

Positive Correlation between Serum Osteocalcin and Testosterone in Male Hyperthyroidism Patients with High Bone Turnover

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Abstract



Purpose: Animal studies suggested that there is an independent bone-osteocalcin-gonadal axis, except of the hypothalamic-pituitary-gonadal axis. Based on this hypothesis, the higher osteocalcin during the high bone turnover should be followed by higher testosterone formation. Yet such clinical evidence is limited. The patients with uncontrolled hyperthyroidism are proper model with high bone turnover. If this hypothesis is true, there should be high testosterone level in patients with uncontrolled hyperthyroidism. Therefore, Graves' disease patients were recruited to study the correlation between osteocalcin and testosterone.

Materials and Methods: 50 male hyperthyroidism patients with Graves' disease and 50 health persons matched by age and gender were enrolled in our cross-section study. Serum markers for thyroid hormone, sex hormone and bone metabolic markers including free tri-

iodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and osteocalcin (OC), C-terminal telopeptide fragments of type I collagen (CTX) were examined. The demographic parameters such as duration of disease were also collected. All data was analyzed by SPSS 20.0.

Results: High testosterone and osteocalcin level was observed in the hyperthyroidism patients (T 36.35±10.72 nmol/l and OC 46.79±26.83 ng/ml). In simple Pearson correlation, testosterone was positively associated with OC ($r=0.486$, $P<0.001$), and this positive relation still existed after adjusted for age, BMI, smoking, drinking, duration of disease, FT3, FT4, LH, FSH, CTX in multi-linear regression analysis (See Model 1–4).

Conclusion: In male hyperthyroidism patients, osteocalcin was positively correlated with serum testosterone, which indirectly supports the hypothesis that serum osteocalcin participates in the regulation of sex hormone.

Introduction



It is well known that testosterone in male is mainly controlled by hypothalamus-pituitary-testis axis, in which circulating levels of luteinizing hormone (LH), a pituitary hormone, acts as an primary upstream regulators of sex steroid [1–3]. However, a round-new finding in animal studies showed that osteocalcin (OC), synthesized and secreted by osteoblasts, could regulate male reproductive functions by promoting testosterone biosynthesis in Leydig cells [4–6], which suggests there is a pancreas-bone-testis axis. Oury's study found that osteocalcin-null (Osteocalcin^{-/-}) male mice's testosterone was lower than wild type(WT) mice. To further iden-

tify whether osteocalcin has positive influence to testosterone, Oury et al. injected osteocalcin to Osteocalcin^{-/-} mice, and then found that the sperm count, testis, epididymal and seminal vesicle weights normalized, and circulating testosterone levels was higher after Osteocalcin^{-/-} mice injected with OC, which suggests osteocalcin can promote testosterone production [4]. Additionally, accumulating evidence in recent years has come up with that skeleton is an endocrine organ [7–9]. It is said that osteocalcin not only regulate bone mineral metabolism, but also participate in lipid metabolism and promote glucose homeostasis by acting in various tissues, such as muscle, liver and fat playing a role in metabolic syndrome [8,9]. What's more, sex steroid hormones play a crucial role during the bone growth spurts of puberty, and for maintenance of bone mass. In view of feedback theory, bone will in turn act on

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gonads. An epidemiological study including 1338 men found that osteocalcin is positively associated with testosterone in general population [10]. Another clinical study also demonstrated that total osteocalcin in serum could predict testosterone level in male type 2 diabetes mellitus [11]. However, more clinical evidence is needed to support this hypothesis.

Osteocalcin, usually employed as bone-forming parameter during bone turnover, was synthesized and secreted by osteoblasts, stored in the bone matrix and can be released into blood. In condition of high bone turnover such as postmenopausal women and hyperthyroidism patients, serum osteocalcin will elevate. Hyperthyroidism is the most common clinical model with high bone turnover. If there is a pancreas-bone-testis axis, there must be a positive relationship between osteocalcin and testosterone. Therefore, a cross section study was performed in male patients with hyperthyroidism to identify whether circulating levels of total testosterone is related with osteocalcin. And then the mechanism how osteocalcin promote testosterone biosynthesis or regulate male reproductive functions will be further explored.

