Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1691 – 1695
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Bone stiffness in men with type 2 diabetes mellitus

Mai Asano, Michiaki Fukui*, Hiroko Hosoda, Emi Shiraishi, Ichiko Harusato, Mayuko Kadono, Muhei Tanaka, Goji Hasegawa, Toshikazu Yoshikawa, Naoto Nakamura

Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

Received 20 May 2008; accepted 29 July 2008

Abstract

Osteoporosis in elderly men as well as women is increasingly recognized, and patients with type 2 diabetes mellitus have higher risk of fracture than nondiabetic subjects. The aim of the present study was to investigate the relationship between bone stiffness and serum testosterone concentration as well as other variables in men with type 2 diabetes mellitus. The relationships between bone stiffness and serum bioavailable testosterone concentrations as well as other variables including age, duration of diabetes, glycemic control (hemoglobin A_{1c}), or body mass index were evaluated in 294 men with type 2 diabetes mellitus. An inverse correlation was found between stiffness index and age. A positive correlation was found between stiffness index and serum bioavailable testosterone concentration ($r = 0.231$, $P = .0005$). Stiffness index was significantly less in current smokers (81.6 ± 17.7) than in past smokers (86.6 ± 17.8, $P = .0396$) or nonsmokers (87.7 ± 15.2, $P = .0426$). Multiple regression analysis demonstrated that serum bioavailable testosterone concentration ($\beta = .271$, $P = .0006$) and smoking status ($\beta = -0.147$, $P = .0408$) were independent determinants of stiffness index. In conclusion, bone stiffness was associated with serum bioavailable testosterone concentration but not associated with hemoglobin A_{1c} or duration of diabetes in men with type 2 diabetes mellitus.

1. Introduction

Both osteoporosis and cardiovascular disease (CVD) are major public health problems that contribute importantly to morbidity and mortality. Accumulating evidence indicates a link between osteoporosis and CVD [1], suggesting some shared pathophysiologic mechanisms underlying both diseases. Although osteoporosis is well known to be common in elderly women, it also is common in elderly men; this high prevalence of osteoporosis in elderly men is increasingly recognized. Available data concerning association of reduced bone mineral density (BMD) with type 2 diabetes mellitus are equivocal; in various studies, type 2 diabetes mellitus has been reported to be associated with increased [2], unchanged [3], or decreased [4] BMD. Buysschaert et al [5] reported low BMD in men with type 2 diabetes mellitus. Patients with type 2 diabetes mellitus have higher risk of fracture than nondiabetic subjects [6]. Risk factors that contribute to increased fracture in diabetic patients include number of falls [7], insulin use [8], functional disability [9], and poor vision [10].

Sex steroids play an important role in the maintenance of bone health. In some studies, low androgen levels were reported to be a predictor of bone loss in men [11]. Serum concentrations of testosterone have been reported to be lower in men with diabetes than in nondiabetic men [12,13]. To our knowledge, the relationship between serum testosterone concentration and bone stiffness has not been assessed in men with type 2 diabetes mellitus. The aim of the present study was to investigate the relationship between bone stiffness and serum testosterone concentration as well as other variables including age, glycemic control, body mass index (BMI), blood pressure, and serum lipid concentration in men with type 2 diabetes mellitus.

2. Subjects and methods

2.1. Subjects

This study consisted of 294 consecutive men with type 2 diabetes mellitus recruited from the outpatient clinic of

* Corresponding author. Tel.: +81 75 251 5505; fax: +81 75 252 3721.
E-mail address: sayarinapm@hotmail.com (M. Fukui).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.07.025

Table 1
Clinical characteristics of men with type 2 diabetes mellitus

	Mean ± SD
n	294
Age (y)	63.1 ± 10.4
Age at onset (y)	50.0 ± 12.3
Duration of diabetes (y)	13.1 ± 11.7
BMI (kg/m^2)	23.1 ± 3.1
HbA_{1c} (%)	7.1 ± 1.1
Systolic blood pressure (mm Hg)	135 ± 15
Diastolic blood pressure (mm Hg)	78 ± 10
Total cholesterol (mmol/L)	5.02 ± 0.83
Triglyceride (mmol/L)	1.53 ± 1.01
HDL cholesterol (mmol/L)	1.34 ± 0.39
Smoking (none/past/current)	59/133/102
Alcohol (none/light-to-moderate/heavy)	156/80/58
Nephropathy (normo-/micro-/macroalbuminuria)	176/85/33
Retinopathy (NDR/SDR/PDR)	214/40/40
CVD (−/+)	247/47
Current treatment (diet/OHA/insulin)	33/187/74
Bioavailable testosterone (pg/mL)	776 ± 380

Data are mean ± SD or number of patients. OHA indicates oral hypoglycemic agent.

