Endocrine Journal 1995, **42** (3), 377–383

Effect of Glycemic Control on Calcium and Phosphorus Handling and Parathyroid Hormone Level in Patients with Non-Insulin-Dependent Diabetes Mellitus

SHOICHIRO NAGASAKA*,**, TORU MURAKAMI*, TORU UCHIKAWA*,
SAN-E ISHIKAWA**, AND TOSHIKAZU SAITO**

**Department of Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo 113, and*
***Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi 329-04, Japan*

Abstract. The present study was undertaken to determine whether improvement of hyperglycemia alters calcium and phosphorus handling, parathyroid hormone (PTH) secretion and bone turnover in patients with non-insulin-dependent diabetes mellitus (NIDDM). We measured serum and urinary mineral levels, serum intact PTH and osteocalcin on admission and at discharge (38 ± 3 days later, Means ± SEM) in 28 patients with poorly-controlled NIDDM (63 ± 2 years old, 13 males and 15 females). During the hospitalization period, glycemic control was markedly improved. Serum calcium levels remained unchanged, but serum phosphorus increased. Urinary calcium and phosphorus excretion decreased. Serum intact PTH decreased from mid-normal (30.0 ± 2.2 ng/*l*) to low normal values (24.0 ± 1.3 ng/*l*) ($P<0.01$, normal values: 10~65 ng/*l*). Serum osteocalcin increased from 4.14 ± 0.35 to 4.92 ± 0.40 μg/*l* ($P<0.01$, normal values: 2.5~13 μg/*l*). On admission, urinary calcium and phosphorus excretion showed a positive correlation with urinary glucose excretion. Serum calcium levels showed a negative correlation with serum intact PTH ($r=-0.46$, $P<0.05$). Moreover, the change in serum calcium during the hospitalization was negatively correlated to the change in serum intact-PTH ($r=-0.45$, $P<0.05$). Serum phosphorus concentrations showed a positive correlation with the renal threshold for phosphorus excretion on admission ($r=0.86$, $P<0.01$). These results indicate that hyperglycemia causes excess urinary calcium and phosphorus excretion in patients with NIDDM. In response to urinary calcium loss, PTH secretion is mildly stimulated. Bone formation seems to be suppressed in the hyperglycemic state in spite of increased PTH secretion.

Key words: Calcium, Phosphorus, Parathyroid hormone, Osteocalcin, Non-insulin-dependent diabetes mellitus

(*Endocrine Journal* **42:** 377–383, 1995)

SEVERAL studies have demonstrated that increased urinary loss of calcium, phosphorus and magnesium may be related to osteopenia [1, 2] or growth retardation [3] in patients with insulin-dependent diabetes mellitus. It is generally accepted that the capacity to secrete parathyroid hormone (PTH) is diminished in patients with diabetes mellitus [4, 5]. An increased concentration of parathyroid hormone-related peptide may play a compensatory role in calcium homeostasis in patients with non-insulin-dependent diabetes mellitus (NIDDM) [6]. With respect to bone metabolism, histomorphometric studies have shown that bone turnover is low in diabetic animals [7, 8]. Also, serum osteocalcin concentrations are low in diabetic animals and human patients [7–10]. Urinary loss of minerals can be corrected by appropriate blood glucose control [11–13], but it remains unclear whether glycemic control has any effect on PTH secretion or bone turnover. The present study

Received: October 13, 1994
Accepted: January 30, 1995
Correspondence to: Dr. Shoichiro NAGASAKA, Division of Endocrinology and Metabolism, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Tochigi 329-04, Japan

was undertaken to determine whether glycemic control alters calcium and phosphorus handling, serum PTH level or bone metabolic markers in patients with poorly-controlled NIDDM.

