Testosterone is an Independent Determinant of Bone Mineral Density in Men with Type 2 Diabetes Mellitus

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Short title; Testosterone and BMD in DT2

List of abbreviations

body mass index (BMI); bone mineral density (BMD); C-reactive protein (CRP); type 2 diabetes mellitus (DT2); follicle-stimulating hormone (FSH); hemoglobin A_{1c} (HbA_{1c}); high-density lipoprotein cholesterol (HDL-C); immunoreactive insulin (IRI); low-density lipoprotein cholesterol (LDL-C); luteinizing hormone (LH); sex hormone-binding globulin (SHBG); total cholesterol (TC); triglyceride (TG); very low-density lipoprotein cholesterol (VLDL-C); qualitative ultrasound (QUS)

Abstract

Background: Although many reports have elucidated pathophysiologic characteristics of abnormal bone metabolism in patients with type 2 diabetes mellitus (DT2), determinants of bone mineral density (BMD) in patients with DT2 are still controversial.

Methods: We examined 168 Belarussian men aged 45 to 60 years. Plasma total cholesterol (TC), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, triglyceride concentrations, hemoglobin A_{1c} (HbA_{1c}), immunoreactive insulin, C-reactive protein were assessed. BMD was measured using dual energy X-ray densitometry in lumbar spine (L_1-L_4). Total testosterone (TT), SHBG were assessed, free testosterone (FT) was calculated. All statistical analyses were performed using SPSS v11.0 software.

Results: By univariate linear regression analysis, bone mineral density of the lumbar spine was significantly correlated with FT ($r=0.32$, $p<0.01$) as well as TT ($r=0.36$, $p<0.01$). By multiple linear regression analysis adjusted for confounding factors, BMD was significantly correlated with total testosterone ($\beta=0.226$, $p<0.001$). On the other hand, age ($\beta=0.005$, $p=0.071$), body mass index (BMI) ($\beta=0.005$, $p= 0.053$), HbA_{1c} ($\beta=-0.002$, $p=0.21$), duration of diabetes ($\beta=0.001$, $p=0.62$) and TC ($\beta=-0.029$, $p=0.005$) were not significantly correlated with BMD.

Conclusions: Our data indicate that androgens are independent determinants of BMD in male patients with DT2.

Keywords: type 2 diabetes mellitus (DT2); free testosterone (FT), total testosterone (TT), bone mineral density (BMD), male.

1. Introduction

Although many reports have elucidated pathophysiologic characteristics of abnormal bone metabolism in patients with type 2 diabetes mellitus (DT2), determinants of bone mineral density (BMD) in patients with DT2 are still controversial (1,2). In recent years the prevalence of osteoporosis in men has been increasing (3). Although the frequency of osteoporosis is lower than that in women, hip and spine fractures in men are associated with higher morbidity and mortality (31%-38% for men vs. 12%-28% for women) (5) and are twice as likely to be institutionalized (4,6).

Sex steroids play an important role in the maintenance of bone metabolism. In a previous study, low androgen levels were reported to be a predictor of bone loss in men (7). Furthermore, serum concentrations of testosterone (T) have been reported to be lower in men with diabetes than in nondiabetic men (8). Hypogonadism, however, is commonly related to age-related changes in sex hormones of the elderly. These changes are usually biochemically defined and generally unaccompanied by clinical evidence of hypogonadism. Estrogen deficiency can also occur in normal older men as a consequence of aging, and serum estradiol appears to have an important effect on bone health in men (9). However, its relative deficiency is less severe than that in

postmenopausal women. Therefore, hypogonadism-induced osteoporosis in men seems to be related to the physiological effects of serum testosterone and estradiol.

In this study, we investigated the determinants of BMD in male patients with DT2 in order to establish future strategies for the prevention of fragility fracture due to osteoporosis in these patients.

