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Association between serum testosterone concentration and collagen degradation fragments in men with type 2 diabetes mellitus

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Abstract

The aim of the present study was to evaluate relationships between serum endogenous androgens and urinary concentration of cross-linked N-telopeptides of type I collagen (NTx), a bone resorption marker, in men with type 2 diabetes mellitus because low androgen concentrations are associated with both osteoporosis and cardiovascular disease. Relationships between serum free testosterone and urinary NTx concentrations were investigated in 246 consecutive men with type 2 diabetes mellitus. In addition, relationships between urinary NTx concentration and other variables including age, duration of diabetes, blood pressure, serum lipid concentration, hemoglobin A_{1c}, and body mass index were evaluated. Urinary NTx concentrations were 27.8 (26.4-29.3) nmol of bone collagen equivalent per millimole of creatinine, correlating inversely with serum free testosterone ($r = -0.263$, $P < .0001$). Multiple regression analysis identified serum free testosterone ($\beta = -.292$, $P < .0001$), hemoglobin A_{1c} ($\beta = .144$, $P = .0404$), and smoking status ($\beta = .143$, $P = .0402$) as independent determinants of urinary NTx. In conclusion, serum free testosterone concentration correlated inversely with urinary NTx concentration, which may partly account for an observed link between osteoporosis and cardiovascular disease in men with type 2 diabetes mellitus.

1. Introduction

Bone loss is a chronic complication of diabetes-associated alterations in mineral and bone metabolism [1-4]. In patients with type 2 diabetes mellitus, osteoblast function was reported to be decreased, whereas osteoclast function was increased [5]. Biochemical markers of bone turnover provide a way to estimate future bone density. Type I collagen, a major component of bone, accounts for more than 90% of the organic matrix. Collagen degradation during osteoclastic bone resorption represents the basis of new clinical bone metabolism assays. Among these assays, urinary concentrations of cross-linked N-telopeptides of type I collagen (NTx) have been reported to be a sensitive and specific marker of bone resorption [6,7]. Bone mineral density (BMD) was reported to be lower, whereas urinary NTx concentrations were higher in patients with type 2 diabetes mellitus than in control subjects [8].

Both osteoporosis and cardiovascular disease (CVD) are major public health problems that contribute importantly to morbidity and mortality. Accumulating evidence indicates that certain shared pathophysiologic mechanisms underlie both diseases [9-11]. In addition to traditional cardiovascular risk factors such as age, dyslipidemia, oxidative stress, hypertension, and diabetes, sex hormone deficiency also influences bone remodeling [12]. Overt hypogonadism results in reduction in BMD and alterations in body composition, mood, cognitive function, sexual function, and several factors promoting CVD [13]. A low serum concentration of testosterone is associated with increased risk of CVD in men [14]. The considerations above raise the possibility of deficient endogenous androgens as causal intermediates linking osteoporosis to CVD.

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Few studies have demonstrated the relationships between serum endogenous androgens and urinary NTx concentrations in men. Khosla et al [15] demonstrated an inverse association between serum testosterone and urinary NTx in the general population of men. To our knowledge, relationships between serum endogenous androgens and urinary NTx concentrations have not been explored in men with type 2 diabetes mellitus. We therefore investigated relationships between serum free testosterone or dehydroepiandrosterone sulfate (DHEA-S) and urinary NTx concentrations, a bone resorption marker, in men with type 2 diabetes mellitus.

2. Subjects and methods

2.1. Subjects

Serum free testosterone concentrations, serum DHEA-S concentrations, and urinary NTx concentrations were measured in 246 consecutive men with type 2 diabetes mellitus. Relationships between serum free testosterone and urinary NTx concentrations and between serum DHEA-S and urinary NTx concentrations were investigated. In addition, relationships between urinary NTx concentration and age, duration of diabetes, blood pressure, serum lipid concentration, glycemic control (hemoglobin A_{1c} [HbA_{1c}]), body mass index (BMI), severity of diabetic retinopathy, severity of diabetic nephropathy defined by urinary albumin excretion, presence of CVD, smoking status, and current treatment of diabetes were evaluated. Serum free testosterone concentrations and serum DHEA-S concentrations were measured by the Coat-A-Count free testosterone and DHEA-S kits (Diagnostic Products, Los Angeles, CA). Intra-assay coefficient of variance (CV) and inter-assay CV for serum free testosterone and DHEA-S concentrations were stated previously [16,17]. Urine samples were collected in the morning. Urinary NTx concentration was measured using an enzyme-linked immunosorbent assay (Osteomark; Ostex International, Seattle, WA) and expressed as nanomoles of bone collagen equivalent per millimole of creatinine (nmol BCE/mmol Cr) [6]. The detection limit for NTx was 7.5 nmol BCE per liter. Intra- and interassay CVs for NTx concentrations of 320 and 1680 nmol BCE per liter were 6.2% and 7.2% for the low concentration and 4.5% and 2.5% for the high concentration, respectively.

