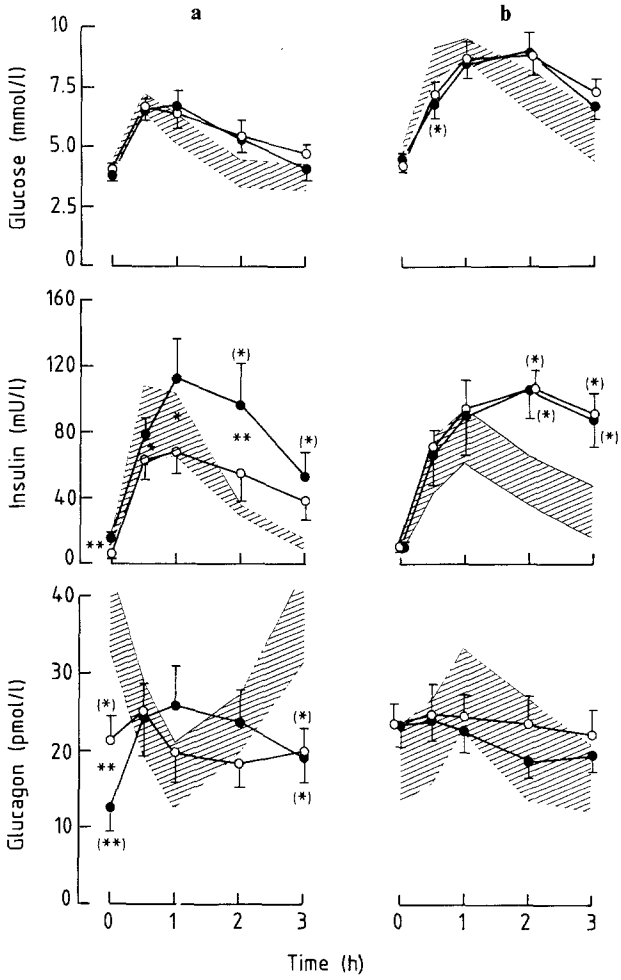


**Fig. 1.** Secretion curves of pancreatic hormones after glucose ingestion before (●—●) and 2 weeks after (○—○) 25-$OHD_3$ treatment in **a** epileptic and **b** geriatric patients. Hatched area represents normal range. *: $p < 0.05$ vs. values before vitamin D administration; **: $p < 0.01$ vs. values before vitamin D administration; (*): $p < 0.05$ vs. control values; (**): $p < 0.01$ vs. control values

the control populations. Half of the epileptic patients and 40% of geriatric patients had 25-$OHD_3$ values in the rachitic range ( < 5 μg/l). The concentrations of total and "free" 1,25-$(OH)_2D_3$ were also decreased in both populations of patients compared with controls, but this decrease was more severe in epileptic patients than in geriatric patients. Other biochemical data were only disturbed in the epileptic patients as shown by decreased serum concentrations of calcium and phosphorus, and by increased levels of parathyroid hormone and alkaline phosphatase activity (Table 1). In the elderly patients only the serum phosphorus concentration was decreased. No clinical signs of osteomalacia were observed. The kidney function, as assessed by serum creatinine, was normal in both study groups. The short term 25-$OHD_3$ treatment restored serum vitamin D metabolites to normal. A significant increase in serum phosphorus concentration and suppression of parathyroid hormone secretion were found in both groups. However, serum calcium and alkaline phosphatase activity remained unchanged. The serum concentration of vitamin D-binding protein remained normal throughout the study.

### *Fasting hormonal levels*

The fasting levels of pancreatic hormones are shown in Table 2. During vitamin D deficiency, fasting plasma glucose and insulin concentrations were normal in both epileptic and geriatric patients.

Glucagon concentration was normal in geriatric patients, but decreased in epileptic patients compared with controls. Vitamin D supplementation did not alter fasting glucose, insulin, or glucagon levels in geriatric patients; however, it decreased insulin and increased glucagon levels in epileptic patients.

### *Response of pancreatic hormones to glucose*

During vitamin D deficiency, glucose tolerance was normal in epileptic patients; however, insulin levels were significantly higher than controls 2 and 3 h after glucose ingestion. Glucose tolerance was impaired and insulin release delayed in geriatric patients (Fig. 1). After vitamin D repletion, the blood glucose curves remained unchanged. Insulin release did not change in geriatric patients, but was reduced by 35% in epileptic patients (Figs. 1 and 2).

In both control subjects and vitamin D-deficient elderly patients, no suppression of glucagon secretion by glucose loading was observed. Vitamin D supplementation in elderly patients did not restore glucagon response to glucose. In younger healthy subjects, glucagon levels decreased after glucose, whereas in vitamin D-deficient epileptic patients they increased. Inhibition of glucagon secretion by glucose tended to be restored after vitamin D supplementation in these patients (Fig. 1).