Kyoto Prefectural University of Medicine. Type 2 diabetes mellitus was diagnosed according to the "Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus" [14]. Retinopathy was graded as follows: no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), or 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. Smoking status was recorded as nonsmoker, past smoker, or current smoker according to a self-administered questionnaire. Patients were divided into 3 groups according to mean ethanol consumption per week: nondrinker, light-to-moderate drinker (<210 g/wk), or heavy drinker (≥210 g/wk). Cardiovascular disease was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history or physical examination. Patients with secondary causes of osteoporosis and those with disorders known to affect mineral metabolism (thyroid dysfunction, liver or kidney disease) were excluded. We also excluded patients medicated with drugs known to interfere with calcium metabolism, such as corticosteroids, thyroid hormone, or vitamin D, and patients who 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).

2.2. Experimental design

Relationships between bone stiffness and age, duration of diabetes, BMI, glycemic control (hemoglobin A_{1c} [HbA_{1c}]), blood pressure, serum lipid concentration, serum bioavailable testosterone concentration, severity of diabetic retinopathy, severity of diabetic nephropathy defined by urinary albumin excretion, presence of CVD, smoking status, alcohol consumption, and current treatment of diabetes were evaluated. Approval for the study was obtained from the local research ethics committee, and informed consent was obtained from all participants.

2.3. Biochemical analyses

Blood samples were obtained in the morning. Bioavailable testosterone was separated using precipitation of testosterone bound to globulins with 50% ammonium sulfate, and serum bioavailable testosterone concentrations were measured by liquid chromatography–tandem mass spectrometry using a modification method based on the use of picolinoyl derivatization [15]. Intraassay and interassay coefficients of variation for serum bioavailable testosterone concentrations at 1 pg/mL were 4.73% and 12.94%, respectively. Hemoglobin A_{1c} was measured by high-performance liquid chromatography. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Urinary albumin and creatinine concentrations were determined in an early morning spot urine. Urinary albumin excretion was measured with an immunoturbidimetric assay. A mean value for urinary albumin excretion was determined from 3 urine collections.

2.4. Quantitative ultrasound evaluation

The quantitative ultrasound (QUS) measurements were carried out with the Achilles EXPRESS ultrasonometer (GE Healthcare Lunar, Madison, WI). The Achilles measures speed of sound (SOS; in meters per second) and broadband ultrasound attenuation (BUA; in decibels per megahertz), a measure of frequency-dependent attenuation of the ultrasound wave passing through the heel. The stiffness index, a variable derived from a combination of SOS and BUA, was calculated by the analysis software according to the following equation: 0.67 BUA + 0.28 SOS − 420 [16]. We evaluated the stiffness index of the right heel using the scanning protocol provided by the manufacturer.

Table 2
Correlation between stiffness index and other variables

	r	*P*
Age	−0.120	.0406
Duration of diabetes	−0.078	.2031
BMI	0.108	.0698
HbA_{1c}	0.024	.6783
Systolic blood pressure	−0.059	.3196
Diastolic blood pressure	0.041	.4853
Total cholesterol	0.002	.9735
Triglyceride	−0.010	.8600
HDL cholesterol	−0.087	.1412

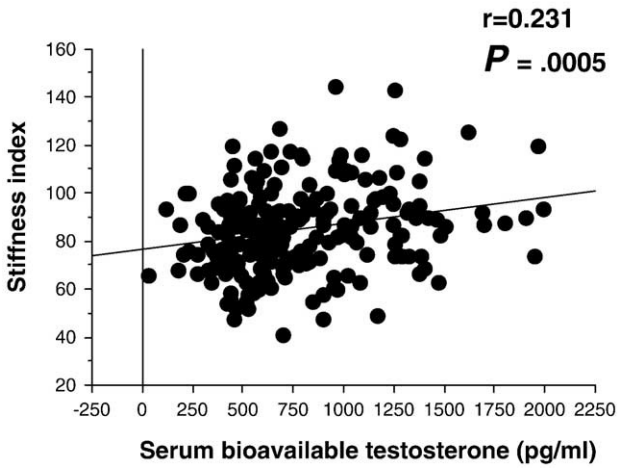

Fig. 1. Correlation between serum bioavailable testosterone concentration and stiffness index in men with type 2 diabetes mellitus.