2. Subjects and methods

2.1. Subjects

Prior to this study, ethical approval was obtained from the special committee of The Republican Research Centre for Radiation Medicine and Human Ecology (Gomel, Republic of Belarus). We investigated 168 Belarussian men with DT2 who consecutively visited this institute. Patients aged 45 to 60 years with DT2, with body mass index (BMI) values between 18.5 and 40 kg/m^2 were included in this study. Criteria for the diagnosis of DT2 were symptoms of diabetes plus the fasting plasma glucose cut-point for Impaired Fasting Glucose (IFG) is remain at 6.1mmol/l and casual plasma glucose concentration ≥11.1 mmol/l. Casual is defined as any time of day without regard to time since last meal.

Patients with thyroid diseases and liver cirrhosis were excluded from the study. Written informed consent was obtained from all men. All patients were treated with diet, oral antidiabetic drugs (biguanides and sulfonylureas) and/or insulin.

Demographic parameters were collected; height and weight were measured and BMI was calculated. Data related to the duration of diabetes and given medications were also

collected. BMD was evaluated using dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, NY, USA). BMD was measured at the lumbar spine (L1-L4).

2.2. Biochemical measurements

After informed consent was obtained, fasting venous blood samples were collected in the morning (before 10 a.m.). Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were assessed using standard enzymatic methods, and low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated by the Friedewald (10) equation. HbA_{1c} in whole blood collected with EDTA was assayed using high-performance liquid chromatography. Immunoreactive insulin (IRI) was measured by RIA using DSL 10-1600 (ACTIV®, Diagnostic Systems Laboratories, Inc., Webster, TX, USA). C-reactive protein (CRP) was measured by a latex particle-enhanced turbidimetric immunoassay on the autoanalyzer.

Serum total testosterone was measured by solid-phase RIA in the morning between 8-11 a.m. [intraassay coefficient of variation (CV) 4.3%, interassay CV 9.8%]. The lower limit of normal for total testosterone is 12 nmol/l. Free testosterone was

calculated from SHBG and total testosterone using the "Free and Bioavailable Testosterone calculator" (11). Sex hormone-binding globulin (SHBG), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by chemiluminescent immunometric assays [SHBG, intraassay CV 3.1% and interassay CV 4.1%; LH, intraassay CV 4.2% and interassay CV 5.5%; FSH, intraassay CV 3.5% and interassay CV 3.7%]. All samples obtained from a subject were assayed in the same run for each hormone to exclude interassay variation from changes in hormone level within subjects.

2.3. Statistical analysis

Data are presented as mean ± standard deviation or median (25^{th} – 75^{th} percentile). Spearman rank correlation analysis was performed to evaluate the association between BMD and other existing parameters. Multivariate linear regression analysis was also performed to evaluate the association between BMD and other existing parameters adjusted for age, BMI, HbA_{1c}, duration of diabetes, TC and log free testosterone. Because free testosterone was distributed in a skewed manner, logarithmic transformation was performed for multivariate linear regression analysis. A p value

<0.05 was considered statistically significant. All statistical analyses were performed using SPSS v11.0 software (SPSS Japan, Tokyo, Japan).

3. Results

Characteristics of the study participants are shown in **Table 1**. The average age was 54.1±4.8 years, duration of diabetes was 7.0 (3.0-12.0) years, and HbA_{1C} was 8.2 (7.0-9.7)%. One hundred and eight patients (64%) had below-normal testosterone levels (<12 nmol/l). Values of LH and FSH were 4.5 (2.9-6.1) mIU/ml and 4.5 (3.2-6.8) mIU/ml, respectively. Forty-three patients (25.6%) had either high LH or high FSH levels (10 had high levels of both LH and FSH, 6 had high LH levels only, and 27 had high FSH levels only). Only 2 patients (1.2%) had high PRL concentrations (23.3 and 24.1 mIU/ml, respectively). Mean BMD was 1.16±0.19 g/cm^2.

