Int J Clin Exp Med 2014;7(4):1145-1149
www.ijcem.com /ISSN:1940-5901/IJCEM1402045

Original Article

Total osteocalcin in serum predicts testosterone level in male type 2 diabetes mellitus

Ran Cui[1*], Bin Su[1*], Chunjun Sheng[1], Xiaoyun Cheng[1], Peng Yang[1], Le Bu[1], Hong Li[1], Jiying Wang[1], Hui Sheng[1,2], Shen Qu[1]

[1]Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China; [2]Advanced Institute of Translational Medicine, Tongji University, Shanghai 200092, China. [*]Equal contributors.

Received February 15, 2014; Accepted March 28, 2014; Epub April 15, 2014; Published April 30, 2014

Abstract: Objective: To investigate the relationship between total osteocalcin (total OC) and testosterone level in the serum of male patients with type 2 diabetes mellitus (T2DM). Methods: 98 male T2DM were recruited in our cross-section study. Their anthropometric parameters were measured and body mass index (BMI) was calculated. Serum markers including glucose, insulin, HbA1c, testosterone, total OC and other bone metabolic markers were examined. Simple Pearson correlation and multiple regression analysis were performed. Results: Poor level of glucose control was found in the patients (HbA1c 9.19±2.53 and FPG 8.20±2.98). In simple Pearson correlation, total OC was positively related with testosterone (r=0.236, P=0.019), and this relation still existed after considering all the parameters of the patients (Model 1, 2 and 3). Conclusion: In male patients with T2DM, total OC was positively correlated with testosterone, so total OC might predict the testosterone level in the serum.

Keywords: Osteocalcin, undercarboxylated osteocalcin, testosterone, type 2 diabetes mellitus

Introduction

In men, testosterone plays a key role in promoting secondary sexual characteristics and maintaining the function of male reproductive tissues such as the testis and prostate. In addition, testosterone is essential for health and well-being [1] as well as the prevention of osteoporosis [2]. While in type 2 diabetes patients (T2DM), including the disorder of glucose metabolism, there was also a lower testosterone level [3]. However, due to economic cause, testosterone was not a parameter regularly examined in T2DM patients.

Increasing evidence has supported that bone is an endocrine organ in recent years [4]. Osteocalcin (OC) is a very critical factor in the crosstalk between bone and energy metabolism [5], especially the undercarboxylated form. Undercarboxylated OC (ucOC) is able to enhance insulin secretion by β-cells, insulin sensitivity and energy expenditure [6]. Moreover, ucOC also affects the testosterone level. Oury et al did experiments with the co-culture assays and found that ucOC was able to induce testosterone production by the testes [7]. In clinical practice, there was also a positive correlation between testosterone and ucOC [8]. However, total OC is the more frequently examined form of OC in clinical practice rather than ucOC.

If there is a relation between total OC and testosterone level in the serum, it will be very useful to predict the testosterone for the physicians with the total OC level. Moreover most clinical and animal studies show it is ucOC that can regulate the male fertility not the total OC, whether total OC level is related with testosterone level in the serum is still not very clear. So, to investigate the association between total OC and testosterone is both on the benefit of clinical application and further mechanism explore of the bone-derived hormone. Thus a cross section study was performed in T2DM male patients to test the level of total OC and testosterone in the serum and analyze their relationship.

Table 1. The baseline of the subjects

Patients	Value	Normal range
Number	98	
Age (years old)	60.28±13.61	-
Duration (months)	(12.00, 123.00)	-
BMI (Kg/m^2)	25.05±4.34	18.50~24.90
HbA1c (%)	9.19±2.53	4.50-6.30
FPG (mmol/l)	8.20±2.98	3.90-6.10
FINS (mU/L)	(8.24, 22.88)	2.60-24.90
Testosterone (nmol/L)	13.51±6.89	9.90-27.80
FSH (IU/L)	8.52±4.88	1.50-12.40
LH (ng/ml)	6.99±3.75	1.70-8.60
TOC (ng/ml)	11.31±3.85	14.00-46.00
CTX (ng/ml)	(0.21, 0.55)	<0.85

Duration: duration of type 2 diabetes mellitus; BMI: body mass index; FPG: fasting plasma glucose; FINS: fasting insulin level; FSH: follicle-stimulating hormone; LH: *luteinizing hormone; TOC: total osteocalcin; CTX*: C-terminal telopeptide of type I collagen. Duration, FINS and CTX were not followed the normal distribution,they were expressed as quantile, others were showed as mean ± SD.

