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Original Article

Poor Glycemic Control Impairs the Response of Biochemical Parameters of Bone Formation and Resorption to Exogenous 1,25-Dihydroxyvitamin D_3 in Patients with Type 2 Diabetes

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Abstract. Osteoblast deficit plays a principal role in the development of diabetic osteopenia. We have previously reported that high glucose conditions impair the function of osteoblast-like MG-63 cells. This study was performed to assess the sensitivity of osteoblasts to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) in patients with type 2 diabetes without insulin deficiency or overt diabetic complications. During stimulation with $1,25(OH)_2D_3$ at 2.0 μg/day for 6 consecutive days in 9 type 2 diabetic patients, serum levels of bone alkaline phosphatase (BALP), osteocalcin (OC) and the carboxyterminal propeptide of type 1 procollagen, and the urinary excretion of pyridinoline and deoxypyridinoline (DPYR), were monitored. As parameters of glycemic control, the mean level of fasting plasma glucose (mFPG) throughout the $1,25(OH)_2D_3$ stimulation test and the level of HbA_{1C} were used. $1,25(OH)_2D_3$ increased serum $1,25(OH)_2D$ significantly by day 2, which was followed by a significant reduction in the serum level of intact parathyroid hormone. The maximal increment of serum OC adjusted for that of $1,25(OH)_2D$ was negatively correlated with both mFPG and HbA_{1C} levels ($p < 0.05$). Furthermore, the magnitude of $1,25(OH)_2D_3$-induced bone resorption, as reflected by the maximal increase in urinary DPYR excretion, was negatively correlated with the mFPG level ($p < 0.05$). Basal BALP tended to be negatively correlated with HbA_{1C}, although not to a significant extent. In conclusion, our findings would indicate that poor glycemic control impairs the responses of osteoblasts and osteoclasts to $1,25(OH)_2D_3$ in normo-insulinemic type 2 diabetic patients.

Keywords: $1,25(OH)_2D$; Diabetes mellitus; Diabetic osteopenia; Osteocalcin

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Introduction

Patients with either type 1 or type 2 diabetes may exhibit various disorders of calcium (Ca) metabolism, such as impairment of Ca absorption [1,2] and loss of Ca from bone [3]. These abnormalities can eventually produce osteopenia [4–8], depending on the quality of diabetic control [9–11], although whether bone loss occurs in type 2 diabetic patients remains to be determined [12]. Impaired bone formation due to osteoblast deficit has been proposed to be a principal factor in the development of diabetic osteopenia [13–15]. Consistent with this hypothesis, serum osteocalcin (OC) levels were found to be significantly decreased in type 2 diabetic patients [16,17]. Since serum levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), a main stimulator of the synthesis and secretion of OC, were not significantly lower in type 2 diabetic patients than in control subjects [9,10], in contrast to type 1 diabetic patients, a primary osteoblast deficit could be present in type 2 diabetic patients. An in vitro study of ours [18] demonstrated that high glucose, but not high mannitol, significantly impaired the $1,25(OH)_2D$-induced secretion of OC from human osteoblast-like MG-63 cells in a concentra-

tion-dependent manner, suggesting that a high glucose level is an independent factor contributing to a significant decrease in the serum OC level in type 2 diabetic patients. It was previously reported that the $1,25(OH)_2D$ stimulation test may provide a sensitive means of assessing osteoblast function in human subjects in vivo [19].

These observations prompted us to examine whether the increase in serum OC level during 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) administration might also be impaired in vivo in type 2 diabetic patients without diabetic complications, and whether the magnitude of any impairment might depend on glycemic control.