There were no significant differences of BMD between smoking patients and non-smoking patients (t-test, p=0.19). By univariate linear regression analysis, BMD was significantly correlated with total testosterone (r=0.36, p<0.01) and free testosterone (r=0.32, p<0.01). BMD also was significantly correlated with SHBG (r=-0.16), TC (r=-0.23), and VLDL-C (r=0.19). On the other hand, age, duration of diabetes, BMI, estradiol, LH, FSH, CRP, IRI, HbA_{1c}, TG, HDL-C, and LDL-C were not significantly correlated with BMD (**Table 2**). By multiple linear regression analysis adjusted for confounding factors, BMD was significantly correlated with log free testosterone

(β=0.23, p<0.001). On the other hand, age (β=0.005, p=0.071), body mass index (BMI) (β=0.005, p= 0.053), HbA_{1c} (β=-0.002, p=0.21), duration of diabetes (β=0.001, p=0.62) and total cholesterol (β=-0.02, p=0.055) were not significantly correlated with BMD.

4. Discussion

In recent years, several studies reported a relationship between reduction in circulating levels of testosterone and bone loss in elderly men (12). Gonadal steroid deprivation increases bone resorption rather than formation, which leads to bone loss (13). Although some authors have identified total testosterone as a strong predictor of BMD, this relationship remains controversial (14,15). In this study, we clearly showed that serum free testosterone concentration is independently correlated with BMD. Testosterone is necessary for the aromatization and formation of estrogen in the testicles and surrounding tissue (16). Previous studies have shown that estrogen is an important determinant of BMD in men and is correlated with testosterone (17). In our current study, however, univariate linear regression analysis showed that BMD was not significantly correlated with total estradiol concentration ($r=-0.13$, $p=0.08$). This discrepancy might be due to our measurement of "total," not "free," estradiol concentration (18), because serum total estradiol concentration does not reflect tissue-level activity, as peripherally formed estradiol is partially metabolized in situ; thus, not all of it enters the general circulation.

Bjornerem et al. reported that low free testosterone levels were positively associated with BMD, whereas low SHBG concentrations were inversely associated with BMD (19). Testosterone levels in men are known to decline with age, and SHBG levels are known to rise in older men and women (20). In this study, we found that SHBG was inversely associated with BMD by univariate correlation, but there was no significant association between BMD and SHBG by multivariate linear regression analysis adjusted for confounding factors.

The relationship of long-term blood glucose control to BMD is still controversial. It has been speculated that long duration of diabetes might cause low BMD because osteoporosis might be a complication of diabetes resulting from the cumulative results of long-term poor control (21). We did not find a relationship between duration of diabetes and BMD or a correlation between HbA_{1c} and BMD. The absence of a relationship with HbA_{1c} might be attributable to the use of a single measurement of HbA_{1c}.

Tanko et al. (22) found a significant inverse correlation between serum cholesterol and BMD in postmenopausal women and explained that estrogen could have direct effects on bone and cholesterol metabolism. It was shown that lipids accumulate around bone vessels (23). Parhami et al. found that oxidized lipids and hyperlipidemia may

inhibit osteoblastic differentiation (24). Because immature osteoblasts are located immediately adjacent to the subendothelial matrix of bone vessels, these cells may be susceptible to damage caused by lipid oxidation products. Additionally, oxidized lipids may induce endothelial expression of Macrophage-Colony Stimulating Factor, which is a potent stimulator of osteoclastic differentiation (25). In a recent work, Kha et al. (26) showed that specific oxysterols—products of cholesterol oxidation—act synergistically with bone morphogenic protein 2 in inducing osteogenic differentiation. In our study, we also found a significant inverse correlation between serum cholesterol and BMD in male patients with DT2, adjusted for confounding factors (β=-0.029, p=0.005). To clarify mechanisms of this relationship, further evaluation will be needed.

