

Vitamin D Metabolites and Their Binding Protein in Adult Diabetic Patients

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SUMMARY

Vitamin D metabolites and vitamin D-binding protein were measured in the serum of nonketotic Bantu and Caucasian insulin-requiring diabetic subjects from Zaire and Belgium, respectively. In Caucasian diabetics, whether untreated ($N = 18$) or insulin treated ($N = 26$), no abnormalities were found. The Bantu diabetics ($N = 20$) were more insulin-deficient and had a poorer glucose control than the Caucasians. They presented, compared with Bantu controls, a significant decrease in the serum concentrations of 25-hydroxyvitamin D₃ (26 ± 10 vs. 35 ± 14 $\mu\text{g/L}$, $P < .01$), 1,25-dihydroxyvitamin D₃ [$1,25(\text{OH})_2\text{D}_3$] (38 ± 15 vs. 58 ± 17 ng/L , $P < .001$), and vitamin D-binding protein (303 ± 55 vs. 356 ± 41 mg/L , $P < .001$). The decreased concentrations of vitamin D metabolites in the adult Bantu diabetic patients may be partly explained by a concomitant decrease in the concentration of vitamin D-binding protein, possibly due to insulin deficiency. The ratio between the molar concentrations of 1,25-(OH)₂D₃ and vitamin D-binding protein, used as an index of the free hormonal level, was also decreased, in association with a decreased serum calcium level.

In conclusion, no abnormalities in vitamin D metabolism were found in Caucasian insulin-dependent diabetics, whereas low serum 1,25(OH)₂D₃ concentrations and hypocalcemia were found in poorly controlled Bantu diabetic subjects. *DIABETES* 1986; 35:911-15.

Alterations of bone and mineral homeostasis frequently occur in both experimental and human diabetes, but the underlying pathogenetic mechanisms remain unclear.¹⁻⁵ In short-term experimental diabetes of the rat, depressed duodenal calcium ab-

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sorption and calcium-binding protein (CaBP), decreased 1,25-dihydroxyvitamin D₃ [$1,25(\text{OH})_2\text{D}_3$] and calcitonin, and increased parathyroid hormone (PTH) levels have been reported by some investigators,⁶⁻⁹ even though denied by others.^{10,11} The serum 1,25(OH)₂D₃ concentration is also depressed in the chronically diabetic rats, but PTH values are decreased, and hyperabsorption of calcium and hypercalcemia develop, possibly due to increased 1,25(OH)₂D₃ receptors.^{12,13} Diabetic rats also present hypercalciuria,^{6,12} despite normal concentrations of renal CaBP.^{14,15} The decreased serum concentrations of 1,25(OH)₂D₃ in diabetic rats have been assigned to a decrease in the renal 25-hydroxyvitamin D₃ [$25(\text{OH})\text{D}_3$]-1 α -hydroxylase activity¹⁶⁻²⁰ or to a decrease in the vitamin D-binding protein (DBP) capacity.²²⁻²⁴

There is much more controversy about the regulation of vitamin D metabolism in human diabetes. Frazer et al.²⁵ reported decreased circulating 1,25(OH)₂D₃ concentrations in diabetic children. In line with this study, Orci et al.^{26,27} reported decreased contents of renal and cerebellar CaBP in diabetic subjects. Most investigators, however, found normal concentrations for 1,25(OH)₂D₃ and normal, decreased, or increased concentrations for 25(OH)D₃, 24,25-dihydroxyvitamin D₃ [$24,25(\text{OH})_2\text{D}_3$], and PTH in insulin-dependent diabetic subjects.²⁸⁻³⁰ There are no reports on the relationship between vitamin D metabolites and their transport protein in human diabetes, and we know of no study of vitamin D metabolism in African diabetics. We therefore measured vitamin D metabolites and their binding protein in insulin-requiring, adult diabetic patients from a Bantu population in parallel with a study in a Caucasian population.

SUBJECTS AND METHODS

Diabetic patients were selected on the basis of the following criteria: 1) symptomatic hyperglycemia and need for insulin for a suitable blood glucose control, 2) no acute ketoacidosis, 3) no evidence for other disease, 4) no intake of drugs known to interfere with calcium metabolism, and 5) normal serum creatinine concentration.