Subjects and methods

Subjects

We screened 40-75 years old male patients with T2DM who visited in our hospital from January 1st 2011 to December 1st 2011. Some of them were excluded as follows: 1) those with liver, heart or kidney function failure and osteoporosis; 2) with conditions known to affect testosterone such as hypogonadism; 3) using drugs that would affect osteocalcin such as vitamin D and bisphosphonate. At last, 98 male patients were enrolled in our cross section study. Human investigation was conducted according to the principles expressed in the Declaration of Helsinki. All subjects agreed to participate in the study and gave written informed consent and the protocol was approved by the Ethics Committee of Shanghai Tenth People's Hospital of Tongji University, School of Medicine.

Anthropometric measurements

Weight measured with a plethysmography scale was scaled when patients wore minimal clothing and height was measured with a stadiometer. Body mass index (BMI) was calculated by dividing the weight by height squared (kg/m^2).

Serum biomarkers

After overnight fasting, serum was collected. Biochemical markers, including fasting plasma glucose (FPG) and fasting insulin level (FINS) were measured with standard biochemical methods. Bone metabolic markers including C-terminal telopeptide fragments of type I collagen (CTX) and parathyroid hormone (PTH) were measured with an ELISA (Roche) method. Testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were examined with Elecsys and Cobas analyzers (Roche) according to the manufacturer's instructions.

Serum total OC was measured using an N-MID Osteocalcin ELISA kit (Elecsys, Roche diagnostic Ltd., Switzerland). Intact osteocalcin is unstable due to protease cleavage between amino acids 43 and 44. The N-MID-fragment, resulting from cleavage, is considerably more stable. This assay detects the stable N-MID-fragment.

Testosterone in blood is present as free testosterone (FT) (1-4%) or bound testosterone. The bound testosterone is loosely bound to albumin or to sex hormone binding globulin (SHBG) (60-70%). The term bioavailable testosterone (BAT) refers to the sum of free testosterone plus albumin-bound testosterone, testosterone bound to SHBG is biologically inactive because of the strong affinity between SHBG and testosterone. The Elecsys Testosterone II assay is based on a competitive test principle using a high affinity monoclonal antibody (sheep) specifically directed against testosterone. Endogenous testosterone released from the sample by 2-bromoestradiol competes with the added testosterone derivative labeled with a ruthenium complex for the binding sites on the biotinylated antibody. (http://www.roche-diagnostics.cz).

Statistical analysis

Nonparametric tests (Kolmogorov-Smimo test) were used to examine whether the baseline parameters were followed with normal distribution. If they followed the normal distribution, they were expressed by mean ± SD, otherwise, and they were shown as quantile. Pearson correlation was used to analyze the relation between testosterone or osteocalcin and other parameters in the male T2DM patients. Multiple

Table 2. Simple Pearson correlation between testosterone (A) or total OC (B) in the serum and other parameters in T2DM

A		
	Testosterone	
	r	P
Age	-0.144	0.156
Log (Duration)	-0.029	0.790
BMI	-0.186	0.077
HbA1c	0.005	0.963
FPG	-0.022	0.839
Log (FINS)	-0.068	0.523
FSH	0.173	0.111
LH	0.197	0.070
TOC	0.236	0.019
Log (CTX)	-0.037	0.732
B		
	TOC	
	r	P
Age	-0.116	0.254
Log (Duration)	-0.111	0.302
BMI	-0.051	0.633
HbA1c	-0.209	0.048
FPG	-0.171	0.104
Log (FINS)	0.064	0.551
FSH	0.034	0.755
LH	0.007	0.949
Testosterone	0.236	0.019
Log (CTX)	0.325	0.002

Duration, FINS and CTX were not followed the normal distribution, so we transformed them with logarithm.

linear regression analysis was used to set up the models between testosterone and other parameters. Statistical Product and Service Solutions 17.0 (SPSS 17.0) were used for all analyses. Differences were considered to be statistically significant where $P < 0.05$.

Results

Basic parameters in the enrolled patients

In these 40-75 years old male patients, the duration of T2DM was from months to years (12.00, 123.00). Poor glucose level was found (HbA1c 9.19±2.53 and FPG 8.20±2.98), while the insulin level of most of them was nearly in the normal range (8.24, 22.88). The average figure of this population was overweight or obesity (BMI: 25.05±4.34). Testosterone (13.51±6.89), FSH (8.52±4.88) and total OC (11.31±3.85) were in lower grade while LH (6.99±3.75) was near the upper layer in the subjects. CTX was in normal range (0.21, 0.55) (**Table 1**).