Subjects and Methods

Twenty-eight diabetic patients (13 males and 15 females) with a mean age of 63 ± 2 years were studied at Tokyo Metropolitan Komagome Hospital from 1991 to 1993. Only one premenopausal female was included. Their Body Mass Index was 22.8 ± 0.7 on admission. They were admitted because of hyperglycemia and diagnosed as having NIDDM, based on their typical clinical courses and urinary C-peptide excretion (23.6 ± 3.2 nmol/day) [14]. Gastrectomized patients and patients with liver diseases, endocrine disorders and those taking drugs affecting bone metabolism were all excluded. Serum creatinine concentrations were below 106 μmol/l in all the patients. Diabetic retinopathy was present in 13 of them, urinary albumin excretion exceeded 30 mg/day in 14 and Achilles tendon reflex was absent in 13. At discharge, 8 patients were treated with diet therapy alone, 10 with sulfonylurea and 10 with insulin. Among 10 insulin-treated patients, 6 patients were newly introduced to insulin therapy. During the hospitalization period, they were given 30 kcal diet per kg of their ideal body weight, containing mean levels of 660 mg calcium, 1130 mg phosphorus and 210 mg magnesium per day. As exercise, they continued walking of 10,000 paces as much as possible. All the patients gave their informed consent to the present study.

On day 2 or 3 of the hospitalization and 3 or 4 days before discharge (38 ± 3 days after the admission), blood samples were taken after an overnight fast to measure plasma glucose, HbA_1c, serum calcium, phosphorus, magnesium, creatinine, intact PTH, 1, 25 dihydroxycholecalciferol (1, $25(OH)_2D_3$), alkaline-phosphatase, leucine amino peptidase and osteocalcin. On the same days as blood sampling, 24-h urine collection was done to measure the daily excretion of calcium, phosphorus, magnesium, creatinine, glucose and C-peptide.

Plasma and urinary glucose concentrations were measured by the glucose-oxidase method, and HbA_1c by HPLC [15]. Serum and urinary calcium, phosphorus, magnesium, creatinine and serum leucine amino peptidase concentrations were measured with an automatic clinical analyzer (Model 736, Hitachi Co., Katsuta, Japan). Serum total alkaline-phosphatase was measured by the method using paranitrophenyl phosphate [16]. Serum intact PTH and osteocalcin levels were measured with immunoradiometric assay kits (Baxter Co. and Mitsubishi Yuka Co., Tokyo, Japan) [17, 18]. Serum 1, $25(OH)_2D_3$ concentrations were measured by a radioreceptor assay [19]. Urinary C-peptide levels were measured with a radioimmunoassay kit (Daiichi-Radioisotope Co., Tokyo, Japan) [20]. The intra- and inter-assay coefficients of variation were 2.6% and 5.8% for intact-PTH, 6.3% and 5.7% for osteocalcin, 7.7% and 7.6% for 1, $25(OH)_2D_3$, and 5.7% and 3.5% for C-peptide, respectively. Serum calcium values (mmol/l) in this paper were converted from the corrected values for calcium (mg/dl); the corrected values for calcium = the measured values for calcium (mg/dl) + 4 − the values for serum albumin (g/dl) [21]. The renal threshold for phosphorus excretion was obtained from the nomogram by Walton [22].

Data are presented as the Means ± SEM. Statistical analysis was performed by using Student's paired t-test and Pearson's correlation coefficient. A P value of less than 0.05 was considered to be significant.

Results

Tables 1~3 show the parameters measured on admission and at discharge. There was significant reduction in fasting plasma glucose, HbA_1c and urinary glucose excretion after the induction of appropriate therapies during the hospitalization. Urinary C-peptide excretion did not show a significant change. Serum calcium concentrations remained unchanged, while serum phosphorus rose significantly. Serum magnesium concentrations were slightly but significantly elevated (Table 1). There was a significant decrease in the ratios of urinary calcium and phosphorus to creatinine, as well as the daily excretion of both minerals. Urinary magnesium excretion did not change. The renal threshold for phosphorus excretion rose significantly (Table 2). Serum intact PTH decreased from mid-normal to low-normal values. Serum 1, $25(OH)_2D_3$ remained unchanged at low-normal values. On admission, serum 1, $25(OH)_2D_3$

concentrations were below the normal range in 10 of the patients (36%). Serum alkaline-phosphatase decreased from high-normal to mid-normal values. Serum leucine amino peptidase showed a similar change. Serum osteocalcin increased slightly but significantly, but remained at low-normal values (Table 3).

