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Increasing Serum Osteocalcin After Glycemic Control in Diabetic Men

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Abstract. The pathogenesis of diabetic osteopenia is unclear. The markers of bone metabolism may show some changes in diabetic patients. In this study, we investigated the effect of glycemic control on serum osteocalcin level and urinary hydroxyproline excretion and the relations of these markers to duration of diabetes, C-peptide status, and body mass index. Twenty-seven men with poorly controlled diabetes mellitus (DM) (HbA1 > 9%, fasting plasma glucose > 7.8 mmol/liter) between ages 25 and 60 years (means ± SD 46.6 ± 10.4) were included in the study. Duration of diabetes was 5.8 ± 4.7 years, body mass index (BMI) was 25.9 ± 3.5 kg/m^2, and fasting C-peptide was 2.33 (1.05–3.21) μg/liter. None of the patients had a disease or were treated with drugs that would interfer with calcium or phosphate metabolism and/or bone structure. They were free from chronic diabetic complications. Of these patients, 11 were lost to follow-up before metabolic control was achieved. The remaining 16 patients obtained good glycemic control (HbA1 < 8.3%, fasting plasma glucose < 7.8 mmol/liter) and completed the study. Serum osteocalcin level and urinary hydroxyproline excretion were determined before and after glycemic control. Urinary hydroxyproline excretion was not significantly changed by glycemic control [17.8 (7.1–23.2) versus 18.1 (10.9–28.1) mg/m^2 day, $P > 0.05$]. However, serum osteocalcin level was significantly elevated (5.04 ± 1.43 versus 4.17 ± 1.83 μg/liter, $P = 0.04$). We found no correlation among fasting plasma glucose, HbA1, and fasting serum C-peptide levels with urinary hydroxyproline excretion. There was also no correlation between serum osteocalcin and fasting plasma glucose or serum C-peptide, but HbA1 was negatively correlated with serum osteocalcin ($P = 0.01$). No correlation was found between DM duration and BMI in the patients with serum osteocalcin level and urinary hydroxyproline excretion. To eliminate the possible effect of exogenous insulin on bone metabolism, the correlation analysis between the markers and C-peptide was further repeated in oral agents-treated patients. Serum C-peptide was not correlated to serum osteocalcin or urinary hydroxyproline in this subgroup of patients. Knowing that serum osteocalcin is a marker of bone formation, we concluded that osteoblast function may improve by glycemic control in diabetic patients; this may be due to correction of metabolic abnormalities associated with insulinopenia.

Key words: Osteocalcin — Urinary hydroxyproline — Diabetes mellitus — C peptide — HbA1.

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Diabetes mellitus (DM) is known to affect the metabolism and structure of bone. Osyteopenia is an established complication of insulin-dependent diabetes mellitus (IDDM) [1–4]. It is also observed in noninsulin-dependent diabetes mellitus (NIDDM) patients [1, 5], however, contradictory results have been observed in some studies [6, 7]. Several mechanisms have been proposed to explain the pathogenesis of this complication, but it is still unclear [8–14].

There are several markers of bone metabolism. Among them, serum osteocalcin is known as a marker of bone formation (synthesized by osteoblasts), and urinary hydroxyproline excretion as a marker of bone resorption [15]. Decreased levels of serum osteocalcin in experimental diabetes have been reported in some previous studies [16–18]. Both decreased or increased urinary hydroxyproline excretion have also been reported in animal models [19, 20]. As for human diabetes, reduced serum osteocalcin levels have been found in IDDM patients [21]. Moreover, it has been reported that improving glycemic control increased serum osteocalcin levels in diabetic children [22]. To our knowledge, there is no previous longitudinal study about the effect of glycemic control on these two markers of bone metabolism in adult diabetic patients. For this reason, we planned to investigate the levels of these markers in diabetic patients and to determine their changes after glycemic control. We also investigated their relations with DM duration, C-peptide status, and weight and body mass index (BMI). To exclude possible confounding effects of age and sex, only male patients between the age of 25 and 60 were included in the study.