Table 3. Multiple linear regression analysis of testosterone and other parameters

	R^2	P
Model 1	0.089	0.011
Model 2	0.076	0.048
Model 3	0.144	0.012

Model 1: age, BMI and TOC were selected. Model 2: age, BMI, Duration, HbA1c and TOC were selected. Model 3: age, BMI, Duration, HbA1c, FSH, LH and TOC were selected.

Correlation between testosterone level and other parameters in male T2DM

In the relation between testosterone and other parameters, simple Pearson correlation analysis showed testosterone level was only related with total OC in the serum (**Table 2A**: r=0.236, P=0.019). While, total OC was also positive related with log (CTX) (**Table 2B**: r=0.325, P=0.002) (**Table 2**).

The relationship between testosterone and total OC was enhanced by the multi-linear regression analysis. Total OC was positively associated with testosterone level in the serum whatever in the model of patients' instinct state (age and BMI involved in Model 1: R^2=0.089, P=0.011), glucose control condition (age, BMI, Duration and HbA1c involved in Model 2: R^2=0.076, P=0.048) or other related hormones (age, BMI, Duration, HbA1c, FSH and LH involved in Model 3: R^2=0.144, P=0.012) (**Table 3**).

Discussion

As compared with the normal range of healthy persons, the subjects recruited in our study manifested higher FPG and HbA1c level, which was consistent with most T2DM reported in China [9]. So we concluded that these subjects could represent the most T2DM individuals of our country.

In our results, testosterone was only related with total OC among all the parameters. Theoretically, testosterone will be regulated by

FSH and LH, and then a negative relation should be found between testosterone and FSH or LH. Dhindsa et al. found T2DM patients revealed a lower LH and FSH level than those in control group; they concluded a hypothalamic rather than a pituitary defect in the patients [10]. As the previous studies, testosterone might associate with age [11] and BMI [12], FPG [13] and HbA1c [14], while, we did not observe these relations. We inferred in this cross section study, the parameters could not be balanced well, for example, the duration of the T2DM had significant difference. And the sample size was not large enough to get statistical results, since all of the studies that demonstrate the interrelations were much more than 100 recruiters. Except testosterone, total OC was negatively associated with log (CTX). Type I collagen is also secreted by osteoblasts as well as OC and degraded into CTX in the bone matrix, so it reflects the level of bone turnover. Thus, the OC has a parallel declination with CTX. Although ucOC was negative with HbA1c or FPG, the relation between total OC and the glucose metabolism did not get an agreement [15].

Total OC, a small γ-carboxyglutamate protein preferentially expressed by mature osteoblasts, which comprises both the carboxylated and the undercarboxylated forms, has been utilized traditionally as a marker of bone formation or bone turnover. The carboxylated OC binds the calcium ions after release in the bone matrix, which results in the mineralization of the bone [16]. For the ucOC form, recent studies have found it could increase the insulin secretion and sensitivity [6] and at the same time insulin and its receptor could regulate bone acquisition and remodeling [17, 18], so ucOC has been considered to play an important role in the crosstalk between bone and energy metabolism. For the phenomenon that testosterone was positively associated with total OC in our study, there would be three explanations. One explanation was that osteocalcin in the serum could affect the testosterone. Among cultured mouse primary Leydig cells in the presence or absence of supernatants of osteoblast cultures or other mesenchymal cell types cultures, they found only osteoblasts could increase the production of testosterone, moreover, osteocalcin was the regulator in the process [7]. Another explanation for the clinical phenomenon was that testosterone affected osteocalcin. Testosterone deficiency could induce bone loss and fragile fracture in senile [19]. Under this condition, the OC secretion of osteoblasts would be affected. At the same time, as the study of ours were a cross section study, we could not exclude that there was other parameter existed that could both affect testosterone and osteocalcin, an example for the parameter was blood glucose level. Low testosterone [3] and low total OC [20] were all reported in T2DM patients. But we found no linear relation between glucose level and testosterone or osteocalcin. So maybe the third consideration could not be supported in the population we studied.

There were some limitations for our study. First, since we focused on the relation between testosterone and total OC, we just did the investigation in patients with diabetes, and we did not examine the population with normal glucose level. Second, there have already been studies about ucOC influencing on testosterone, we did not test the ucOC in the serum as a comparison. Lastly, the number of population is not very large, but after the reference literature review, 98 patients were enough to get the conclusion logically.