A mean value for urinary albumin excretion was determined from 3 urine collections. Plasma total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A_{1c} was assayed using high-performance liquid chromatography. Mean values for biochemical parameters obtained during the previous year were used for statistical analysis.

Type 2 diabetes mellitus was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [18]. Retinopathy was graded as follows: no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), and proliferative diabetic retinopathy (PDR). Nephropathy was graded as follows: normoalbuminuria, urinary albumin excretion less than 30 mg/g Cr; microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, more than 300 mg/g Cr. Hypertension was defined as blood pressure of at least 140/90 mm Hg or use of antihypertensive medication. Sitting blood pressure was measured after a 5-minute rest. Hyperlipidemia was defined as total cholesterol exceeding 220 mg/dL, triglyceride exceeding 150 mg/dL, or use of antihyperlipidemic medication. Cardiovascular disease was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history or physical examination. Smoking status was recorded as nonsmoker, past smoker, or current smoker according to a self-administered questionnaire. Patients were excluded if they had been castrated for treatment of testicular or prostate cancer, or were taking any medications known to affect sex hormone concentrations (eg, antiandrogenic agents for prostate cancer). None had taken oral calcium supplementation, vitamin D, vitamin K, or steroid hormones. In addition, patients with macroalbuminuria were excluded because advanced diabetic nephropathy may influence urinary excretion of NTx. Approval for the study was obtained from the local research ethics committee, and informed consent was obtained from all participants.

2.2. Statistical analysis

Means and frequencies of potential confounding variables were calculated, and unpaired Student *t* tests or analyses of variance were conducted, to assess statistical significance of differences between groups using the StatView software (version 5.0; SAS Institute, Cary, NC). Because urinary albumin excretion showed a skewed distribution, logarithmic (log) transformation of these values was carried out before performing correlation analysis. Relationships between serum free testosterone or DHEA-S concentration and urinary NTx concentrations, and between urinary NTx concentration and age, duration of diabetes, glycemic control, and other variables were examined by Pearson correlation analyses. All continuous variables are presented as the mean (95% confidence interval). Multiple regression analysis was performed to assess the combined influence of variables on urinary NTx concentration. To examine the effects of various factors on urinary NTx concentration, we considered the following factors as independent variables: serum free testosterone concentration, age, duration of diabetes, BMI, systolic blood pressure, HbA_{1c}, and smoking status. A P value of less than .05 was considered statistically significant.

3. Results

Characteristics of the 246 men with type 2 diabetes mellitus enrolled in this study are shown in Table 1. The mean (confidence interval) concentration of urinary NTx

Table 1
Clinical characteristics of patients with diabetes

	Mean (95% CI)
n	246
Age (y)	64. 0 (62.8-65.3)
Age at onset (y)	50.6 (49.1-52.0)
Duration of diabetes (y)	13.3 (11.9-14.6)
BMI (kg/m^2)	23.3 (22.9-23.7)
HbA_{1c} (%)	7.1 (7.0-7.3)
Systolic blood pressure (mm Hg)	134 (132-136)
Diastolic blood pressure (mm Hg)	77 (76-79)
Total cholesterol (mg/dL)	192 (188-195)
Triglyceride (mg/dL)	134 (124-144)
HDL cholesterol (mg/dL)	52 (50-53)
Smoking (none/past/current)	57/113/76
Retinopathy (NDR/SDR/PDR)	186/34/26
Nephropathy (normo-/microalbuminuria)	153/93
CVD (−/+)	205/41
Current treatment (diet/OHA/insulin)	26/148/72
Free testosterone (pg/mL)	9.3 (9.0-9.7)
DHEA-S (ng/mL)	1130 (1039-1223)
Urinary NTx (nmol BCE/mmol creatinine)	27.8 (26.4-29.3)

Data are expressed as mean (95% confidence interval) or number of patients. CI indicates confidence interval; HDL, high-density lipoprotein; OHA, oral hypoglycemic agents.