Patients and Methods

Between June 1992 and July 1993, 27 men with poorly controlled DM (HbA1 > 9%) who attended our outpatient clinic were selected according to the inclusion criteria (see below) and participated in the study on a voluntary basis. The following persons were excluded from the study: patients suffering from diseases or treated with drugs able to interfere with calcium or phosphate metabolism, patients with chronic diabetic complications, patients younger than 25 or older than 60 years of age. All patients gave informed consent. Of these selected 27 patients, 11 were lost to follow-up before metabolic control was achieved. The remaining 16 patients obtained good glycemic control and completed the study.

Clinical diagnosis was NIDDM in 20 and IDDM in 7 patients. We could not determine islet cell antibodies in our patients, and age of onset of the disease is relatively late in our IDDM patients, ranging from 19 to 38 years (median 30). So, the clinical diagnosis of IDDM was based primarily on insulin dependency, abrupt onset of the disease, history of ketoacidosis, and leanness of the patients. However, these criteria were insufficient to make a precise discrimination between the types of diabetes. We also measured fasting serum C-peptide values, but this was higher than 1 μg/liter in three patients with a clinical diagnosis of IDDM and lower than 0.8 μg/liter in two

Table 1. The clinical characteristics and baseline biochemical data on the patients

n	27	
Age (years)	46.6 ± 10.4	
DM duration (years)	5.8 ± 4.7	
Therapy (oral agents/insulin)	16/11	
Weight (kg)	75.3 ± 11.6	
BMI (kg/m^2)	25.9 ± 3.5	
Fasting plasma glucose (mmol/liter)	12.8 ± 4.2	Normal range: 3.3–5.1
HbA1 (%)	10.1 (9.5–10.6)	Normal range: 5.8–7.8
Fasting serum C-peptide (μg/liter)	2.33 (1.05–3.21)	Normal range: 0.80–4.00
Serum calcium (mmol/liter)	2.4 ± 0.1	Normal range: 2.2–2.6
Serum phosphorus (mmol/liter)	1.0 ± 0.1	Normal range: 0.8–1.5
Serum alkaline phosphatase (U/liter)	79.8 ± 26.9	Normal range: 41–133
Serum creatinine (μmol/liter)	74.2 ± 19.4	Normal range: 45–96
Serum osteocalcin (μg/liter)	3.99 ± 1.46	Normal range: 2.00–12.00
Urinary hydroxyproline (mg/m^2/day)	20.8 (10.8–29.9)	Normal range: 6.0–22.0

Values are expressed as either means ± SD or as median and interquartile range (in parenthesis) if not normally distributed

patients with a clinical diagnosis of NIDDM, making a discrimination more difficult. On the other hand, in the NIDDM patients, seven had a C-peptide value less than 2 μg/liter, suggesting relative hypoinsulinism and four had a C-peptide value higher than 3 μg/liter, suggesting relative hyperinsulinism. One of our objectives was to investigate the relation between bone markers and C-peptide which is a marker of endogenous insulin secretion. To accomplish this, we decided to evaluate the group as a whole and we used the C-peptide as a common denominator instead of separating the group in two, as IDDM and NIDDM, and relying on imprecise clinical diagnosis.

The complications of DM were screened in the following manner: (1) Nephropathy: urinary albumin excretion was determined by a double-antibody radioimmunoassay (Diagnostic Product Corporation, LA; interassay coefficient of variations: 2.3–3.5%) in a 24-hour collection of urine. Serum creatinine was determined by an autoanalyser. All included patients were normoalbuminuric (<20 mg/24 hours) and their serum creatinine levels were below 96 μmol/liter. (2) Retinopathy: fundoscopic examination; patients with proliferative or preproliferative retinopathy were excluded. (3) Neuropathy: autonomic and sensorial symptoms, meticulous neurological examination, and cardiovascular autonomic function tests as described by Ewing [23]. Only patients with no symptoms, normal neurological examination, and normal cardiovascular tests were included in the study.