Subjects and Methods

Subjects and Design

Nine patients with type 2 diabetes were enrolled in the study. Their diabetes was diagnosed according to the criteria of the World Health Organization [20]. They were on dietary management alone or taking oral hypoglycemic agents for the control of their condition. To avoid effects of diabetic complications, age, and menstrual cycle, only males aged under 60 years old without overt diabetic complications were recruited. To avoid the effects of insulin deficiency, individuals with fasting morning immunoreactive insulin (IRI) or daily urinary excretion of C-peptide reactivity (CPR) below lower normal limits were also excluded. The other exclusion criteria were renal disease possibly causing secondary hyperparathyroidism, endocrine disorder, liver disease, malnutrition (serum albumin $\leqslant$ 3.0 g/dl), any other disease and the taking of any medication that might affect bone or mineral metabolism. The $1,25(OH)_2D_3$ stimulation test was performed as described [19], with a slight modification. Patients received 2.0 μg of $1,25(OH)_2D_3$ (Rocaltrol; Roche, Nutley, NJ) orally, once daily at 2200 hours for 6 consecutive days, to avoid as far as possible the effect of $1,25(OH)_2D_3$ on blood Ca levels. Blood samples were drawn at 0800 hours after an overnight fast, and daily 24h urine samples were collected. Biochemical parameters of bone and mineral metabolism were measured on days 1, 2, 4 and 7. Stimulation was initiated at 2200 hours on day 1. Values at 0800 hours on day 1 were used as the prestimulation values. The basal level of serum OC was determined in sex- and age-matched control subjects (M/F, 10/0; age, 53.2 ± 4.25 years; n = 10).

Biochemical Parameters

Ca, phosphate (P), glucose and creatinine (Cr) levels were measured in serum and urine with an autoanalyzer. As parameters of glycemic control, HbA_{1C} was measured on day 1 and the mean level of fasting plasma glucose (mFPG) was determined from measurements on days 1, 2, 4 and 7. FPG was determined by the glucose oxidase method, and HbA_{1C} by high-pressure liquid chromatography [21]. The former and latter parameters were assumed to indicate glycemic control of longer and shorter duration, respectively. Serum parathyroid hormone (PTH)(1–84) was measured by an immunoradiometric assay (Allegro Intact PTH, Nichols Institute, San Juan, Capistrano, CA). This assay measures only active intact PTH and not degradation products resulting from its cleavage [22]. Serum $1,25(OH)_2D$ was measured with a kit obtained from the Nichols Institute. $1,25(OH)_2D$ was extracted with acetonitrile, purified with a Sep Pak C18-OH column, and finally measured by a competitive protein-binding assay [23]. Serum carboxyterminal propeptide of human type 1 procollagen (P1CP) was measured using a radioimmunoassay kit [24]. Urinary total pyridinoline (PYR) (both pyridinoline and deoxypyridinoline) and deoxypyridinoline (DPYR) levels were measured in 24-h urine samples using Pyrilinks and Pyrilinks-D assay kits (Metra Biosystems, CA) [25]. The urinary excretion of Ca, PYR and DPYR was expressed in each case as a ratio to urinary Cr excretion. Serum OC, also known as bone Gla-protein, was measured with a two-site immunoradiometric assay kit from Mitsubishi Kagaku Bio-Clinical Laboratories (Tokyo, Japan) [26]. Serum bone alkaline phosphatase (BALP) was measured by enzyme immunoassay [27,28] and polyacrylamide gel electrophoresis [29].

Bone Densitometry

Bone mineral density was measured in the lumbar spine (L2–4) and nondominant radius at the 33% (midshaft) site by dual-energy X-ray absorptiometry (DXA; Hologic QDR 1000W, Waltham, MA). An age-matched comparison (Z-score) was calculated relative to a Hologic database of healthy Japanese men.

Statistical Analysis

Values are means ± SEM unless otherwise indicated. Statistical analysis was performed by ANOVA followed by Scheffé's test. Findings of $p < 0.05$ were considered significant.

Results

Biochemical Profiles of Type 2 Diabetic Patients

Biochemical profiles of the 9 type 2 diabetic patients enrolled in the present study are shown in Table 1. The mean FPG level was 174.9 ± 16.0 mg/dl, with a daily urinary glucose excretion of 1.54 ± 0.80 g. Serum HbA_{1C} ranged from 6.6% to 13.5%. Fasting morning IRI was 5.00 ± 0.71 μU/ml and the daily urinary CPR was 118.7 ± 19.0 μg/day. The daily urinary excretion of albumin was 13.0 ± 9.7 mg, ranging from 0 to 90 mg. The basal