2.5. Statistical analysis

Means and frequencies of potential confounding variables were calculated. Unpaired Student *t* tests or analysis of variance was conducted to assess statistical significance of differences between groups using StatView software (version 5.0; SAS Institute, Cary, NC). Relationships between stiffness index and age, duration of diabetes, HbA_{1c}, serum bioavailable testosterone concentration, and other variables were examined by Pearson correlation analysis. Multiple regression analysis was performed to assess the combined influence of variables on stiffness index. To examine the effects of various factors on stiffness index, we considered the following factors as independent variables: age, duration of diabetes, BMI, HbA_{1c}, systolic blood pressure, serum total cholesterol concentration, serum bioavailable testosterone concentration, and smoking status. All continuous variables are presented as the mean ± SD. A *P* value less than .05 was considered statistically significant.

3. Results

The characteristics of the 294 men with type 2 diabetes mellitus enrolled in this study are shown in Table 1. The mean stiffness index was 84.3 ± 17.7. Correlation between stiffness index and other variables are shown in Table 2. An inverse correlation was found between stiffness index and age. A positive correlation was found between stiffness index and serum bioavailable testosterone concentration ($r = 0.231$, $P = .0005$) (Fig. 1). Multiple regression analysis demonstrated that serum bioavailable testosterone concentration ($\beta = .271$, $P = .0006$) and smoking status ($\beta = -0.147$, $P = .0408$) were independent determinants of stiffness index. Stiffness index was significantly less in current smokers (81.6 ± 17.7) than in past smokers (86.6 ± 17.8, $P = .0396$) or nonsmokers (87.7 ± 15.2, $P = .0426$; Fig. 2A). Stiffness index did not differ according to the amount of alcohol consumption (84.7 ± 16.3 vs 86.4 ± 18.9 vs 90.6 ± 18.0 for nondrinkers, light-to-moderate drinkers, and heavy drinkers, respectively; Fig. 2B).

Stiffness index was significantly less in patients treated (79.5 ± 19.3) than in patients not treated with insulin (85.9 ± 16.8, $P = .0070$). Stiffness index did not differ according to severity of diabetic nephropathy (84.7 ± 19.0 vs 84.9 ± 15.2 vs 82.7 ± 17.0 for patients with normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively) or according to severity of diabetic retinopathy (85.8 ± 17.1 vs 83.9 ± 17.4 vs 82.0 ± 16.3 for patients with NDR, SDR and PDR, respectively). Stiffness index did not differ between patient with and without CVD (84.0 ± 20.4 vs 84.8 ± 16.9, $P = .7933$).