Blood was drawn after an overnight fast for measurement of levels of glucose, calcium, phosphorus, alkaline phosphatase, C-peptide, and HbA1. Plasma glucose was measured by the glucose oxidation technique using an autoanalyzer. Serum calcium, phosphorus, and alkaline phosphatase were also determined by automated techniques (normal values: Ca; 2.2–2.6 mmol/liter; *P;* 0.8–1.5 mmol/liter; ALP, 41–133 U/liter). Serum C-peptide level was measured by radioimmunoassay (DSL, Texas) (intraassay CVs: 3.3–7.9%, interassay CVs: 2.4–5.3%). HbA1 was determined by the cation-exchange chromatography method with commercially available sets (Eagle Diagnostics, Texas) (within run % CVs, 1.7–2.7, run to run % CVs, 4.1–4.6; normal range in our laboratory is 5.8–7.8%). All patients included in the study had a HbA1 value >9% and fasting plasma glucose >7.8 mmol/liter. In these patients, serum osteocalcin level and 24-hour urinary hydroxyproline excretion were studied. All blood samples for serum osteocalcin were taken between 8 and 9 a.m. after an overnight fast. Serum osteocalcin level was determined by radioimmunoassay (DSL, Texas) (intraassay CVs, 5.4–8.1%; interassay CVs, 5.5–14.7%, expected values; 2–12 μg/liter). Patients received a collagen-free diet about 24 hours before and also during the collection of the urine, and urinary hydroxyproline excretion was determined by the resin-exchange method following the manufacturer's instructions (Hypronosticon, Organon Teknika) (normal values, 6–22 mg hydroxyproline/24 hours/m^2 for 22–65 years). Patients were treated by oral hypoglycemics or insulin according to the clinical and/or biochemical status, and after glycemic control was obtained (HbA1 < 8.3%), serum osteocalcin and urinary hydroxyproline measurements were repeated.

Statistical analysis included Wilcoxon signed-rank test and Spearman correlation coefficients.

Results

The clinical characteristics and baseline biochemical data on the patients are listed in Table 1. As can be seen, mean serum calcium, phosphorus, alkaline phosphatase, and creatinine levels and median value of urinary hydroxyproline excretion were in the normal range. Mean serum osteocalcin level was near the lower limit of normal.

Fasting plasma glucose (FPG), HbA1, serum osteocalcin levels, and 24-hour urinary hydroxyproline excretion before and after glycemic control in 16 patients are shown in Table 2. Urinary hydroxyproline excretion was not significantly changed by glycemic control [median and interquartile ranges: 17.8 (7.1–23.2) versus 18.1 (10.9–28.1), $P > 0.05$]. However, serum osteocalcin level was significantly elevated (5.04 ± 1.43 versus 4.17 ± 1.83 μg/liter, $P = 0.04$).

The correlation analysis between various parameters as performed using baseline values. We found no correlation between FPG, HbA1, and fasting serum C-peptide levels with urinary hydroxyproline excretion. There was also no correlation between FPG and serum C-peptide with serum osteocalcin, but HbA1 was negatively correlated with serum osteocalcin ($r = -0.51$, $P = 0.009$), (Fig. 1). No correlation was found among age, DM duration, weight, and BMI of the patients with serum osteocalcin level and urinary hydroxyproline excretion. There was also no correlation between serum osteocalcin and urinary hydroxyproline. Our objective was to investigate the relation between endogenous insulin and bone markers using C-peptide as an indicator of insulin secretion. In the patients treated with exogenous insulin, C-peptide would not be a good indicator for whole-body insulin status. So, the correlation analysis between the bone markers and C-peptide was repeated further in oral agents-treated patients. No correlation was found between these parameters in this subgroup of patients.

Discussion

IDDM in humans is frequently associated with osteoporosis [1–4, 14]. Studies in animal models have also demonstrated decreased bone growth and strength [18, 19, 24, 25]. The relationship between NIDDM and bone mass has been more

Table 2. Fasting plasma glucose, HbA1, serum osteocalcin levels, and 24 hourly hydroxyproline excretion of 16 patients before and after glycemic control

Variable	Before control	After control	*P* value
Fasting plasma glucose (mmol/liter)	11.8 ± 3.9	7.0 ± 0.8	0.0004
HbA1 (%)	9.8 (9.2–10.5)	7.8 (7.5–8.2)	0.0004
Serum osteocalcin (μg/liter)	4.17 ± 1.63	5.04 ± 1.43	0.04
Urinary hydroxyproline (mg/m^2/liter)	18.1 (10.9–28.1)	17.8 (7.1–23.2)	n.s.