Table 1. Biochemical profile of 9 type 2 diabetic patients

	Diabetic patients		Normal range
	mean ± SEM	Range	
n	9		
Age (years)	56.0 ± 1.43	51–62	
mFPG (mg/dl)	174.9 ± 16.0	108.0–271.8	70–105[a]
HbA_{1C} (%)	9.81 ± 0.79	6.6–13.5	4.0–5.5[a]
24-h urinary glucose (g/day)	1.54 ± 0.80	0–7.34	0[a]
Fasting IRI (μU/ml)	5.00 ± 0.72	1.9–7.7	0–12.5[b]
24-h urinary CPR (μg/day)	118.7 ± 19.0	59.8–252.0	43.0–146.0[b]
Albumin (g/dl)	4.03 ± 0.10	3.5–4.4	3.5–5.0[a]
Cr (mg/dl)	0.80 ± 0.06	0.6–1.1	0.6–1.5[a]
24-h urinary albumin (mg/day)	13.0 ± 9.7	0–90	2.6–16.6[b]
Ca (mg/dl)	9.09 ± 0.06	8.6–9.6	8.5–10.5[a]
P (mg/dl)	3.84 ± 0.24	2.8–4.9	2.5–4.5[a]
BALP (EIA) (U/l)	18.0 ± 2.95	12.4–40.1	10.0–27.0[b]
BALP (PAGE) (IU/l)	68.3 ± 10.6	40.0–141.0	39.2–66.8[b]
intact PTH (pg/ml)	20.9 ± 1.69	14.0–28.0	10.0–65.0[b]
$1,25(OH)_2D$ (pg/ml)	31.3 ± 5.75	8.7–63.5	27.5–68.7[b]
OC (ng/ml)	2.13 ± 0.34	1.0–4.3	5.2–9.6[b]
P1CP (ng/ml)	92.8 ± 16.9	47.4–215.0	30–182[b]
Urinary PYR (pmol/μmol Cr)	27.2 ± 9.24	16.8–47.6	17.7–41.9[b]
Urinary DPYR (pmol/μmol Cr)	4.14 ± 0.67	2.3–8.7	2.2–6.1[b]
L2–4 BMD (Z-score)	−0.164 ± 0.308	−1.600–1.210	
Radius 33% BMD (Z-score)	−0.118 ± 0.353	−1.830–1.250	

[a] Normal ranges obtained by our hospital laboratory as those of healthy Japanese adults
[b] Normal ranges supplied by the manufacturer as those of healthy Japanese adults.

level of serum OC in the type 2 diabetic patients was 2.13 ± 0.34 ng/ml, significantly less than that in the sex- and age-matched controls (5.33 ± 1.80 ng/ml, $n = 10$, $p < 0.05$). Type 2 diabetic patients exhibited normal bone mineral density in both the lumbar spine and radius 33%.

Changes in Ca and P Metabolism During the 1,25(OH)$_2$D$_3$ Stimulation Test

Since $1,25(OH)_2D_3$ was administered orally at 2200 hours, without measurement of serum Ca and P levels until 0800 hours the next morning, serum levels of Ca and P appeared unchanged during the $1,25(OH)_2D_3$ stimulation test (data not shown). In contrast, the daily urinary excretion of Ca, normalized for Cr excretion, increased significantly from 0.044 ± 0.013 to 0.111 ± 0.019 (mg/mg).

Time Courses of Serum Levels of 1,25(OH)$_2$D, OC and intact PTH During the 1,25(OH)$_2$D$_3$ Stimulation Test

Serum concentrations of $1,25(OH)_2D$, OC and PTH during $1,25(OH)_2D_3$ stimulation are shown in Fig. 1. Stimulation with $1,25(OH)_2D_3$ (2.0 μg/day) induced a significant 2-fold increase in serum $1,25(OH)_2D$ from 31.3 ± 5.75 to 60.7 ± 8.02 pg/ml on day 2, and levels remained elevated throughout the administration of $1,25(OH)_2D_3$. The increase in serum $1,25(OH)_2D$ appeared to be followed by a reduction in serum PTH, since a significant reduction from 20.9 ± 1.69 to 15.4 ± 1.94 ng/ml was detected initially on day 4. Serum OC levels tended to increase progressively until day 4 during $1,25(OH)_2D_3$ stimulation.

Relationship Between Basal Levels of Serum BALP and OC and Parameters of Glycemic Control

Serum BALP tended to be negatively correlated with the serum HbA_{1C} level ($r = -0.630$, $p = 0.069$ by EIA; $r = -0.613$, $p = 0.080$ by PAGE), but not with the serum mFPG ($r = -0.505$, $p = 0.166$ by EIA; r= −0.443, $p = 0.233$ by PAGE). Serum BALP did not change appreciably during the $1,25(OH)_2D_3$ stimulation test (data not shown). Serum OC was correlated with neither mFPG ($r = -0.497$, $p = 0.174$) nor HbA_{1C} ($r = -0.545$, $p = 0.129$). Even when serum OC was normalized against the serum $1,25(OH)_2D$ level, the serum OC/$1,25(OH)_2D$ ratio was not significantly negatively correlated with either mFPG or HbA_{1C} (data not shown).

