Vitamin D Metabolites and Their Binding Protein in Adult Diabetic Patients

B. L. NYOMBA, R. BOUILLON, M. BIDINGIJA, K. KANDJINGU, AND P. DE MOOR

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 D_3 (26 ± 10 vs. 35 ± 14 μg/L, $P < .01$), 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] (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-$(OH)_2D_3$ 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$(OH)_2D_3$ 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 absorption and calcium-binding protein (CaBP), decreased 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] 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$(OH)_2D_3$ 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$(OH)_2D_3$ 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$(OH)_2D_3$ in diabetic rats have been assigned to a decrease in the renal 25-hydroxyvitamin D_3 [25$(OH)D_3$]-1α-hydroxylase activity[16–20] 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$(OH)_2D_3$ 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$(OH)_2D_3$ and normal, decreased, or increased concentrations for 25$(OH)D_3$, 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$], 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

From the Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Belgium (B.L.N., R.B., P.D.M.); and Département de Médecine Interne, Université de Kinshasa, Zaire (M.B., K.K.).
Address reprint requests to Prof. Dr. Roger Bouillon, Laboratorium voor Experimentele, Geneeskunde en Endocrinologie, Campus Gasthuisberg, B-3030 Leuven, Belgium.
Received for publication 18 November 1985 and in revised form 18 February 1986.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/8/911/354490/35-8-911.pdf by guest on 15 April 2024

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_1 (%)	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 $1,25(OH)_2D_3$ were measured by radioimmunoassay.[33,34] Serum $25(OH)D_3$ and DBP were measured by competitive protein-binding assay[35] and by radial immunodiffusion,[36] respectively. The free $1,25(OH)_2D_3$ index was calculated as the ratio between the molar concentrations of this metabolite and DBP.[37] C peptide was determined according to Heding[38] 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_1) 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 $1,25(OH)_2D_3$ were elevated in normal Bantu subjects compared with Caucasians (Table 3).

In Caucasian diabetic subjects, serum calcium, albumin, DBP, $25(OH)D_3$ and $1,25(OH)_2D_3$ 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.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/8/911/354490/35-8-911.pdf by guest on 15 April 2024

TABLE 3
Serum DBP, $25(OH)D_3$, $1,25(OH)_2D_3$, and PTH in insulin-treated diabetic patients

	DBP (mg/L)	$25(OH)D_3$ (μg/L)	$1,25(OH)_2D_3$ (ng/L)	Free $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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, $25(OH)D_3$, and total $1,25(OH)_2D_3$ were all reduced below control levels (Table 3). The free $1,25(OH)_2D_3$ 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 $25(OH)D_3$, $1,25(OH)_2D_3$, 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 $1,25(OH)_2D_3$ were higher in Bantu than in Caucasian healthy subjects. Because the concentrations of $25(OH)D_3$ were similar in both healthy populations, due to blood sampling in the summer in Belgium, the difference in $1,25(OH)_2D_3$ cannot be related to sun exposure but is possibly due to low dietary calcium intake in the Bantu people.[40,41]

The serum $1,25(OH)_2D_3$ concentration was decreased in Bantu insulin-requiring diabetic subjects relative to controls from the same population. The decreased $25(OH)D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 thyroxin-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 $1,25(OH)_2D_3$ 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 $25(OH)D_3$-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)	$1,25(OH)_2D_3$ (ng/L)	Free $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ index calculated as in Table 3.
*$P < .05$ diabetics versus controls.
†$P < .05$ before versus after 1 yr of insulin treatment.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/8/911/354490/35-8-911.pdf by guest on 15 April 2024

TABLE 5
Correlations among biochemical parameters in Bantu subjects

	Albumin	DBP	$25(OH)D_3$	$1,25(OH)_2D_3$
DBP	0.434*			
$25(OH)D_3$	0.445*	0.470†		
$1,25(OH)_2D_3$	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 secretion[44] 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 $1,25(OH)_2D_3$ associated with a mild hypocalcemia.