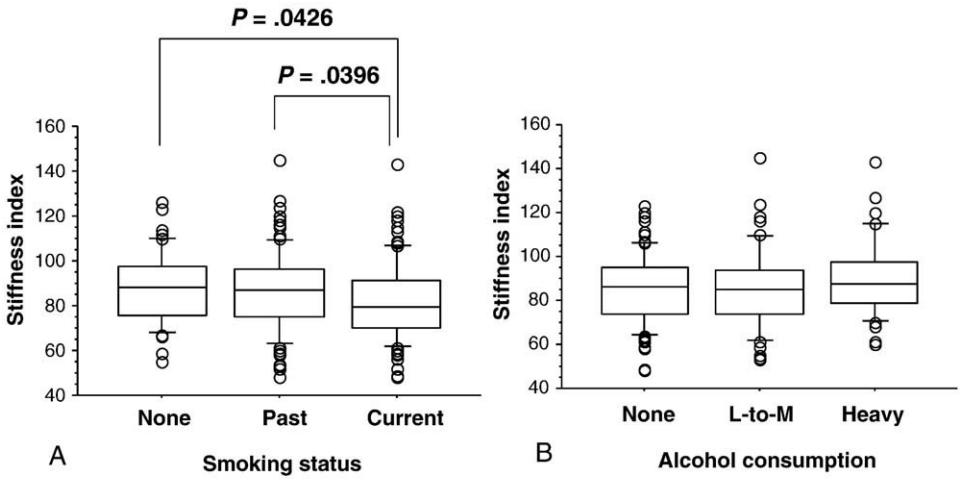

Fig. 2. Associations between smoking status and stiffness index (A) and between alcohol consumption and stiffness index (B) in men with type 2 diabetes mellitus. Data are presented as medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers). Stiffness indices (mean ± SD) were 87.7 ± 15.2, 86.6 ± 17.8, and 81.6 ± 17.7 in nonsmokers, past smokers, and current smokers, respectively. Stiffness indices (mean ± SD) were 84.7 ± 16.3, 86.4 ± 18.9, and 90.6 ± 18.0 in nondrinkers, light-to-moderate drinkers, and heavy drinkers, respectively. L-to-M indicates light-to-moderate drinker.

4. Discussion

Although osteoporosis is well known to be common in elderly women, it also is common in elderly men; this high prevalence of osteoporosis in elderly men is increasingly recognized. Fragility fracture in men is also an important health issue. Previous report shows more than one third of all hip fractures occur in men [17]. Compared with women, men have higher rates of 1-year mortality after hip fracture (31%-38% for men vs 12%-28% for women) [18,19] and are twice as likely to be institutionalized [20]. Although many reports have elucidated pathophysiologic characteristics of abnormal bone metabolism in patients with type 2 diabetes mellitus, 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 [2], unchanged [3], or decreased [4] BMD; however, patients with type 2 diabetes mellitus have higher risk of fracture than nondiabetic subjects [6].

Sex steroids are important for bone growth and the maintenance of the skeleton [21]. Hypogonadisim is one of the most important risk factors for osteoporosis in men [22]. Although some studies have reported a weak but significant association between free testosterone and BMD [23], conflicting data have been presented regarding the predictive value of testosterone for BMD in elderly men [11,24]. However, 1 study showed a significant association between low serum testosterone concentration and increased fracture risk in men [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]. In the present study, we found a weak but significant positive correlation between serum bioavailable testosterone concentration and stiffness index in men with type 2 diabetes mellitus. Stiffness index was also correlated significantly with serum free testosterone concentration measured by radioimmunoassay ($r = 0.209$, $P = .0007$). Furthermore, multiple regression analysis also identified serum bioavailable testosterone concentration as a determinant of stiffness index, independently of age and other variables. Diabetes is an important consideration here because men with diabetes have lower serum concentrations of testosterone than nondiabetic men [12,13]. Recent studies suggest that serum estrogen concentrations also influence bone density in men [28]; however, stiffness index was not correlated significantly with total estradiol concentration measured by an electrochemiluminescence immunoassay ($r = 0.086$, $P = .2574$) in this study population. Possible explanations for this discrepancy are that we evaluated bone stiffness—not BMD by dual x-ray absorptiometry (DXA) but stiffness index by QUS—and that our data were not serum free estradiol concentration [29] but serum total estradiol concentration. Moreover, serum estradiol concentrations do not necessarily reflect tissue-level activity, as peripherally formed estradiol is partially metabolized in situ; thus, not all enter the general circulation.

In this study, we evaluated the correlation between stiffness index and other variables including age, duration of diabetes, blood pressure, serum lipid concentration, HbA_{1c}, and BMI. We did not find a significant correlation between stiffness index and HbA_{1c}. 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 hypercalcinuria, may lead to an increase in net bone resorption [31]. A possible explanation of this discrepancy is that stiffness index evaluated by QUS reflects not only the amount of mineral component but also the qualitative properties of the bone [32]. Smoking [33], heavy alcohol consumption [34], and low body weight [35] are generally recognized as the risk factors for osteoporosis and fracture. In keeping with such consensus, we found that stiffness index is less in current smokers than in past smokers or nonsmokers in the present study. Stiffness index tended to positively correlate with BMI, although it did not reach statistical significance; however, stiffness index correlated significantly with body weight ($r = 0.136$, $P = .0216$). No significant correlation was found between stiffness index and alcohol consumption. In this study, stiffness index was significantly less in patients treated than in those not treated with insulin. A lack of endogenous proinsulin and disturbed insulin-like growth factor systems might contribute to bone loss in patients with type 1 diabetes mellitus [36]. Hypercalcinuria has long been noted in patients with poorly controlled insulin-dependent diabetes [37]. Low stiffness index value in patients treated with insulin might be accounted for by these mechanisms.