Materials and Methods

Subjects

This study was conducted in the Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine from July 2014 to July 2015. Fifty male patients diagnosed as Graves' disease (Age: 48.96 ± 5.86 years), were recruited from the inpatients department of Shanghai Tenth People's Hospital. Fifty non-hyperthyroidism male participants matched by age and gender, were taken as control.

Inclusion criteria and exclusion criteria

Inclusion criteria: 1) male patients first diagnosed as Graves' disease, $FT3 \geq -6.5$ pmol/l (normal range 3.5–6.5 pmol/l), $FT4 \geq 31$ pmol/l (normal range, 10.2–31 pmol/l), $TSH \leq 0.35$ mU/L (normal range 0.35–5.5 mU/L). Exclusion criteria: 1) the patients with isotope therapy; 2) the patients taking drugs affecting bone metabolism such as anti-osteoporosis agents: bisphosphonates, SERMs, calcitonin, androgen, strontium and parathyroid hormone; 3) the patients with primary or secondary hypogonadism; 4) the patients with diseases affecting bone metabolism, such as recent fracture, chronic liver or renal insufficiency and hyperparathyroidism. Finally, 50 male patients were enrolled in this study. Human investigation was performed according to the principles of the Declaration of Helsinki. All subjects agreed to participate in the study with written informed consent and the protocol was approved by the Ethics Committee of Shanghai Tenth People's Hospital, Tongji University School of Medicine.

The demographic parameters

Age, duration of disease, Weight, height, alcohol use and smoking history was collected. And body mass index (BMI) was calculated by dividing the weight by height squared (kg/m^2). Current smoking was defined as having smoked 100 cigarettes in one's lifetime and currently smoking cigarettes [12]. Current drinking was defined as alcohol intake more than once per month during the past 12 months [12].

Serum biomarkers

Fasting blood samples was collected after overnight (early in the morning after an overnight fast, with the last meal generally

10h before the blood draw). Thyroid hormone, including $FT3$, $FT4$ and TSH concentration were examined with standard biochemical methods. Bone metabolic markers including C-terminal telopeptide fragments of type I collagen (CTX) and parathyroid hormone (PTH), as well as sex hormone, including testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were tested with electrochemical luminescence (ELISA) method by Roche cobase601 immune analyzer. Serum total OC was measured using its more stable breakdown fragment form, the large N-terminal-mid-region fragment (N-Mid fragment; amino acids 1–43), for intact osteocalcin is unstable. The stable N-MID-fragment was measured using an N-MID Osteocalcin ELISA kit (Elecsys, Roche diagnostic Ltd., Switzerland). Testosterone in blood was tested by The Elecsys Testosterone II assay based on a competitive test principle using a high affinity monoclonal antibody (sheep) specifically directed against testosterone. (<http://www.roche-diagnostics.cz>).

Statistical analysis

The normality of the distribution was tested by the Kolmogorov-Smirnov statistical. Data were presented as mean \pm standard deviation (SD). Comparisons of baseline demographic and biochemical parameters between groups were done by paired Student's t-test. The correlation between testosterone and osteocalcin or other parameters in the male hyperthyroidism patients was analyzed with Pearson correlation or multiple linear regression analysis. All analysis was calculated by Statistical Product and Service Solutions 20.0 (SPSS 20.0). Statistical significance was set at $P < 0.05$.