## Discussion

We investigated glucose tolerance and secretion of pancreatic hormones before and after vitamin D repletion in mildly vitamin D-deficient patients. Low serum 25-$OHD_3$ and 1,25-$(OH)_2D_3$ concentrations were found in both groups of patients. Vitamin D deficiency was more severe in the epileptic patients, as their serum calcium, alkaline phosphatase and parathyroid hormone concentrations were also disturbed. In both groups the serum concentration of 1,25$(OH)_2D_3$ increased rapidly to normal after 25-$OHD_3$ supplementation (Table 1). This clearly demonstrates the presence of a functioning renal 25-$OHD_3$-1$\alpha$-hydroxylase activity, and further supports the conclusion that the 25-$OHD_3$ concentration before vitamin D treatment was insufficient for optimal production of the vitamin D hormone. The increase in serum phosphorus and the decrease in

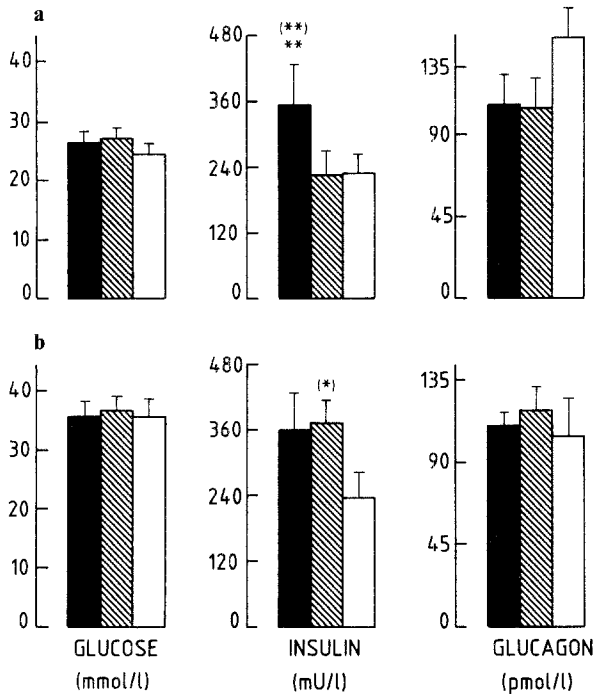


**Fig. 2.** Total sum of blood glucose and pancreatic hormones during oral glucose tolerance test in **a** epileptic and **b** geriatric patients before (■) and after (▨) vitamin D treatment, and in normal subjects (□). **: $p < 0.01$ vs. values before vitamin D treatment; (*): $p < 0.05$ vs. control values; (**): $p < 0.01$ vs. control values

parathyroid hormone after vitamin D repletion also suggest pre-existent vitamin D deficiency.

Recent animal studies have shown that vitamin D is essential for the normal function of pancreatic B cells [5–9]. In the present study, however, we were unable to observe an increase in basal or glucose-stimulated insulin secretion after correction of vitamin D deficiency. This discrepancy could be explained by the much more pronounced vitamin D deficiency obtained in experimental animals. The decreased insulin secretion after vitamin D treatment in epileptic patients is not explained. An effect of diphenylhydantoin is unlikely, since the same amount of drug was administered throughout the study [18]. Other data also indicate that vitamin D administration is not always followed by increased insulin secretion and may even be associated with decreased release of this hormone. In a study by Kawashima et al. [19], insulin concentrations were decreased in obese ob/ob mice treated with 1$\alpha$-hydroxyvitamin D, but remained unchanged in lean animals. Chertow et al. [20] found inhibitory effects of high doses of 1,25-$(OH)_2D_3$ on insulin secretion by isolated rat pancreatic islets, but no effect of physiological doses of this sterol. Likewise, Frankel et al. [21] found no effect of 1,25-$(OH)_2D_3$ on insulin secretion by mouse pancreatic islets.

In contrast to the hypoinsulinaemia of severely vitamin D-deficient animals, we observed a delayed hypersecretion of insulin in mildly vitamin D-deficient epileptic patients. Vitamin D repletion normalized their insulin secretion. This could thus be an indication that vitamin D deficiency decreased the peripheral insulin sensitivity of the epileptic patients. The insulin secretion of the geriatric vitamin D-depleted patients was also delayed and increased above normal; however, it was not corrected by short-term vitamin D repletion and is therefore less likely to be due to vitamin D deficiency.

Vitamin D-deficient patients presented a lack of responsiveness of the A cells to glucose. Indeed, fasting glucagon level was markedly lower in D-deficient epileptic patients than in control subjects, and ingestion of glucose by these patients paradoxically increased glucagon levels instead of suppressing them (Fig. 1), a situation previously reported in diabetic and prediabetic states [10, 22, 23]. Vitamin D treatment tended to restore the glucose sensitivity of the A cells in these patients. Glucagon levels in geriatric patients were comparable to control values, and were non-suppressible by glucose.

Our observations confirm the high frequency of subclinical vitamin D deficiency in epileptic and home-confined geriatric patients. We investigated the secretion of pancreatic hormones comparing each group of patients with controls, and before and during vitamin D supplementation. These longitudinally collected data demonstrate that vitamin D deficiency is associated with a delayed insulin response and a resistance of glucagon secretion to glucose. Vitamin D repletion did not increase insulin secretion or improve glucose tolerance in man.

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Professor Roger Bouillon
Laboratorium Experimentele Geneeskunde en Endocrinologie
Onderwijs en Navorsing
Gasthuisberg
B-3000 Leuven
Belgium