Clinical management of osteoporosis should be important for the preservation of quality of life in elderly men as well as women with type 2 diabetes mellitus, considering that metabolic derangements resulting from diabetes are related to high risk of fracture. Limitations of the present study include the cross-sectional design and a relatively small number of patients. We could not compare the stiffness index between diabetic men and age-matched nondiabetic men. Furthermore, we have evaluated bone stiffness by QUS rather than DXA. Bone mineral density assessed by DXA is an established marker for osteoporosis; however, in some geographical areas, there are clear limitations in the accessibility to this technique [38]. In recent years, QUS has emerged as a possible alternative to DXA because ultrasound measurement is inexpensive, more convenient than DXA, and free from radiation exposure. Moris et al [39] demonstrated that significant correlation was found between QUS of the calcaneus and DXA of the lumbar spine. Moreover, it has been reported that QUS parameters may reflect not only bone density but also other qualitative properties of bone (elasticity, structure, microarchitecture) [32]. Some previous studies showed the relationship between low QUS parameters and increased fracture risk independently of age and other variables [38].

Despite these limitations, this study, for the first time, has investigated the factors that correlated with bone stiffness evaluated by QUS in men with type 2 diabetes mellitus. Large prospective trials and intervention studies are needed to better assess the factors that influence bone stiffness in men with type 2 diabetes mellitus. In conclusion, bone stiffness was associated with serum bioavailable testosterone concentration but not associated with HbA_{1c} or duration of diabetes in men with type 2 diabetes mellitus.

References

[1] Fyre MA, Melton 3rd LJ, Bryant SC, et al. Osteoporosis and calcification of the aorta. Bone Miner 1992;19:185-94.

[2] van Daele PL, Stork RP, Burger H, et al. Bone density in non–insulin-dependent diabetes mellitus. The Rotterdam Study. Ann Intern Med 1995;122:409-14.

[3] Wakasugi M, Wakao R, Tawata M, Gan N, Koizumi K, Onaya T. Bone mineral density measured by dual energy x-ray absorptiometry in patients with non–insulin-dependent diabetes mellitus. Bone 1993;14: 29-33.

[4] Ishida H, Seino Y, Matsukura S, et al. Diabetic osteopenia and circulating levels of vitamin D metabolism in type 2 (noninsulin-dependent) diabetes. Metabolism 1985;34:797-801.

[5] Buysschaert M, Cauwe F, Jamart J, et al. Proximal femur density in type 1 and 2 diabetic patients. Diabetes Metab 1992;18:32-7.

[6] Lipscombe LL, Jamal SA, Booth GL, Hawker GA. The risk of hip fractures in older individuals with diabetes: a population-based study. Diabetes Care 2007;30:835-41.

[7] Schwartz AV, Hillier TA, Sellmeyer DE, et al. Older women with diabetes have an increased risk of falls: a prospective study. Diabetes Care 2002;25:1749-54.

[8] Forsén L, Meyer HE, Midthjell K, Edna TH. Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trøndelag Health Survey. Diabetologia 1999;42:920-5.

[9] Ramnemark A, Nilsson M, Borssén B, Gustafson Y. Stroke, a major and increasing risk factor for femoral neck fracture. Stroke 2001;31: 1572-7.

[10] Ivers RQ, Cummings RG, Mitchell P, Peduto AJ. Diabetes and risk of fracture: the Blue Mountains Eye Study. Diabetes Care 2001;24: 1198-203.

[11] Center JR, Nguyen TV, Sambrook PN, Eisman JA. Hormonal and biochemical parameters in the determination of osteoporosis in elderly men. J Clin Endocrinol Metab 1999;84:3626-35.

[12] Andersson B, Marin P, Lissner L, Vermeuren A, Bjorntorp P. Testosterone concentrations in women and men with NIDDM. Diabetes Care 1994;17:405-11.

[13] Fukui M, Soh J, Tanaka M, et al. Low serum testosterone concentration in middle-aged men with type 2 diabetes. Endocr J 2007;54:871-7.