Eighteen newly diagnosed and 26 insulin-treated Caucasian diabetic subjects were selected during the summer

TABLE 1
Characteristics of Bantu and Caucasian diabetic subjects

	N	Sex (M:F)	Age (yr)	Duration of diabetes (yr)	Daily insulin dose (U)	Blood glucose (mg/dl)	HbA _{1c} (%)	C peptide (nmol/l)	Creatinine (mg/dl)
Bantu	20	13:7	46 ± 14	4.1 ± 3.6	41 ± 20	328 ± 156		0.06 ± 0.05	0.85 ± 0.24
Caucasians									
Insulin treated	26	15:11	47 ± 16	9.6 ± 11.0	30 ± 24	204 ± 101	10.6 ± 2.3	0.18 ± 0.20	0.93 ± 0.19
Newly diagnosed	18	14:4	29 ± 15	0	0	280 ± 78	13.2 ± 3.2	0.21 ± 0.10	1.09 ± 0.24

months at the St. Rafael University Hospital of Leuven, Belgium. Their mean age was 29 ± 15 (SD) yr and 47 ± 16 (SD) yr, respectively. The insulin-treated patients were receiving a once- or twice-daily insulin regimen depending on their needs. Twenty Bantu patients, aged 46 ± 14 yr, were selected in December and January at the University Hospital of Kinshasa, Zaire. These patients presented no clinical evidence for protein malnutrition, but their insulin treatment was irregular due to socioeconomic factors. The control groups consisted of 36 healthy Bantu (29 males, 7 females) and 26 Caucasian (14 males, 12 females) ambulatory subjects selected in the general population and aged 45 ± 11 yr and 44 ± 12 yr, respectively. The newly diagnosed Caucasian diabetic patients were compared with a subgroup of the Caucasian control subjects aged 31 ± 8 yr. The main characteristics of the patients are given in Table 1.

The postprandial serum C-peptide concentrations in Bantu diabetics and in insulin-treated or newly diagnosed Caucasian diabetics (0.06 ± 0.05, 0.18 ± 0.20, and 0.21 ± 0.10 nmol/L, respectively) were markedly lower than the fasting values in normal Bantu and Caucasian subjects (0.36 ± 0.19 and 0.45 ± 0.16 nmol/L, respectively), indicating insulin deficiency in the diabetic subjects. The Bantu diabetics, however, had the lowest C-peptide values.

Blood sampling was made between 0800 and 1000 h, 1–2 h after breakfast. Serum samples from Leuven were either immediately used or stored at –20°C until used. Samples from Kinshasa were packed on ice, and the package was transported as hand luggage by aircraft, kept at 4°C during an 8-h journey. The samples were then kept at –20°C until assay. Note that transporting samples at even higher temperatures has been shown not to be harmful to the blood constituents measured in our study.^{31,32}

Serum PTH and 1,25(OH)₂D₃ were measured by radioim-

munoassay.^{33,34} Serum 25(OH)D₃ and DBP were measured by competitive protein-binding assay³⁵ and by radial immunodiffusion,³⁶ respectively. The free 1,25(OH)₂D₃ index was calculated as the ratio between the molar concentrations of this metabolite and DBP.³⁷ C peptide was determined according to Heding³⁸ with a human C-peptide radioimmunoassay kit (Novo, Bagsvaerd, Denmark). Automated methods were used to measure glucose, creatinine, calcium, and alkaline phosphatase (Technicon, Tarrytown, NY). Glycosylated hemoglobin (HbA_{1c}) was measured by cation-exchange chromatography with commercially available microcolumns and reagents (Isolab, Akron, OH). Serum albumin was measured by radial immunodiffusion with rabbit anti-human albumin immunoglobulins (Dako, Copenhagen, Denmark). Serum calcium was corrected for albumin levels according to Berry et al.³⁹ Statistical analysis was performed by the Student's *t* test, the Mann-Whitney test, and linear regression analysis. Data are presented as mean ± SD.