Acknowledgements

This paper was supported by China Sholarship Council (File No. 201306260063), NSFC (30900698) and Ph.D. Programs Foundation of Ministry of Education of China (20090072120020), and Shanghai Municipal Natural Science Foundation (13ZR1432100).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hui Sheng, Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China; Advanced Institute of Translational Medicine, Tongji University, Shanghai 200092, China. E-mail: shenghui@tongji.edu.cn; Dr. Shen Qu, Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China. E-mail: qushencn@hotmail.com

References

[1] Spitzer M, Basaria S, Travison TG, Davda MN, DeRogatis L and Bhasin S. The effect of testosterone on mood and well-being in men with erectile dysfunction in a randomized, placebo-controlled trial. Andrology 2013; 1: 475-482.

[2] Mosekilde L, Vestergaard P and Rejnmark L. The pathogenesis, treatment and prevention of osteoporosis in men. Drugs 2013; 73: 15-29.

[3] El Baba K and Azar ST. Low testosterone and diabetes. Curr Diabetes Rev 2013; 9: 418-421.

[4] Fukumoto S and Martin TJ. Bone as an endocrine organ. Trends Endocrinol Metab 2009; 20: 230-236.

[5] Neve A, Corrado A and Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. J Cell Physiol 2013; 228: 1149-1153.

[6] Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P and Karsenty G. Endocrine regulation of energy metabolism by the skeleton. Cell 2007; 130: 456-469.

[7] Oury F, Sumara G, Sumara O, Ferron M, Chang H, Smith CE, Hermo L, Suarez S, Roth BL, Ducy P and Karsenty G. Endocrine regulation of male fertility by the skeleton. Cell 2011; 144: 796-809.

[8] Kanazawa I, Tanaka K, Ogawa N, Yamauchi M, Yamaguchi T and Sugimoto T. Undercarboxylated osteocalcin is positively associated with free testosterone in male patients with type 2 diabetes mellitus. Osteoporos Int 2013; 24: 1115-1119.

[9] Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G and He J. Prevalence of diabetes among men and women in China. N Engl J Med 2010; 362: 1090-1101.

[10] Dhindsa S, Prabhakar S, Sethi M, Bandyopadhyay A, Chaudhuri A and Dandona P. Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. J Clin Endocrinol Metab 2004; 89: 5462-5468.

[11] Biswas M, Hampton D, Newcombe RG and Rees DA. Total and free testosterone concentrations are strongly influenced by age and central obesity in men with type 1 and type 2 diabetes but correlate weakly with symptoms of androgen deficiency and diabetes-related quality of life. Clin Endocrinol (Oxf) 2012; 76: 665-673.

[12] Dhindsa S, Miller MG, McWhirter CL, Mager DE, Ghanim H, Chaudhuri A and Dandona P. Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes Care 2010; 33: 1186-1192.

[13] Shin JY, Park EK, Park BJ, Shim JY and Lee HR. High-normal Glucose Levels in Non-diabetic and Pre-diabetic Men Are Associated with Decreased Testosterone Levels. Korean J Fam Med 2012; 33: 152-156.

[14] Ho CH, Yu HJ, Wang CY, Jaw FS, Hsieh JT, Liao WC, Pu YS and Liu SP. Prediabetes is associated with an increased risk of testosterone deficiency, independent of obesity and metabolic syndrome. PLoS One 2013; 8: e74173.

[15] Hwang YC, Jee JH, Jeong IK, Ahn KJ, Chung HY and Lee MK. Circulating osteocalcin level is not associated with incident type 2 diabetes in middle-aged male subjects: mean 8.4-year retrospective follow-up study. Diabetes Care 2012; 35: 1919-1924.

[16] Hoang QQ, Sicheri F, Howard AJ and Yang DS. Bone recognition mechanism of porcine osteocalcin from crystal structure. Nature 2003; 425: 977-980.

[17] Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P and Karsenty G. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 2010; 142: 296-308.

[18] Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, Faugere MC, Aja S, Hussain MA, Bruning JC and Clemens TL. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell 2010; 142: 309-319.

[19] Vanderschueren D, Sinnesael M, Gielen E, Claessens F and Boonen S. Testosterone and bone. Testosterone: Action, Deficiency, Substitution 2012; 177.

[20] Kim SH, Lee JW, Im JA and Hwang HJ. Serum osteocalcin is related to abdominal obesity in Korean obese and overweight men. Clin Chim Acta 2010; 411: 2054-2057.