Several limitations need to be acknowledged and addressed regarding the present study. First, a relatively small sample size is one of the limitations of the study. Also, we evaluated BMD not by ultrasound but by dual-energy X-ray absorptiometry. It is known that BMD assessed by DXA is an established marker for osteoporosis; however, in recent years, ultrasound absorptiometry has emerged as a possible alternative to DXA because ultrasound measurement is free from radiation exposure (27). On the other hands, CT imaging of the lumbar spine provides superior anatomic imaging of the osseous (bony) structures of the spine and for patients when more detailed imaging of

the bony architecture is important, CT imaging is recommended. In addition, we could not evaluate bone formation markers, physical activity, or alcohol consumption. Further studies are needed to clarify the contribution of androgens to BMD in patients with DT2.

In conclusion, we showed that androgens are of importance for bone health of male patients with DT2. In order to fully exploit opportunities for hormone-related management of osteoporosis in men, a better understanding of the precise nature of their action on BMD is needed.

Acknowledgment

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan through the Nagasaki University Global COE program. We would also like to thank Miss Miho Yoshida for her technical assistance.

Declaration of interest

All authors have no conflict of interest.

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Table 1. Clinical characteristics of men with type 2 diabetes mellitus (n=168)

Age, yrs	54.1±4.8
Duration of diabetes, y	7.0 (3.0-12.0)
Body mass index, kg/m^2	29.9±5.5
Hemoglobin A1c, %	8.2 (7.0-9.7)
Total cholesterol, mmol/L	5.2±1.4
Triglyceride, mmol/L	1.8 (1.2-2.6)
High-density lipoprotein cholesterol, mmol/L	1.3±0.6
Low-density lipoprotein cholesterol, mmol/L	2.5 (1.8-3.5)
Very low-density lipoprotein cholesterol, mmol/L	0.8 (0.5-1.2)
Smoking, none/current	104/62
Current treatment, diet/OHA/insulin/OHA and insulin	2/85/55/26
Total testosterone, nmol/L	9.6 (5.4-14.5)
Free testosterone, nmol/L	0.18 (0.12-0.44)
Sex hormone-binding globulin, nmol/L	41.0 (30.5-52.2)
Estradiol, IU/L	0.16 (0.10-0.20)
Luteinizing hormone, IU/L	4.5 (2.9-6.1)
Follicle-stimulating hormone, IU/L	2.4 (1.2-4.6)
C-reactive protein, mg/L	2.4 (1.2-4.6)
Immune-reactive insulin, mU/L	9.2 (6.0-17.3)
Bone mineral density of lumbar spine, g/cm^2	1.16±0.19

Values are mean ± standard deviation or median (25th - 75th percentile).

Table 2. Univariate correlation between BMD and other variables

	r	p
Age	0.08	0.25
Duration of diabetes	0.09	0.09
Body mass index	-0.08	0.23
Total testosterone[b]	0.36	<0.001
Free Testosterone[b]	0.32	<0.001
Sex hormone-binding globulin[a]	-0.16	0.04
Estradiol	-0.13	0.08
Luteinizing hormone	0.10	0.21
Follicle-stimulating hormone	-0.009	0.91
C-reactive protein	-0.003	0.21
Immune-reactive insulin	0.01	0.89
Hemoglobin A_{1c}	-0.06	0.4
Total cholesterol[b]	-0.23	0.003
Triglyceride	-0.09	0.24
High-density lipoprotein cholesterol	0.06	0.57
Low-density lipoprotein cholesterol	-0.1	0.17
Very low-density lipoprotein cholesterol[a]	0.19	0.03

r: correlation coefficients. [a]$p<0.01$, [b]$p<0.05$.

Table 3. Multivariate linear regression analysis for putative predictors of BMD

Variables	β	95% CI	p
Age	0.005	0, 0.010	0.071
Body mass index	0.005	0, 0.011	0.053
Hemoglobin A_{1c}	-0.002	-0.015, 0.010	0.72
Duration of diabetes	0.001	-0.004,0.006	0.62
Total cholesterol	-0.029	-0.040,0	0.005
Log free testosterone	0.23	0.13, 0.32	<0.001

All variants are adjusted for the analysis. β: standardized regression coefficient; CI: confidence interval

R^2=0.49