ACKNOWLEDGMENTS

The technical assistance of Mr. W. Coopmans, I. Jans, J. Laureys, and E. Van Herck is gratefully acknowledged.

This work was supported by the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Grants 3.0019.81 and 3.0029.85).

REFERENCES

[1] Ramamurthy, N. S., Zebrowski, E. J., Baker, C., and Golub, L. M.: Alloxan diabetes and reduced bone density in rat mandibule. Res. Commun. Chem. Pharmacol. 1973; 5:614–20.

[2] Levin, M. E., Boisseau, V. C., and Avioli, L. V.: Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. N. Engl. J. Med. 1976; 294:241–45.

[3] Rosenbloom, A. L., Lezotte, D. C., Weber, T. F., Gudat, J., Heller, D. R., Weber, M. L., Klein, S., and Kennedy, B. B.: Diminution of bone mass in childhood diabetes. Diabetes 1977; 26:1052–55.

[4] McNair, P., Madsbad, S., Christensen, M. S., Christiansen, C., Faber, O. K., Binder, C., and Transbol, I.: Bone mineral loss in insulin-treated diabetes mellitus: studies on pathogenesis. Acta Endocrinol. 1979; 90:463–72.

[5] Hough, S., Avioli, L. V., Bergfeld, M. A., Fallon, M. D., Slatopolsky, E., and Teitelbaum, S. L.: Correction of abnormal bone and mineral metabolism in chronic streptozotocin-induced diabetes mellitus in the rat by insulin therapy. Endocrinology 1981; 108:2228–34.

[6] Schneider, L. E., and Schedl, H. P.: Diabetes and intestinal calcium absorption in the rat. Am. J. Physiol. 1972; 223:1319–23.

[7] Schneider, L. E., Wilson, H. D., and Schedl, H. P.: Intestinal calcium binding protein in the diabetic rat. Nature 1973; 245:327–28.

[8] Schneider, L. E., Schedl, H. P., McCain, T., and Haussler, M. R.: Experimental diabetes reduces circulating 1,25-dihydroxyvitamin D in the rat. Science 1977; 196:1452–54.

[9] Schedl, H. P., Heath, H., and Wenga, J.: Serum calcitonin and parathyroid hormone in experimental diabetes: effects of insulin treatment. Endocrinology 1978; 103:1368–73.

[10] Charles, M. A., Tirunaguru, P., and Morrissey, R. L.: Duodenal calcium transport and calcium binding protein levels in experimental diabetes mellitus. Min. Electrolyte Metab. 1981; 5:15–22.

[11] Ishida, H., Seino, Y., Tsuda, K., Takemura, J., Nishi, S., Ishizuka, S., and Imura, H.: Effects of streptozotocin-induced diabetes on circulating levels of vitamin D metabolites. Acta Endocrinol. 1983; 104:96–102.

[12] Hough, S., Russel, J. E., Teitelbaum, S. L., and Avioli, L. V.: Calcium homeostasis in chronic streptozotocin-induced diabetes mellitus in the rat. Am. J. Physiol. 1983; 242:E451–56.

[13] Seino, Y., Sierre, R. I., Sonn, Y. M., Jafair, A., Birge, S. J., and Avioli, L. V.: The duodenal $1\alpha,25$-dihydroxyvitamin D_3 receptor in rats with experimentally induced diabetes. Endocrinology 1983; 113:1721–25.

[14] Weissman, J., Schneider, L. E., and Schedl, H.: Renal calcium binding protein in the diabetic and vitamin D–deficient rat. Biochim. Biophys. Acta 1977; 497:358–68.

[15] Schedl, H. P., Christakos, S., Wilson, H. D., Malkowitz, L., and Horst, R. L.: Diabetes and renal calcium binding protein in the rat. Proc. Soc. Exp. Biol. Med. 1984; 177:176–79.