Values are expressed as either means ± SD or as median and interquartile range (in parenthesis) if not normally distributed

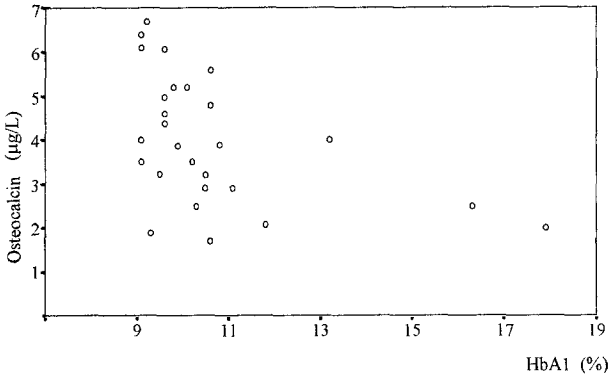

Fig. 1. Correlation between HbA1 and serum osteocalcin in 27 diabetic men ($r = -0.51$, $P = 0.009$).

controversial. Bone mass has been reported to be increased, normal, or decreased in NIDDM [6, 7, 26]. The mechanism and pathogenesis of diabetic osteopenia remains unclear. Increased urinary calcium loss, decreased intestinal calcium absorption, disturbed vitamin D metabolism, hyperparathyroidism, reduced serum calcitonin concentrations, disturbed bone vascularity due to diabetic microangiopathy, and inflammation-mediated osteopenia have been proposed as possible pathogenetic mechanisms primarily based on experimental animal models [8–13]. But all these mechanisms are doubtful and there are insufficient data to prove them. It has been reported that there is no derangement of calcium metabolism in adults with insulin-requiring diabetes and it has been suggested that the diabetic rat model is not useful for determining the pathogenesis of diabetic osteopenia in humans [27].

Absence of insulin has been implicated in the pathogenesis of the accelerated bone loss in IDDM patients [28]. It has been reported that circulating insulin levels are related to bone density in normal postmenopausal women [29]. It has been suggested that endogenous insulin protects against the loss of bone mass, and that osteoporosis in diabetics is secondary to the metabolic abnormalities associated with insulinopenia [30]. Bone formation and osteoid volume have been found to be reduced in experimental diabetes [31]. It has been reported that diabetic osteopathy may result from decreased osteoblast number and this is probably due to deficiency of insulin or insulin-dependent growth factors [18]. Specific high-affinity insulin receptors have been demonstrated in the rat osteoblastic cells [32]. On the other hand, it has been suggested that increased adipose tissue frequently seen in NIDDM patients yields metabolically active steroid hormones and insulin-like growth factors which may stimulate bone formation [14].

The circulating level of osteocalcin and the urinary excretion of hydroxyproline in diabetic animals have been investigated in some previous studies. These suggest that serum concentration of osteocalcin—a marker of bone formation—is decreased in diabetic animals [17, 18]. The reports on the urinary hydroxyproline excretion—a marker of bone resorption—are more controversial. It has been reported to be decreased or increased [19, 20]. In human diabetes, it has been reported that serum osteocalcin is reduced in IDDM patients and increased by glycemic control in diabetic children [21, 22].

In the present study, we have found that serum osteocalcin level increased significantly with improvement of glycemic control in diabetic men, but urinary hydroxyproline excretion did not change significantly. Serum osteocalcin was negatively correlated with HbA1. These results suggest that chronic hyperglycemia in diabetic patients may result in decreased bone formation, as reflected by serum osteocalcin level; this can be reversed by improvement of glycemic control. According to the results of urinary hydroxyproline excretion, it may be reasonable to think that bone resorption is not much affected by glycemic control. However, due to the relatively small number of patients and nonhomogeneous distribution, we are unable to definitely rule out a false-negative result. No correlation was found between weight or BMI with both markers. This is not in accord with the hypothesis that increased adipose tissue may stimulate bone formation. There was also no correlation between fasting serum C-peptide and serum osteocalcin levels in the whole group and in the subgroup of patients treated with oral hypoglycemic agents. This finding does not support the idea that insulin has a direct effect on osteoblast function independent of its metabolic effects. It seems that the improvement of osteoblast function is related to the correction of metabolic abnormalities associated with insulinopenia.

In conclusion, osteoblast function may improve by glycemic control in diabetic patients; this may be due to the correction of metabolic abnormalities rather than a direct effect of insulin on bone cells.

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