was 27.8 nmol BCE/mmol Cr (26.4-29.3 nmol BCE/mmol Cr). Correlations between urinary NTx concentration and other variables are shown in Table 2. An inverse correlation was found between serum free testosterone and urinary NTx concentrations ($r = -0.263$, $P < .0001$) in men with type 2 diabetes mellitus (Fig. 1). No significant correlation was found between serum DHEA-S and urinary NTx concentrations ($r = -0.052$, $P = .4611$). Urinary NTx concentration was significantly greater in patients with lower concentrations of free testosterone (<10 pg/mL) than in patients with higher concentrations of free testosterone (29.2 [27.1-31.4] vs 25.5 [23.8-27.3] nmol BCE/mmol Cr, $P = .013$). Urinary NTx concentration was significantly greater in current smokers (31.1 [28.0-34.3] nmol BCE/mmol Cr) than in past smokers (27.0 [24.9-29.0] nmol BCE/mmol Cr, $P = .0176$) or nonsmokers (25.1 [22.4-27.8] nmol BCE/mmol Cr,

Table 2
Correlation between urinary NTx concentration and other variables

	r	P
Age	−0.006	.9195
Age at onset	0.060	.3647
Duration of diabetes	−0.079	.2331
BMI	−0.104	.1076
HbA_{1c}	0.082	.2032
Systolic blood pressure	−0.024	.7108
Diastolic blood pressure	−0.113	.0788
Total cholesterol	−0.126	.0515
Triglyceride	−0.083	.1952
HDL cholesterol	−0.015	.8168
DHEA-S	−0.052	.4611
Log (urinary albumin excretion)	0.023	.7253

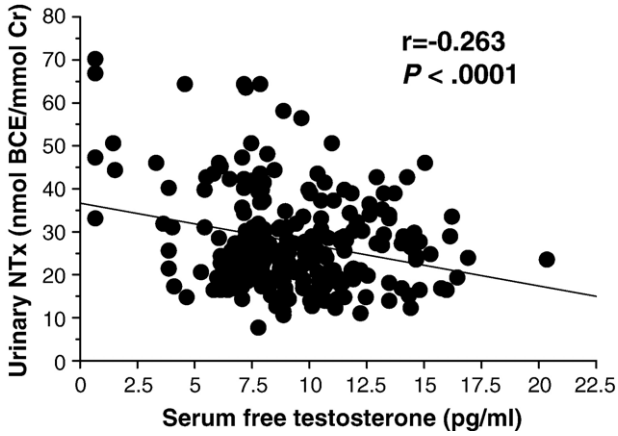

Fig. 1. Correlation between serum free testosterone and urinary NTx concentrations in men with type 2 diabetes mellitus.

$P = .0037$). Multiple regression analysis demonstrated that serum free testosterone concentration ($\beta = -.292$, $P < .0001$), HbA_{1c} ($\beta = .144$, $P = .0404$) and smoking status ($\beta = .143$, $P = .0402$) were independent determinants of urinary NTx concentration. Urinary NTx concentration did not differ between patients with and those without CVD (28.3 [24.1-32.6] vs 27.6 [26.1-29.2] nmol BCE/mmol Cr, $P = .7216$). In addition, urinary NTx concentration did not differ according to severity of diabetic nephropathy (28.1 [26.2-30.1] vs 27.6 [25.2-30.0] nmol BCE/mmol Cr for patients with normoalbuminuria and microalbuminuria, respectively) or according to severity of diabetic retinopathy (28.3 [26.5-30.1] vs 26.2 [22.5-30.0] vs 25.4 [20.3-30.5] nmol BCE/mmol Cr for patients with NDR, SDR, and PDR, respectively). Urinary NTx concentration did not differ between patients with and those without hypertension (27.4 [25.5-29.3] vs 28.5 [26.1-30.8] nmol BCE/mmol Cr, $P = .4884$) or between patients with and those without hyperlipidemia (26.9 [24.9-29.0] vs 28.9 [26.8-30.9] nmol BCE/mmol Cr, $P = .1993$). Urinary NTx concentration did not differ between patients treated with insulin and patients treated without insulin (26.0 [23.4-28.6] vs 28.6 [26.9-30.3] nmol BCE/mmol Cr, $P = .1027$).

4. Discussion

Available data concerning association of reduced BMD with type 2 diabetes mellitus are equivocal; in various studies, type 2 diabetes mellitus has been reported to be associated with increased [19], unchanged [20], or decreased [21,22] BMD. Buysschaert et al [23] reported low BMD in men with type 2 diabetes mellitus. Although osteoporosis is well known to be common in elderly women, it also is common in elderly men; this high prevalence of osteoporosis in older men is increasingly recognized.