[16] Spencer, E. M., Khalil, M., and Tobiassen, O.: Experimental diabetes in the rat causes an insulin reversible decrease in renal 25-hydroxyvitamin D_3-1-hydroxylase activity. Endocrinology 1980; 107:300–305.

[17] Wongsurawat, N., Armbrecht, H. J., Zenser, T. V., Davis, B. B., Thomas, M. L., and Forte, L. R.: 1,25-Dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 production by isolated renal slices is modulated by diabetes and insulin in the rat. Diabetes 1983; 32:302–306.

[18] Hough, S., Fausto, A., Sonn, Y., Dong, J. O. O. K., Birge, S. J., and Avioli, L. V.: Vitamin D metabolism in the chronic streptozotocin-induced diabetic rat. Endocrinology 1983; 113:790–96.

[19] Henry, H. L.: Insulin permits parathyroid hormone stimulation of 1,25-dihydroxyvitamin D_3 production in cultured kidney cells. Endocrinology 1981; 108:733–35.

[20] Wongsurawat, N., and Armbrecht, H. J.: Insulin modulates the stimulation of renal $1,25-(OH)_2D_3$ production by parathyroid hormone. Acta Endocrinol. 1985; 109:243–48.

[21] Wilson, H. D., Horst, R. L., and Schedl, H. P.: Calcium intake regulates 1,25-dihydroxyvitamin D formation in the diabetic rat. Diabetes 1982; 31:401–405.

[22] Nyomba, B. L., Bouillon, R., and De Moor, P.: Vitamin D metabolites and vitamin D–binding protein in streptozotocin-induced and spontaneous diabetes of rat. Abstract. Diabetologia 1984; 27:315A–16A.

[23] Nyomba, B. L., Bouillon, R., Lissens, W., Van Baelen, H., and De Moor, P.: 1,25-Dihydroxyvitamin D and vitamin D–binding protein are both decreased in streptozotocin-diabetic rats. Endocrinology 1985; 116:2483–88.

[24] Nyomba, B. L., Bouillon, R., and De Moor, P.: Decreased serum 1,25-dihydroxyvitamin D in diabetes: possible role of vitamin D–binding protein. *In* Vitamin D. A Chemical, Biochemical and Clinical Update. Norman, A. W., Schaefer, K., Grigoleit, H.-G., and Herrath, D. V., Eds. Berlin, de Gruyter, 1985:869–70.

[25] Frazer, T. E., White, N. H., Hough, S., Santiago, J. V., McGee, B. R., Bryce, G., Mallon, J., and Avioli, L. V.: Alterations in circulating vitamin D metabolites in the young insulin-dependent diabetic. J. Clin. Endocrinol. Metab. 1981; 53:1154–59.

[26] Orci, L., Brown, D., Roth, J., and Norman, A. W.: Vitamin-D–dependent calcium binding protein content is reduced in kidneys of juvenile-onset diabetics. Lancet 1982; 2:102–103.

[27] Bruhlmann-Papazyan, M., Garcia-Segura, L. M., Pizzolato, G. P., Norman, A. W., and Orci, L.: Immunohistochemically detectable vitamin D–dependent calcium-binding protein is reduced in cerebellum of diabetic subjects. Diabetes 1984; 33:917–22.

[28] Heath, H., Lambert, P. W., Service, F. J., and Arnaud, S. B.: Calcium homeostasis in diabetes mellitus. J. Clin. Endocrinol. Metab. 1979; 49:462–66.

[29] Christiansen, C., Christensen, M. S., Nielsen, B., and Madsbad, S.: Vitamin D metabolites in diabetic patients: decreased serum concentration of 24,25-dihydroxyvitamin D. Scand. J. Clin. Lab. Invest. 1982; 42:487–91.

[30] Witt, M. F., White, N. H., Santiago, J. V., Seino, Y., and Avioli, L. V.: Use of oral calcium loading to characterize the hypercalcemia of young insulin-dependent diabetes. J. Clin. Endocrinol. Metab. 1983; 57:94–100.