On admission, urinary calcium and phosphorus excretion showed a positive correlation with urinary glucose excretion (Fig. 1). Serum calcium

Table 1. Indices of glycemic control and serum electrolytes on admission and at discharge in patients with poorly-controlled non-insulin-dependent diabetes mellitus

	Normal values	Diabetic patients		(n)	*P* values
		On admission	At discharge		
Fasting plasma glucose (mmol/*l*)	3.3 ~ 5.6	12.3 ± 0.5	7.4 ± 0.2	(28)	<0.01
HbA_1c (%)	5.0 ~ 6.7	11.1 ± 0.3	9.3 ± 0.2	(28)	<0.01
24 h urinary glucose (mmol)		223 ± 37.7	26.0 ± 14.4	(27)	<0.01
Ratio of urinary glucose to Cr[a)]		49.5 ± 8.4	5.5 ± 2.8	(27)	<0.01
24 h urinary C-peptide (nmol)	13.7 ~48.3	23.6 ± 3.2	19.0 ± 3.3	(26)	NS
Serum calcium (mmol/*l*)	2.10~ 2.42	2.22 ± 0.02	2.24 ± 0.01	(28)	NS
Serum phosphorus (mmol/*l*)	0.77~ 1.45	1.12 ± 0.03	1.21 ± 0.03	(28)	<0.01
Serum magnesium (mmol/*l*)	0.66~ 0.91	0.79 ± 0.02	0.82 ± 0.01	(28)	<0.01

Means ± SEM are shown. [a)]Cr : Creatinine.

Table 2. Urinary electrolytes, creatinine clearance and TmP/GFR on admission and at discharge in patients with poorly-controlled non-insulin-dependent diabetes mellitus

	Normal values	Diabetic patients		(n)	*P* values
		On admission	At discharge		
24 h urinary calcium (mmol)	<4.99	3.64±0.49	2.86± 0.28	(27)	<0.05
Ratio of urinary calcium to Cr[a)]		0.18±0.02	0.15± 0.01	(27)	<0.05
24 h urinary phosphorus (mmol)	9.69 ~19.4	21.4 ±1.50	16.4 ± 0.96	(27)	<0.01
Ratio of urinary phosphorus to Cr[a)]		0.83±0.04	0.67± 0.04	(27)	<0.01
24 h urinary magnesium (mmol)	2.06 ~8.23	3.47±0.26	3.23± 0.23	(27)	NS
Ratio of urinary magnesium to Cr[a)]		0.11±0.01	0.11± 0.01	(27)	NS
Creatinine clearance (ml/min)	75 ~102	84.6 ±7.19	78.1 ± 5.11	(27)	NS
TmP/GFR[b)] (mmol/*l*)	0.81 ~1.36	0.98±0.04	1.15± 0.05	(27)	<0.01

Means ± SEM are shown. [a)]Cr : Creatinine, [b)]TmP/GFR : The renal threshold for phosphorus excretion.

Table 3. Bone metabolic markers on admission and at discharge in patients with poorly-controlled non-insulin dependent diabetes mellitus

	Normal values	Diabetic patients		(n)	*P* values
		On admission	At discharge		
Serum intact-PTH (ng/*l*)	10 ~65	30.0 ±2.2	23.9 ±1.3	(28)	<0.01
Serum 1, 25$(OH)_2D_3$[a)] (ng/*l*)	20 ~76	24.8 ±2.0	25.2 ±2.2	(28)	NS
Serum alkaline-phosphatase (IU/*l*)	34 ~88	85.1 ±3.2	71.8 ±3.0	(28)	<0.01
Serum leucine amino peptidase (IU/*l*)	28 ~45	41.5 ±2.9	37.4 ±1.8	(16)	<0.05
Serum osteocalcin (μg/*l*)	2.5~13	4.14±0.35	4.92±0.40	(28)	<0.01

Means ± SEM are shown. [a)]1, 25$(OH)_2D_3$: 1, 25 dihydroxycholecalciferol.

levels revealed a negative correlation with serum intact PTH. Similarly, the change in serum calcium between the values on admission and those at discharge showed a negative correlation with the change in serum intact PTH (Fig. 2). On admission, serum phosphorus showed a positive