Influence of Glycemic Control on the 1,25(OH)$_2$D$_3$-Induced Increment in Serum OC Level

The maximal increment in serum OC during $1,25(OH)_2D_3$ stimulation tended to be negatively correlated with both serum HbA_{1C} ($r = -0.662$, $p = 0.050$) and mFPG ($r = -0.612$, $p = 0.080$) (Figs 2, 3). Taking into consideration (i) the large individual differences in the increment of serum $1,25(OH)_2D$ observed during

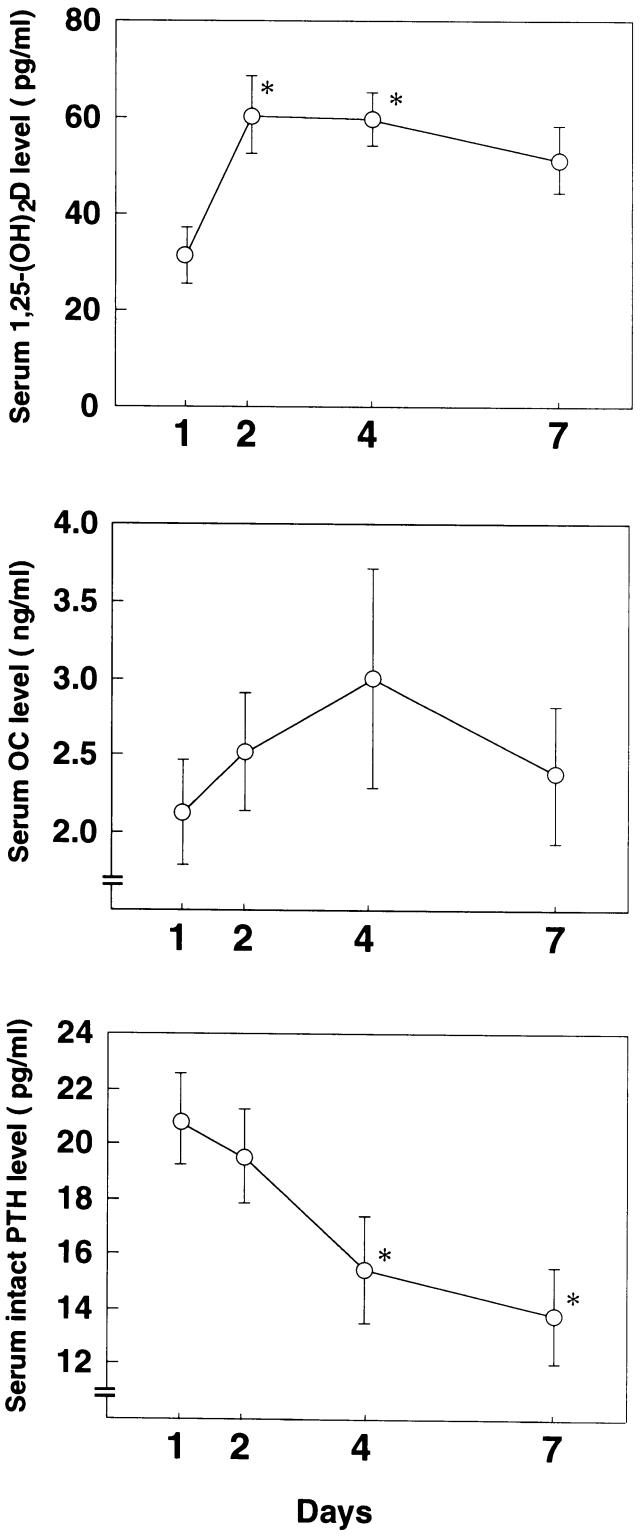

Fig. 1. Changes in serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$; *upper panel*), osteocalcin (OC, *middle panel*) and parathyroid hormone (PTH, *lower panel*) levels during $1,25(OH)_2D_3$ stimulation test in type 2 diabetic patients. *Circles* and *error bars* denote means ± SEMs of 9 type 2 diabetic patients. *$p < 0.05$ versus prestimulation value.