[14] The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2002;25:S5-S20.

[15] Yamashita K, Okuyama M, Watanabe Y, Honma S, Kobayashi S, Numazawa M. Highly sensitive determination of estrone and estradiol in human serum by liquid chromatography–electrospray ionization tandem mass spectrometry. Steroids 2007;72:819-27.

[16] Hirota T, Kusu T, Hirota K. Improvement of nutrition stimulates bone mineral gain in Japanese school children and adolescents. Osteoporos Int 2005;16:1057-64.

[17] Cooper C, Campion G, Melton III LJ. Hip fractures in the elderly: a world-wide projection. Osteoporos Int 1992;2:285-9.

[18] Forsen L, Sogaard AJ, Meyer HE, Edna T, Kopiar B. Survival after hip fracture: short- and long-term excess mortality according to age and gender. Osteoporos Int 1999;10:73-8.

[19] Jiang HX, Majumder SR, Dick DA, et al. Development and initial validation of a risk score for predicting in-hospital and 1-year mortality in patients with hip fracture. J Bone Miner Res 2005;20:494-500.

[20] Osnes EK, Lofthus CM, Meyer HE, et al. Consequences of hip fracture on activities of daily life and residential needs. Osteoporos Int 2004;15: 567-74.

[21] Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocr Rev 2004;25:389-425.

[22] Howell S, Shalet S. Testosterone deficiency and replacement. Horm Res 2001;56:86-92.

[23] Murphy S, Khaw KT, Cassidy A, Compson E. Sex hormones and bone mineral density in elderly men. Bone Miner 1993;20:133-40.

[24] Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC. Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. J Clin Invest 1997;100:1755-9.

[25] Leifke E, Wichers C, Gorenoi V, Brabant G. Low serum levels of testosterone in men with minimal traumatic hip fractures. Exper Clin Endocr Diabetes 2005;113:208-13.

[26] Maillefert JF, Sibilia J, Michael F, Saussine C, Javier RM, Tavernier C. Bone mineral density in men treated with synthetic gonadotropin-releasing hormone agonists for prostatic carcinoma. J Urol 1999;161: 1219-22.

[27] Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DL, Anderson EJ, Klinbaski A. Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab 1996;81:4358-65.

[28] Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994; 331:1056-61.

[29] van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 2000;85: 3276-82.

[30] Kayath MJ, Dib SA, Vieiaa JG. Prevalence and magnitude of osteopenia associated with insulin-dependent diabetes mellitus. J Diabetes Complications 1994;97:97-104.

[31] Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM. Bone loss and bone turnover in diabetes. Diabetes 1995; 44:775-82.

[32] Gonnelli S, Cepollaro C, Gennari L, et al. Quantitative ultrasound and dual-energy X-ray absorptiometry in the prediction of fragility fracture in men. Osteoporos Int 2005;16:963-8.

[33] Hannan MT, Felson DT, Dawson-Hughs B, et al. Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res 2000;15:710-20.

[34] Orwoll ES, Bevan L, Phipps KR. Determinants of bone mineral density in older men. Osteoporos Int 2000;11:815-21.

[35] Huuskonen J, Väisänen SB, Kröger H, et al. Determinants of bone mineral density in middle aged men: a population-based study. Osteoporos Int 2000;11:702-8.

[36] Jehle PM, Jehle DR, Mohan S, Böhm BO. Serum levels of insulin-like growth factor system components and relationship to bone metabolism in type 1 and type 2 diabetes mellitus patients. J Endocrinol 1998;159: 297-306.

[37] Gertner JM, Tamborlane WV, Horst RL, Sherwin RS, Felig P, Genel M. Mineral metabolism in diabetes mellitus: changes accompanying treatment with a portable subcutaneous insulin infusion system. J Clin Endocrinol Metab 1980;50:862-6.

[38] Marín F, González-Macías J, Díez-Pélez A, Palma S, Delgado-Rodríguez M. Relationship between bone quantitative ultrasound and fractures: a meta analysis. J Bone Miner Res 2006;21:1126-35.

[39] Moris M, Peretz A, Tjeka R, Negaban N, Wouters M, Bergmann P. Quantitative ultrasound bone measurements: normal values and comparison with bone mineral density by dual x-ray absorptiometry. Calcif Tissue Int 1995;57:6-10.