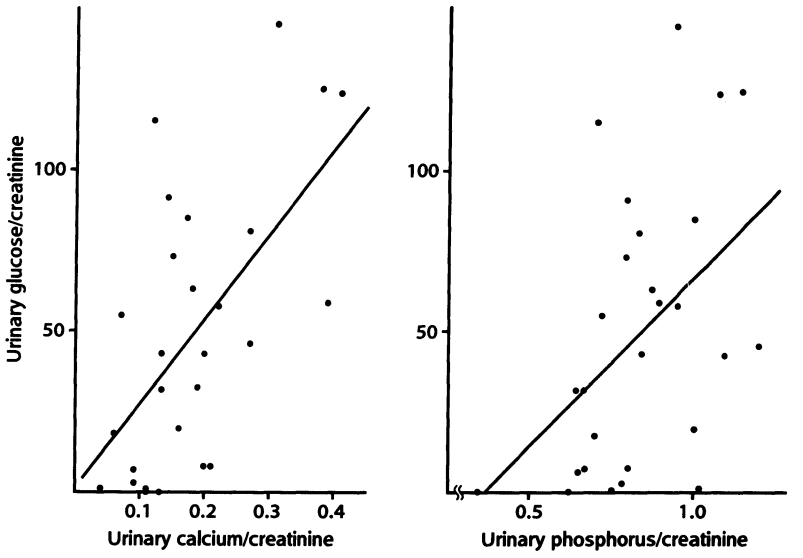

Fig. 1. Positive correlation between urinary calcium, phosphorus and glucose excretion on admission in patients with poorly-controlled non-insulin-dependent diabetes mellitus (left side: calcium *vs.* glucose, r=0.61, $P<0.01$; right side: phosphorus *vs.* glucose, r=0.46, $P<0.05$, n=27). Urinary excretion is expressed as the ratio to the urinary creatinine concentration.

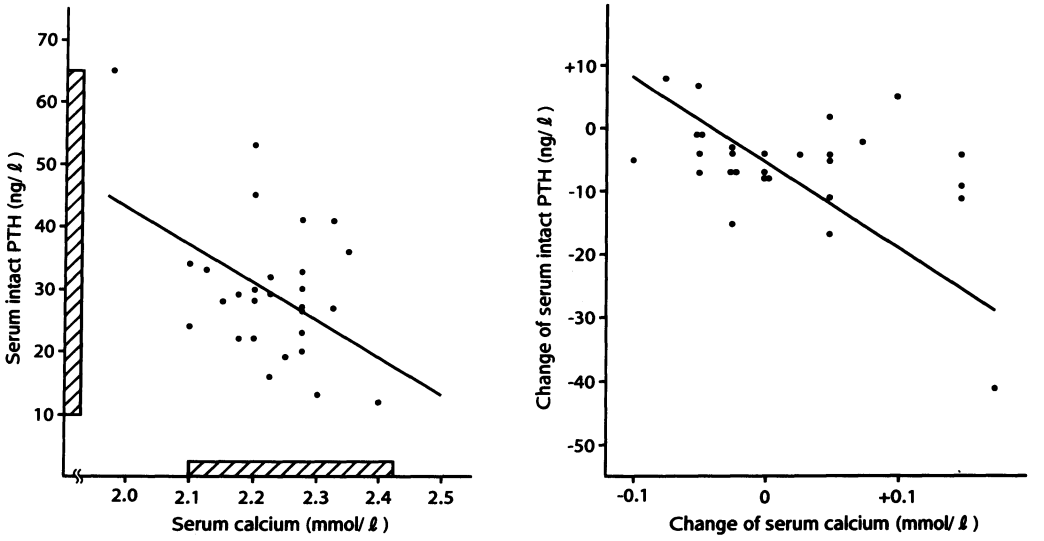

Fig. 2. Negative correlation between serum calcium and intact parathyroid hormone (intact PTH) in patients with poorly-controlled non-insulin-dependent diabetes mellitus (left side: correlation on admission, r=−0.46, $P<0.05$; right side: correlation between change in serum calcium and that in serum intact PTH, the change is calculated by subtracting the values on admission from those at discharge, r=−0.45, $P<0.05$, n=28). Hatched bars show the normal ranges in our laboratory.