Results

Comparisons of basic parameters between the hyperthyroidism and control group

The baseline anthropometric and biochemical characteristics of hyperthyroidism and control group were presented in **Table 1**. In these male hyperthyroidism patients, the population of currently smoking account for 56% ($n=28$) and currently drinking account for 22% ($n=11$). Corresponding ratio among the control group is separately 54% ($n=27$) and 26% ($n=13$). The hyperthyroidism group had significantly higher thyroid hormone ($P < 0.001$) and lower TSH ($P < 0.001$) than the control group, which was consistent with the diagnostic criteria of hyperthyroidism patients. The other adjusted variable for testosterone like PTH, VitD, LH and FSH had no significant difference between the 2 groups. The average BMI of this population was smaller (BMI: 21.56 ± 2.75 , $P=0.001$) than the control. In addition, total cholesterol (TC 3.44 ± 0.94 mmol/l, $P < 0.001$) were lower than the control. Whereas the bone metabolism markers like CTX (2.01 ± 2.31 ng/ml vs. 0.42 ± 0.27 ng/ml; $P < 0.001$) and osteocalcin levels (46.79 ± 26.83 ng/ml vs. 13.39 ± 5.23 ng/ml; $P < 0.001$) as well as the male fertility marker testosterone (36.35 ± 10.72 nmol/l vs. 13.63 ± 5.79 nmol/l; $P < 0.001$) were significantly higher in the hyperthyroidism group (**Table 1**).

Association between testosterone level and other parameters in male hyperthyroidism

In the simple Pearson correlation analysis, the correlation between testosterone and respective parameter showed that testosterone level was negatively related with age ($r = -0.354$, $P = 0.012$), and positively related with OC ($r = 0.486$, $P < 0.001$)

Table 1 Comparison of the baseline between the hyperthyroidism group and the control group.

Patients	hyperthyroidism group	control group	P
Number	50	50	
Age (years old)	48.96 ± 5.86	48.96 ± 5.86	
Current smoking (n/%)	28 (56%)	27 (54%)	
Current drinking (n/%)	11 (22%)	13 (26%)	
Duration (years)	6.06 ± 9.23	0	
BMI (kg/m ²)	21.56 ± 2.75	26.25 ± 6.46	0.001 *
TC (mmol/l)	3.44 ± 0.94	4.93 ± 1.35	<0.001 *
Osteocalcin (ng/ml)	46.79 ± 26.83	13.39 ± 5.23	<0.001 *
CTX (ng/ml)	2.01 ± 2.31	0.42 ± 0.27	<0.001 *
25 (OH)VitD (mmol/l)	57.3 ± 20.32	56.46 ± 19.64	>0.05
PTH (pmol/l)	4.22 ± 2.67	4.06 ± 2.64	>0.05
Testosterone (nmol/l)	36.35 ± 10.72	13.62 ± 5.79	<0.001 *
FSH (IU/L)	9.63 ± 7.12	9.09 ± 8.05	>0.05
LH (ng/ml)	10.65 ± 5.57	7.89 ± 4.92	>0.05
FT3 (pmol/l)	15.67 ± 8.42	4.88 ± 0.59	<0.001 *
FT4 (pmol/l)	43.07 ± 28.39	16.84 ± 3.50	<0.001 *
TSH (Mu/l)	0.40 ± 1.49	1.71 ± 0.85	<0.001 *

* * *: the difference has statistical significance. BMI: Body mass index; TC: Total Cholesterol; CTX: C-terminal telopeptide fragments of type I collagen; PTH: parathyroid hormone; FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone; FT3: Free thyroxine; FT4: Free Thyroxine; TSH: Thyroid Stimulating Hormone

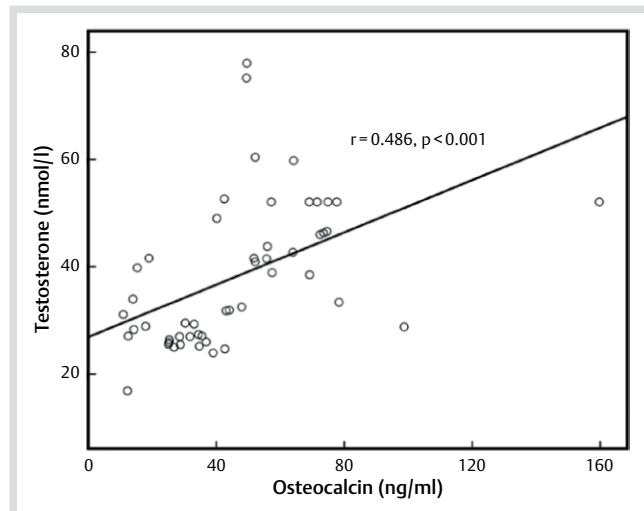


Fig. 1 The association between testosterone and osteocalcin in hyperthyroidism group. In simple Pearson correlation, testosterone was positively associated with Osteocalcin ($r = 0.486$, $P < 0.001$).