Both osteoporosis and CVD are major public health problems that adversely impact morbidity and mortality. Accumulating evidence indicates a link between osteoporosis

and CVD [9-11], suggesting some shared pathophysiologic mechanisms underlying both diseases. In addition to traditional cardiovascular risk factors such as age, dyslipidemia, oxidative stress, hypertension, and diabetes, sex hormone deficiency also regulate bone remodeling [12]. Hypogonadism is one of the most important risk factors for osteoporosis in men [13]. Some studies have shown a weak but significant association between free testosterone and BMD [24,25]. Gonadal steroid deprivation increases bone resorption relative to formation, which leads to bone loss [26]. On the other hand, androgen replacement clearly increases BMD in hypogonadal men [27]. Although many reports have elucidated pathophysiologic characteristics of abnormal bone metabolism in patients with type 2 diabetes, the relationship between serum free testosterone or DHEA-S and urinary NTx concentrations, a bone resorption marker, has never been explored in men with type 2 diabetes mellitus. Then, we evaluated the relationships between endogenous androgens and urinary NTx concentrations in men with type 2 diabetes mellitus. We found a weak but significant inverse association between serum free testosterone and urinary NTx concentrations. Multiple regression analysis identified free testosterone as an independent predictor of urinary NTx concentration.

Low testosterone concentrations also appear to be associated with increased risk of CVD in men [14]. Inverse association between serum free testosterone and urinary NTx concentrations may partly account for the link between osteoporosis and CVD in men with type 2 diabetes mellitus.

A decline in testosterone concentration with advancing age may partly explain the age-related increases in the risk of osteoporosis as well as CVD. Diabetes is an important consideration here, as men with diabetes have significantly lower plasma concentrations of free and total testosterone than nondiabetic men [28,29]. Men with low concentrations of testosterone are candidates for testosterone therapy to prevent osteoporosis as well as atherosclerosis.

Some studies have found an association between BMD decrease and degree of glycemic control [30]. Metabolic effects of poor glycemic control in patients with type 2 diabetes mellitus, such as hypercalciuria, may lead to an increase in net bone resorption [2]. In the present study, multiple regression analysis identified HbA_{1c} as a determinant of urinary NTx concentration, so poor control may lead to decreased BMD. We evaluated associations between urinary NTx concentration and the degree of diabetic macroangiopathy or microangiopathy. Urinary NTx concentrations did not differ between patients with CVD and those without despite an apparent link between osteoporosis and CVD. We also previously have demonstrated that serum testosterone concentrations did not differ significantly between patients with and those without CVD [16]. However, serum free testosterone concentrations correlated significantly with ultrasonographically evaluated mean intima-media thickness and plaque score, early preclinical markers of atherosclerosis. Urinary NTx concentrations also might be correlated with these preclinical markers. No association was found between urinary NTx concentrations and diabetic microangiopathy (retinopathy and nephropathy). Clausen et al [31] reported that BMD was reduced in male patients with increased urinary albumin excretion, considering diabetic osteopenia to be a progressive disorder related to the development of diabetic nephropathy, associated with decreased creatinine clearance and consequently increased parathyroid hormone. However, no significant correlations were found between urinary NTx concentration and log (urinary albumin excretion) in men with type 2 diabetes mellitus in the present study. A possible explanation of this discrepancy is that we excluded patients with macroalbuminuria because such advanced diabetic nephropathy appears likely to influence urinary NTx excretion. Also, surrogate markers differed between the 2 studies (BMD vs urinary NTx concentration).

Serum free testosterone concentration inversely correlated with urinary NTx concentration as well as age (data not shown). However, age did not correlate with urinary NTx concentration. One possible explanation is that a clearer correlation of serum free testosterone and urinary NTx concentrations was found in older men (>65 years; $r = -0.335$, $P = .0001$) than in younger men (<65 years; $r = -0.156$, $P = .0984$).

Clinical management of osteoporosis should be important for the preservation of quality of life in elderly male as well as female patients with type 2 diabetes mellitus, considering that metabolic derangements resulting from diabetes are related to high risk of fracture [32]. Diabetes might also increase fracture risk by mechanisms in addition to osteopenia, such as increased likelihood of falling [33].

Limitations of the present study include a cross-sectional design and a relatively small number of patients. In addition, we did not evaluate bone formation markers or BMD. Greater muscle strength and physical activity have been associated with greater bone mass, whereas radial bone mass was less in cigarette smokers and subjects with moderate alcohol intake [34]. We did not evaluate physical activity or alcohol consumption, both of which could affect bone metabolism. Despite these limitations, this study demonstrated that serum free testosterone concentration was inversely associated with urinary NTx concentration, representing part of the link between osteoporosis and CVD in men with type 2 diabetes mellitus. Urinary NTx concentration is a marker of ongoing bone resorption, whereas BMD quantitates the present state of osteoporosis. Large prospective trials and intervention studies are needed to better assess possible benefits of testosterone on bone metabolism.

In conclusion, serum free testosterone concentration correlated inversely with urinary NTx concentration, which may partly account for an observed link between osteoporosis and CVD in men with type 2 diabetes mellitus.

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