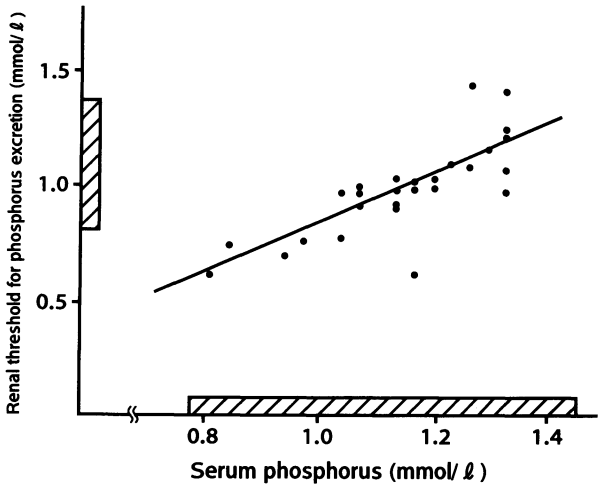

Fig. 3. Positive correlation between serum phosphorus and the renal threshold for phosphorus excretion on admission in patients with poorly-controlled non-insulin-dependent diabetes mellitus (r=0.86, $P<0.01$, n=27). Hatched bars show the normal ranges in our laboratory.

correlation with the renal threshold for phosphorus excretion (Fig. 3). Serum osteocalcin levels were neither correlated with serum alkaline-phosphatase (r=0.36, $P>0.05$) nor with the ratio of urinary calcium to creatinine (r=0.34, $P>0.05$).

Discussion

In the present study, we compared several parameters of bone and mineral metabolism in patients with poorly-controlled NIDDM to those after they achieved improved glycemic control. Our results revealed that urinary excretion of calcium and phosphorus increased relatively when the patients were poor in metabolic control. The enhanced urinary loss of calcium and phosphorus, related to urinary glucose excretion [11–13], became reduced in parallel with the improvement in glycemic control. The lowered serum phosphorus levels on admission were d ue to urinary loss of phosphorus, since they were positively correlated with the renal threshold for phosphorus excretion. Serum magnesium concentrations increased significantly, but remained within the normal range. Urinary magnesium excretion also remained within the normal range. It seems that there was no magnesium deficiency in our diabetic patients.

In general, plasma levels of PTH have been reported to be low or low-normal in patients with diabetes mellitus [4–6, 23]. Low-normal PTH concentrations after the achievement of glycemic control in the present study were consistent with previous reports, but we demonstrated that PTH concentrations were slightly but significantly higher in the hyperglycemic state than those in the improved glycemic state. Serum 1, $25(OH)_2D_3$ concentrations did not change in accordance with intact PTH. The low or low-normal levels of active vitamin D suggest that intestinal calcium absorption was diminished in the subjects studied. Also, the decreased active vitamin D concentrations may contribute in part to the low-normal serum osteocalcin, since 1, $25(OH)_2D_3$ is known to stimulate osteocalcin synthesis in the osteoblast-like cell line [24].

Serum alkaline-phosphatase and osteocalcin are markers of bone formation [25]. Osteocalcin is also a marker of bone turnover, when bone formation and resorption are coupled [25]. An increase in serum alkaline-phosphatase, derived from bone, is frequently found in diabetic patients [26, 27], but in the present study serum leucine amino peptidase also decreased after glycemic control. It is not concluded that the decrease in serum total alkaline-phosphatase was due to the change in its iso-enzyme from bone. Low-normal osteocalcin, observed in our diabetic patients, was consistent with previous reports [9, 10]. The function of osteoblasts is suppressed in diabetic animals and human patients [7–10]. After glycemic control, serum osteocalcin increased in spite of the decrease in serum intact PTH. As PTH stimulates bone turnover by directly acting on osteoblasts [28], the response of osteoblasts to PTH may be impaired in the hyperglycemic state. On the other hand, insulin has an anabolic effect on osteoblasts [29]. Urinary C-peptide excretion did not change during the hospitalization period. Insulin therapy was newly introduced in some of the patients. The improvement in insulin sensitivity and adequate supplement of insulin might have a beneficial effect on the function of osteoblasts.