RESULTS

In normal subjects from both Bantu and Caucasian populations, the serum concentration of calcium was similar. The concentration of albumin was lower and the alkaline phosphatase activity higher in Bantu than in Caucasians (Table 2). The concentration of DBP was also similar in both control groups. By contrast, the concentrations of total and free 1,25(OH)₂D₃ were elevated in normal Bantu subjects compared with Caucasians (Table 3).

In Caucasian diabetic subjects, serum calcium, albumin, DBP, 25(OH)D₃ and 1,25(OH)₂D₃ concentrations were all normal, and the alkaline phosphatase and PTH levels were slightly increased in comparison with control values (Tables 2–4). In Bantu diabetic subjects, however, total serum calcium and albumin concentrations were both significantly de-

TABLE 2
Serum albumin, calcium, and alkaline phosphatase in insulin-treated diabetic patients

	Albumin (g/dl)	Total calcium (mg/dl)	Protein-corrected calcium (mg/dl)	Alkaline phosphatase (U/L)
Bantu				
Controls	3.7 ± 0.3*	9.2 ± 0.6	10.3 ± 0.4	85 ± 19§
Diabetics	2.9 ± 0.6†	8.0 ± 0.6‡	9.6 ± 0.9‡	94 ± 28
Caucasians				
Controls	4.1 ± 0.5	9.5 ± 0.3	10.1 ± 0.6	65 ± 21
Diabetics	3.9 ± 0.4	9.5 ± 0.4	10.0 ± 0.4	76 ± 21‡

**P* < .05 between Bantu and Caucasian controls.

†*P* < .001 diabetics versus controls from the same population.

‡*P* < .05 diabetics versus controls from the same population.

§*P* < .01 between Bantu and Caucasian controls.

TABLE 3
Serum DBP, 25(OH)D₃, 1,25(OH)₂D₃, and PTH in insulin-treated diabetic patients

	DBP (mg/L)	25(OH)D ₃ (μg/L)	1,25(OH) ₂ D ₃ (ng/L)	Free 1,25(OH) ₂ D ₃ index	PTH (mU/L)
Bantu					
Controls	356 ± 41	35 ± 14	58 ± 17†	2.3 ± 0.6§	44 ± 32
Diabetics	303 ± 55*	26 ± 10†	38 ± 15*	1.6 ± 0.6*	65 ± 29
Caucasians					
Controls	364 ± 45	33 ± 9	47 ± 9	1.7 ± 0.4	52 ± 34
Diabetics	373 ± 69	34 ± 16	45 ± 11	1.6 ± 0.4	69 ± 28

Free 1,25(OH)₂D₃ index was calculated as the ratio ($\times 10^5$) between molar concentrations of this metabolite and DBP 37.

**P* < .001 diabetics versus controls from the same population.

†*P* < .01 diabetics versus controls from the same population.

‡*P* < .01 between Bantu and Caucasian controls.

§*P* < .001 between Bantu and Caucasian controls.

||*P* < .05 diabetics versus controls from the same population.

creased, whereas protein-corrected serum calcium was slightly but significantly decreased (Table 2). In these patients, the serum values for DBP, 25(OH)D₃, and total 1,25(OH)₂D₃ were all reduced below control levels (Table 3). The free 1,25(OH)₂D₃ concentration was also reduced, together with reduced protein-corrected serum calcium and increased PTH concentrations.

Correlation coefficients were calculated for vitamin D metabolites, DBP, albumin, and calcium. In Bantu subjects, positive interrelations were found among these factors (Table 5). Low DBP concentrations were associated with low 25(OH)D₃, 1,25(OH)₂D₃, total calcium, and albumin. Correlation studies showed no influence of glucose control or duration of diabetes on the concentrations of vitamin D metabolites or DBP.

DISCUSSION

Our data show that the circulating concentrations of total and free 1,25(OH)₂D₃ were higher in Bantu than in Caucasian healthy subjects. Because the concentrations of 25(OH)D₃ were similar in both healthy populations, due to blood sampling in the summer in Belgium, the difference in 1,25(OH)₂D₃ cannot be related to sun exposure but is possibly due to low dietary calcium intake in the Bantu people.^{40,41}

The serum 1,25(OH)₂D₃ concentration was decreased in Bantu insulin-requiring diabetic subjects relative to controls from the same population. The decreased 25(OH)D₃ level in these patients might reflect a less frequent exposure to sunshine by patients with less active lives. The concentration of this precursor sterol, however, was still widely sufficient and

therefore cannot account for the decreased level of 1,25(OH)₂D₃ in these diabetic patients.