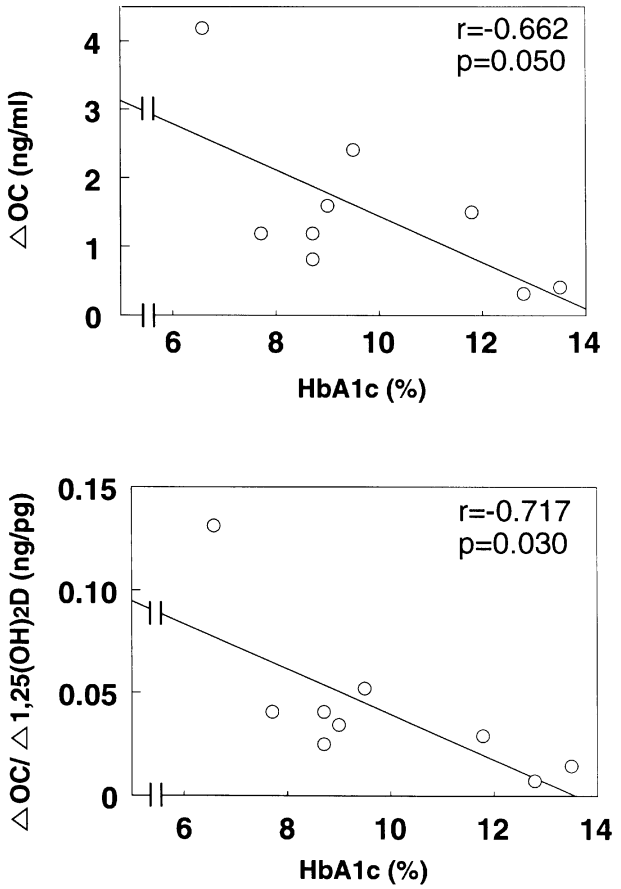

Fig. 2. Relationship between the incremental increase in serum OC level (ΔOC) and HbA_{1C} in 9 type 2 diabetic patients. Probably due to wide individual variation in the incremental increase in serum $1,25(OH)_2D$ ($\Delta 1,25(OH)_2D$), ΔOC failed to correlate significantly with HbA_{1C} ($r = -0.662$, $p = 0.050$). When ΔOC was adjusted for $\Delta 1,25(OH)_2D$, $\Delta OC/\Delta 1,25(OH)_2D$ significantly correlated with serum HbA_{1C} ($r = -0.717$, $p = 0.030$).

$1,25(OH)_2D_3$ stimulation and (ii) the strong dependence of the serum OC level on $1,25(OH)_2D$, the incremental response in serum OC was adjusted for the increment in serum $1,25(OH)_2D$, as previously described [19]. The increment in serum OC adjusted for that of serum $1,25(OH)_2D$ was significantly negatively correlated with both serum HbA_{1C} ($r = -0.717$, $p = 0.030$) and mFPG ($r = -0.682$, $p = 0.043$).

Influence of Glycemic Control on the $1,25(OH)_2D_3$-Induced Increment in Urinary DPYR Excretion

As shown in Fig. 4, the basal excretion of DPYR in urine was not correlated with the mFPG level. Neither PYR nor DPYR excretion increased significantly during $1,25(OH)_2D_3$ stimulation (data not shown). However, both the maximal increment in DPYR excretion and the rate of maximal increment during $1,25(OH)_2D_3$ stimulation were significantly correlated with the mFPG level.