The association between serum calcium and PTH concentrations seems to have been well maintained in our diabetic patients. Both serum PTH levels and urinary calcium excretion decreased with the improvement in glycemic control. Therefore, urinary calcium loss, associated with glycosuria, seems to stimulate PTH secretion in the hyperglycemic

state. If enhanced urinary calcium excretion were secondarily caused by exaggerated bone resorption associated with hyperglycemia, secretion of PTH would have been suppressed on admission. And when the decrease in urinary calcium excretion were mainly due to the restricted calcium intake during hospitalization, secretion of PTH would be stimulated at discharge. But our results were to the contrary. The excess urinary calcium on admission seems to be derived from bone, since there are no factors that increase intestinal calcium absorption. Mildly stimulated PTH secretion may contribute to calcium efflux from bone. However, it remains unclear whether the calcium is supplied by active bone resorption. The exact role of PTH and vitamin D in altering bone metabolism in hyperglycemia remains to be elucidated.

In conclusion, hyperglycemia exaggerates urinary loss of calcium and phosphorus in patients with NIDDM. Hyperphosphaturia decreases serum phosphorus. In response to urinary calcium loss, PTH secretion is mildly but significantly stimulated to maintain serum calcium concentrations. Excess urinary calcium seems to be derived from bone. The dysfunction of osteoblasts seems to be partly corrected by glycemic control. The present results indicate that glycemic control alters calcium and phosphorus handling, PTH level and bone turnover in patients with NIDDM.

References

1. McNair P, Madsbad S, Christensen MS, Christensen C, Faber OK, Binder C, Transbol I (1979) Bone mineral loss in insulin-treated diabetes mellitus: studies on pathogenesis. *Acta Endocrinol (Copenh)* 90: 463–472.
2. Giacca A, Fassina A, Caviezel F, Cattaneo AG, Caldirola G, Pozza G (1988) Bone mineral density in diabetes mellitus. *Bone* 9: 29–36.
3. Malone JI, Lowitt S, Duncan JA, Shah SC, Vargas A, Root AW (1986) Hypercalciuria, hyperphosphaturia, and growth retardation in children with diabetes mellitus. *Pediatrics* 78: 298–304.
4. Kawagishi T, Morii H, Nakatsuka K, Sasao K, Kawasaki K, Miki T, Nishizawa Y (1991) Parathyroid hormone secretion in diabetes mellitus. In: Morii H (ed) Calcium-Regulating Hormones. I. Role in Disease and Aging. Basel, Karger, Contrib Nephrol, vol 90: 217–222.
5. Schwarz P, Sorensen HA, Momsen G, Friis T, Transbol I, McNair P (1992) Hypocalcemia and parathyroid responsiveness in diabetes mellitus: a tri-sodium-citrate clamp study. *Acta Endocrinol (Copenh)* 126: 260–263.
6. Ishida H, Suzuki K, Someya Y, Nishimura M, Sugimoto C, Goto M, Taguchi Y, Kasahara H, Kadowaki S, Imura H, Seino Y (1993) Possible role of parathyroid hormone-related peptide on maintenance of calcium homeostasis in patients with non-insulin-dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 129: 519–524.
7. Ishida H, Seino Y, Taminato T, Usami M, Takeshita N, Seino Y, Tsutsumi C, Moriuchi S, Akiyama Y, Hara K, Imura H (1988) Circulating levels and bone contents of bone γ-carboxyglutamic acid-containing protein are decreased in streptozocin-induced diabetes. Possible marker for diabetic osteopenia. *Diabetes* 37: 702–706.
8. Verhaeghe J, Suiker AMH, Nyomba BL, Visser WJ, Einhorn TA, Dequeker J, Bouillon R (1989) Bone mineral homeostasis in spontaneously diabetic BB rats. II. Impaired bone turnover and decreased osteocalcin synthesis. *Endocrinology* 124: 573–582.
9. Rico H, Hernandez ER, Carbranes JA, Gomez-Castresana F (1989) Suggestion of a deficient osteoblastic function in diabetes mellitus: the possible cause of osteopenia in diabetics. *Calcif Tissue Int* 45: 71–73.
10. Pedrazzoni M, Ciotti G, Pioli G, Girasole G, Davoli L, Palummeri E, Passeri M (1989) Osteocalcin levels in diabetic subjects. *Calcif Tissue Int* 45: 331–336.
11. Raskin P, Stevenson MRM, Barilla DE, Pak CYC (1978) The hypercalciuria of diabetes mellitus: its amelioration with insulin. *Clin Endocrinol (Oxf)* 9: 329–335.
12. Gertner JM, Tamborlane WV, Horst RL, Sherwin RS, Felig P, Genel M (1980) Mineral metabolism in diabetes mellitus: changes accompanying treatment with a portable subcutaneous insulin infusion system. *J Clin Endocrinol Metab* 50: 862–866.
13. Raskin P, Pak CYC (1981) The effect of chronic insulin therapy on phosphate metabolism in diabetes mellitus. *Diabetoligia* 21: 50–53.
14. Matsuda A, Kuzuya T (1985) Urine C-peptide in relation to the degree of insulin dependence in diabetic patients. *Diabetes Res Clin Pract* 1: 65–72.
15. Cole RA, Soeldner JS, Dunn PJ, Bunn HF (1978) A rapid method for the determination of glycosylated hemoglobins using high pressure liquid chromatography. *Metabolism* 27: 289–301.
16. Tietz NW, Rinker AD, Shaw LM (1983) IFCC meth-