We have recently shown that DBP concentration is decreased in insulinopenic rats and proposed this abnormality as a pathogenetic mechanism for the decreased 1,25(OH)₂D₃ level in these animals.²²⁻²⁴ This explanation may, at least partly, apply to our Bantu diabetic subjects, because their C-peptide levels were very low and their DBP concentration was significantly decreased relative to controls. In analogy with our observations, a decrease in thyroxine-binding protein concentration was associated with decreased total thyroxine, triiodothyronine, and reverse triiodothyronine in insulin-dependent diabetic subjects.⁴² In view of the lower serum albumin level in the Bantu diabetics, however, a mild protein malnutrition as the origin of low DBP concentration cannot be excluded. However, the observed decrease in free 1,25(OH)₂D₃ concentration in these patients cannot be explained by a decrease in DBP and could be the result of insulinopenia and poor glucose control. Indeed, experimental insulin deficiency in rats is associated with a reduced 25(OH)D₃-1-hydroxylase activity,¹⁶⁻¹⁸ and insulin permits the stimulation of this enzyme by PTH or low calcium intake.¹⁹⁻²¹ These results agree with the observations of Frazer et al.²⁵ in insulin-dependent diabetic children and with the recent results of Ishida et al.⁴³ in Japanese insulin-dependent diabetic subjects.

Adult Caucasian diabetics showed slight alterations in PTH and alkaline phosphatase levels, but we found no abnormalities of vitamin D metabolism in these patients, in ac-

TABLE 4
Biochemical measurements in Caucasian diabetic subjects before and 1 yr after insulin treatment

	Albumin (g/dl)	Total calcium (mg/dl)	Protein-corrected calcium (mg/dl)	Alkaline phosphatase (U/L)	DBP (mg/l)	25(OH)D ₃ (μg/L)	1,25(OH) ₂ D ₃ (ng/L)	Free 1,25(OH) ₂ D ₃ index	PTH (mU/L)
Diabetics									
Before	4.6 ± 0.3	9.8 ± 0.4	9.8 ± 0.5	94 ± 25*	344 ± 61	30 ± 12	44 ± 13	1.8 ± 0.6	44 ± 14
After					366 ± 66†	31 ± 15	45 ± 12	1.7 ± 0.7	48 ± 13
Controls	4.2 ± 0.4	9.5 ± 0.3	10.0 ± 0.6	62 ± 16	369 ± 45	33 ± 9	47 ± 9	1.8 ± 0.4	54 ± 35

Free 1,25(OH)₂D₃ index calculated as in Table 3.

**P* < .05 diabetics versus controls.

†*P* < .05 before versus after 1 yr of insulin treatment.

TABLE 5
Correlations among biochemical parameters in Bantu subjects

	Albumin	DBP	25(OH)D ₃	1,25(OH) ₂ D ₃
DBP	0.434*			
25(OH)D ₃	0.445*	0.470†		
1,25(OH) ₂ D ₃	0.461*	0.474†	0.332‡	
Total calcium	0.542†	NS	0.389‡	0.438*

NS, not significant.

Significance of correlation coefficients: * $P < .01$; † $P < .001$; ‡ $P < .05$.

cordance with previous reports.²⁸⁻³⁰ In these studies and our study, the patients probably presented a still sufficient endogenous insulin secretion⁴⁴ and/or a sufficient exogenous insulin supply. Levy et al.⁴⁵ recently showed that diabetic rats with subnormal insulin levels can maintain normal calcium, vitamin D, and bone metabolism, despite elevated blood glucose.

In conclusion, insulin-deficient Caucasian diabetics without ketoacidosis or moderately controlled with insulin have no detectable abnormalities in vitamin D metabolism. More severely insulinopenic Bantu diabetics with poorer glucose control demonstrated a significant decrease in total and free 1,25(OH)₂D₃ associated with a mild hypocalcemia.

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