[31] Berg, B., Estborn, B., and Tryding, N.: Stability of serum and blood constituents during mail transport. Scand. J. Clin. Lab. Invest. 1981; 41:425–30.

[32] Lissner, D., Mason, R. S., and Posen, S.: Stability of vitamin D metabolites in human blood serum and plasma. Clin. Chem. 1981; 27:773–74.

[33] Bouillon, R., Koninckx, P., and De Moor, P.: A radioimmunoassay for serum parathyroid hormone: methods and clinical application. *In* Radioimmunoassay and Related Procedures in Medicine, Vol 1. Vienna, International Atomic Energy Agency, 1974:353–65.

[34] Bouillon, R., De Moor, P., Baggiolini, E. G., and Uskokovic, M. R.: A radioimmunoassay for 1,25-dihydroxycholecalciferol. Clin. Chem. 1980; 26:562–67.

[35] Bouillon, R., Van Kerkhove, P., and De Moor, P.: Measurement of 25-hydroxyvitamin D_3 in serum. Clin. Chem. 1976; 22:364–68.

[36] Bouillon, R., Van Baelen, H., and De Moor, P.: The measurement of vitamin D–binding protein in human serum. J. Clin. Endocrinol. Metab. 1977; 45:225–31.

[37] Bouillon, R., Van Assche, F. A., Van Baelen, H., Heyns, W., and De Moor, P.: Influence of the vitamin D–binding protein on the serum concentration of 1,25-dihydroxyvitamin D_3. Significance of the free 1,25-dihydroxyvitamin D_3 concentration. J. Clin. Invest. 1981; 67:589–96.

[38] Heding, L. G.: Radioimmunological determination of human C-peptide in serum. Diabetologia 1975; 11:541–48.

[39] Berry, E. M., Gupta, M. M., Turner, S. J., and Burns, R. R.: Variations in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. Br. Med. J. 1973; 4:640–43.

[40] Pettifor, J. M., Ross, F. P., Travers, R., Glorieux, F. H., and DeLuca,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/8/911/354490/35-8-911.pdf by guest on 15 April 2024

H. F.: Dietary calcium deficiency: a syndrome associated with bone deformities and elevated serum 1,25-dihydroxyvitamin D concentrations. Metab. Bone Dis. Rel. Res. 1981; 2:301–305.

[41] Lambrechts, A., and Holemans, K.: Le problème du phosphore et du calcium en zone rurale au Congo belge. Ann. Soc. Belg. Med. Trop. 1958; 38:459–86.

[42] Bernasconi, S., Vanelli, M., Nori, G., Siracusano, M. F., Marcellini, C., Butturini, A., and De Luca, F.: Serum TSH, T4, T3, FT4, FT3, rT3, and TBG in youngsters with non-ketotic insulin-dependent diabetes mellitus. Hormone Res. 1984; 20:213–17.

[43] Ishida, H., Seino, Y., Taminato, Y., Nishi, S., Ikeda, M., Yawata, M., Moridera, K., Tsuda, K., Goto, Y., and Imura, H.: Osteopenia and circulating levels of vitamin D metabolites in diabetic patients. Abstract. International Diabetes Federation Meeting, Madrid, 1985. Diabetes Res. Clin. Pract. 1985; S262 (Suppl 1).

[44] Gonen, B., Goldman, J., Baldwin, D., Goldberg, R. B., Ryan, W. G., Blix, P. M., Schanzli, D., Fritz, K. J., and Rubenstein, A. H.: Metabolic control in diabetic patients. Effect of insulin-secretory reserve (measured by plasma C-peptide levels) and circulating insulin antibodies. Diabetes 1979; 28:749–53.

[45] Levy, J., Teitelbaum, S. L., Gavin, J. R., III, Fausto, A., Kurose, H., and Avioli, L. V.: Bone calcification and calcium homeostasis in rats with non-insulin-dependent diabetes induced by streptozotocin. Diabetes 1985; 34:365–72.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/8/911/354490/35-8-911.pdf by guest on 15 April 2024