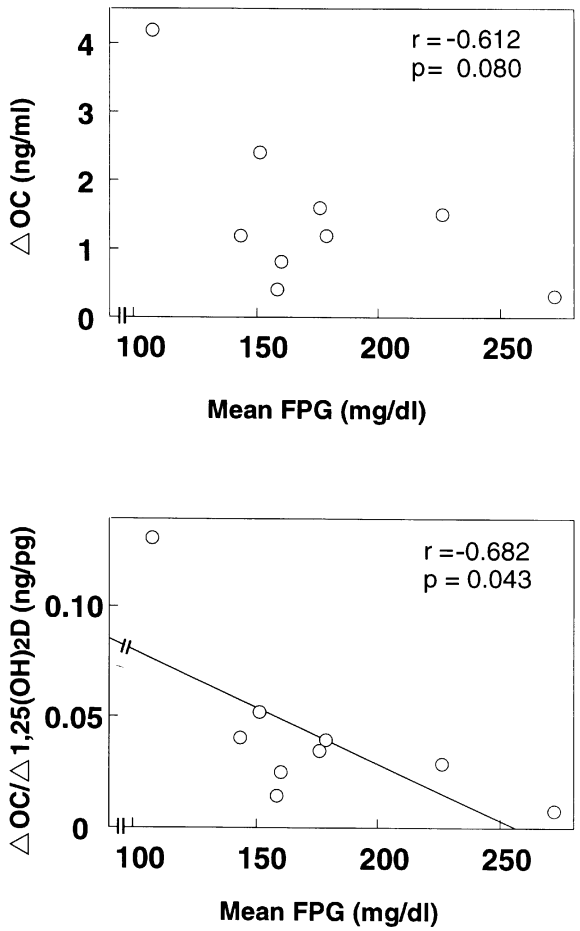

Fig. 3. Relationship between incremental increase in serum OC level (ΔOC) and mean fasting plasma glucose (mFPG) for 9 type 2 diabetic patients. Probably due to wide individual variation in the incremental increase in serum $1,25(OH)_2D$ ($\Delta 1,25(OH)_2D$), ΔOC failed to correlate significantly with mFPG ($r = -0.612$, $p = 0.080$). When ΔOC was adjusted for $\Delta 1,25(OH)_2D$, $\Delta OC/\Delta 1,25(OH)_2D$ significantly correlated with mFPG ($r = -0.682$, $p = 0.043$).

Discussion

Previous studies have demonstrated that the number of osteoblasts is significantly decreased in type 2 diabetic patients with overt diabetic complications [13,30], and deficiencies of insulin and insulin-like growth factor-I have been established as major factors explaining the occurrence of osteoblast dysfunction in the diabetic state [31,32]. Since the present study was designed to examine the early development of osteoblast dysfunction in patients with type 2 diabetes, only patients with neither overt diabetic complications nor overt insulin deficiency were enrolled, in order to avoid the effects of these conditions on Ca metabolism. Furthermore, since the patients in our study exhibited a normal bone mineral density, probably because of the short duration of their diabetes, secondary effects of osteopenia on Ca metabolism can also be ruled out.

We demonstrated that, in these patients with neither overt complications nor insulin deficiency, osteoblast