ods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase. *J Clin Chem Clin Biochem* 21: 731–748.
17. Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY, Keutmann HT, Wang CA, Potts JT, Segre GV (1987) Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. *Clin Chem* 33: 1364–1367.
18. Kawaguchi H, Matsumoto T, Kurokawa T, Orimo H, Mizunashi K, Takuwa Y, Niimi H, Shiraki M, Ohara T, Shishiba Y, Tsuchiya Y, Takahashi H, Takatsuki K, Seino Y, Morii H, Fujita T, Okamoto S, Ogata E (1990) Serum levels of BGP determination by two-site immunoradiometric assay (IRMA) using monoclonal antibodies. *Hormone to Rinsho* 38: 1291–1296 (In Japanese).
19. Reinhardt TA, Horst RL, Orf JW, Hollis BW (1984) A microassay for 1, 25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J Clin Endocrinol Metab* 58: 91–98.
20. Kaneko T, Oka H, Munemura M, Oda T, Yamashita K, Susuki S, Yanaihara N, Hashimoto T, Yanaihara C (1975) Radioimmunoassay of human proinsulin C-peptide using synthetic human connecting peptide. *Endocrinol Japon* 21: 141–145.
21. Payne RB, Little AJ, Williams RB, Milner JR (1973) Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J* 4: 643–646.
22. Walton RJ, Bijvoet OL (1975) Nomogram for derivation of renal threshold phosphate concentration. *Lancet* 2: 309–310.
23. Saggese G, Federico G, Bertelloni S, Baroncelli GI, Calisti L (1991) Hypomagnesemia and the parathyroid hormone-vitamin D endocrine system in children with insulin-dependent diabetes mellitus: effects of megnesium administration. *J Pediatr* 118: 220–225.
24. Price PA, Baukol SA (1980) 1, 25-dihydroxyvitamin D_3 increases synthesis of the vitamin K-dependent bone protein by osteosarcoma cells. *J Biol Chem* 255: 11660–11663.
25. Delmas PD (1993) Biochemical markers of bone turnover I: Theoretical considerations and clinical use in osteoporosis. *Am J Med* 95 (Suppl 5A): 5A–11A.
26. Stepan J, Havranek T, Formankova J, Skrha J, Skrha F, Pacovsky V (1980) Bone isoenzyme of serum alkaline phosphatase in diabetes mellitus. *Clin Chim Acta* 105: 75–81.
27. Maxwell DB, Fisher EA, Ross-Clunis III HA, Estep HL (1986) Serum alkaline phosphatase in diabetes mellitus. *J Am Coll Nutr* 5: 55–59.
28. Fitzpatrick LA, Coleman DT, Bilezikian JP (1992) The target tissue actions of parathyroid hormone. In: Coe FL, Favus MJ (eds) Disorders of Bone and Mineral Metabolism. Raven Press, New York, 123–148.
29. Kream BE, Smith MD, Canalis E, Raisz LG (1985) Characterization of the effect of insulin on collagen synthesis in fetal rat bone. *Endocrinology* 116: 296–302.