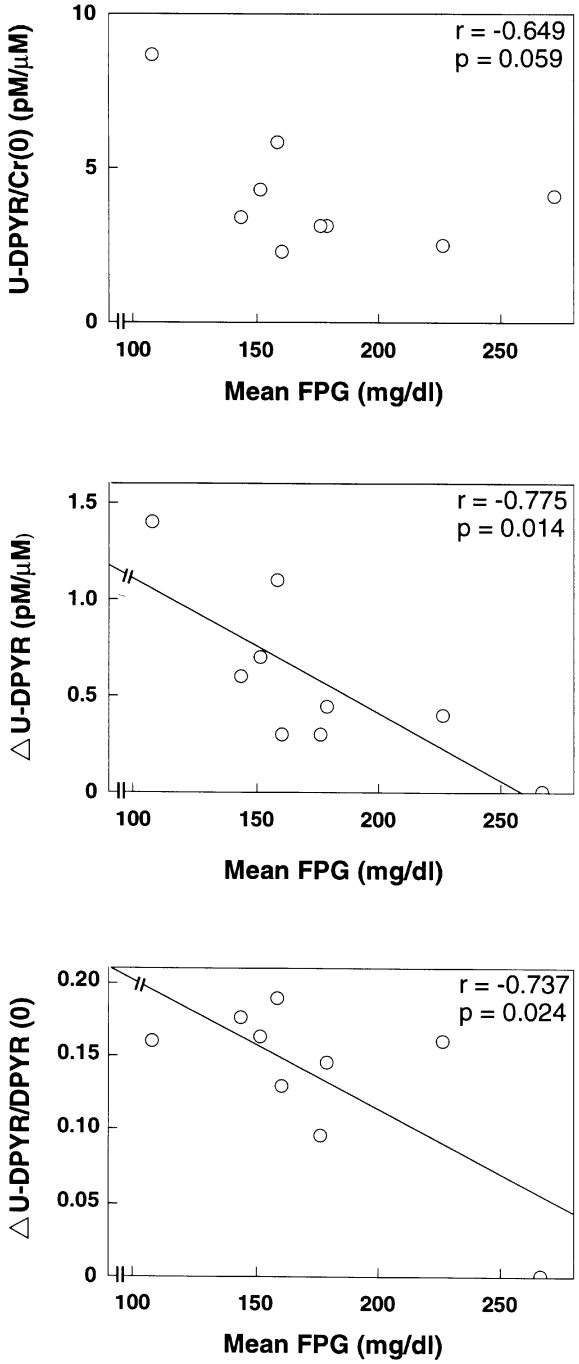

Fig. 4. Relationship between basal urinary excretion of deoxypyridinoline (DPYR, *upper panel*), maximal increment of DPYR excretion during $1,25(OH)_2D_3$ stimulation (*middle panel*) or maximal rate of increment (*lower panel*) and mFPG. mFPG was significantly negatively correlated with $1,25(OH)_2D_3$-induced increment in urinary DPYR excretion ($r = -0.775$, $p = 0.014$) and the rate of increment ($r = -0.737$, $p = 0.024$), but not with basal urinary excretion of DPYR ($r = -0.649$, $p = 0.059$).

function was significantly impaired secondary to glycemic control. First, serum OC levels were indeed lower in the type 2 diabetic patients than in age- and sex-matched controls. Second, although not significant, probably because of the small number of patients,

serum BALP tended to be negatively correlated with serum HbA_{1C}. Lastly, the increments in urinary DPYR excretion and serum OC during $1,25(OH)_2D_3$ stimulation were dependent on glycemic control. Furthermore, each of three patients who underwent $1,25(OH)_2D_3$ stimulation tests both before and after the introduction of dietary therapy, showed greater increments in serum OC as their serum HbA_{1C} decreased (data not shown).

These findings are in good agreement with our in vitro study, which showed that sustained 7-day exposure to high concentrations of glucose significantly impaired $1,25(OH)_2D_3$-induced OC secretion by human osteoblast-like MG-63 cells [18]. We also observed that high-glucose-treated MG-63 cells had a significantly impaired response to PTH as determined by increases in intracellular cAMP and Ca^{2+} levels [33]. Furthermore, an aldose reductase inhibitor, epalrestat, restored the positive correlation between the serum OC level (bone formation marker) and urinary DPYR excretion (bone resorption marker) and the number of osteoblasts in the tibia of galactose-fed rats, suggesting the importance of intracellular galactitol accumulation in the impairment of osteoblast function (data not shown). These findings together show that sustained high glucose by itself may contribute to the development of impaired osteoblast function via the intracellular accumulation of sorbitol. Supporting this hypothesis in man is a report that long-term change in the Z-score for bone mineral density of the radius in type 2 diabetic patients is significantly related to the severity of diabetic retinopathy (-0.23 ± 1.26 for moderate or severe vs 0.92 ± 1.29 for mild or no retinopathy, $p = 0.01$) [34], in which the intracellular accumulation of sorbitol is known to play a major role.

A previous study demonstrated that serum OC was indeed lower in type 2 diabetic patients than in an age- and sex-matched group, and that the relative decrease in serum OC became smaller with the normalization of HbA_{1C} [35]. Interestingly, $1,25(OH)_2D_3$ treatment suppressed PTH secretion, showing that negative feedback on PTH secretion by $1,25(OH)_2D_3$ is fully operative in type 2 diabetic patients, although it failed significantly to increase serum OC in these patients. These results confirm the previous findings of tissue-specific loss of $1,25(OH)_2D_3$ responsiveness of bone in spontaneously diabetic BB rats. [36]. In type 2 diabetic patients, BALP appears to be a significant predictor of the log-transformed change in Z-score for bone mineral density in the radius [34]. In the present study, serum BALP levels tended to be correlated negatively with HbA_{1C} but not with mFPG, suggesting that the serum BALP level may be suppressed by a high glucose exposure of longer, but not shorter duration. Taken collectively, these findings suggest that sustained exposure to high glucose suppresses osteoblast secretion of BALP and simultaneously attenuates age-related bone loss by reducing bone turnover. Furthermore, osteoblast transmission of $1,25(OH)_2D_3$ stimulation to activate osteoclastic bone resorption might be impaired by brief high glucose exposure, as suggested by the finding that the maximal increment of DPYR excretion during $1,25(OH)_2D_3$ stimulation and the rate of its maximal increase were significantly correlated with the mFPG level.

In summary, our findings suggest that the $1,25(OH)_2D_3$ stimulation test is useful for estimating osteoblast function in humans, and that poor glycemic control can impair osteoblast function either directly or indirectly as an independent factor in type 2 diabetic patients with neither overt diabetic complications nor insulin deficiency.

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