(**Fig. 1**), FT3 ($r = 0.59$, $P = 0.000$) and FT4 ($r = 0.575$, $P < 0.001$) in the serum (**Table 2a**), but not related with smoking, drinking, BMI, duration of disease, PTH, CTX, VitD and TSH ($P < 0.05$). While in the correlation between OC and respective parameter, OC was also positive related with serum testosterone ($r = 0.486$, $P < 0.001$), FT3 ($r = 0.464$, $P = 0.003$) and FT4 ($r = 0.401$, $P = 0.013$) and negative related with age ($r = -0.523$, $P = 0.001$) (**Table 2b**). The correlation between testosterone and OC was strengthened by the multi-linear regression analysis. OC was positively related with serum testosterone concentration whichever in the model of patients' anthropometric state (Age, BMI, Current smoking, Current drinking and OC involved in Model 1: $R^2 = 0.428$, $P = 0.002$), thyroid hormone (Age, BMI, Current smoking, Current drinking, Duration of disease, FT3, FT4 and OC involved in Model

2: $R^2 = 0.587$, $P < 0.001$), related sex hormones (Age, BMI, Current smoking, Current drinking, Duration of disease, FT3, FT4, FSH, LH and OC involved in Model 3: $R^2 = 0.409$, $P = 0.015$), or other bone metabolism markers (Age, BMI, Current smoking, Current drinking, Duration of disease, FT3, FT4, FSH, LH, CTX and OC involved in Model 4: $R^2 = 0.454$, $P = 0.008$) (**Table 3**).

Discussion

Compared with the control group, the bone resorption marker CTX and the bone formation marker osteocalcin in the serum were significantly higher in the hyperthyroidism group ($p < 0.05$), which suggested male hyperthyroidism patients in this assay were indeed with high bone turnover. In addition, total testosterone level is higher in the hyperthyroidism group than the control.

In this study, testosterone was negatively correlated with age, and positively related with serum total OC, CTX, FT3 and FT4 in simple Pearson correlation analysis, but in multi-linear regression analysis from model 1 to model 4, testosterone was only positively correlated with serum total OC. It is reported that testosterone will decline with aging [13], increasing BMI [14] and alcohol use [15] and that smoking will increase testosterone production [16]. We only observed that there is a relationship between testosterone and age in simple Pearson correlation analysis (**Table 2a**), and that testosterone was only positively correlated with serum total OC after adjusted for age, BMI, smoking, drinking, duration of disease as well as OC in multi-linear regression analysis (**Table 3 – Model1**). Some studies revealed that testosterone might relate with FT3 and FT4 in hyperthyroidism patients [17]. We indeed observed it had some relationship with FT3 and FT4, when simple Pearson correlation analysis was performed in our study (**Table 2a**). To adjust the influence of thyroid hormone itself to testosterone, FT3, FT4 along with age, BMI, smoking, drinking, duration of disease and OC were induced in multi-linear regression analysis, testosterone was only positively correlated with serum total OC (**Table 3 – Model2**). In theory, testosterone is regulated by FSH and LH, so a negative association should be found between testosterone and LH or FSH, whereas such association was not observed in the face of high serum level of osteocalcin in our study (**Table 3 – Model3**). It is well known that bone metabolism markers contain bone formation marker OC and bone resorption marker CTX. However, the association between serum testosterone and CTX had no statistical significance in simple Pearson correlation analysis (**Table 2a**). In addition, when CTX was considered by model 4 in the multiple regression analysis, serum OC instead of CTX had statistical significance.

Taking the above together, we infer in our study that when OC was high, it would play a primary role in mediating testosterone biosynthesis and male reproductive functions regardless of the mediation function of LH, FT3 and FT4 to testosterone. What's more, our findings of significant association between testosterone and osteocalcin level in the face of LH (**Table 3 – Model3**) were consistent with previous report in mice [4] and with some other recent studies [18]. For example, in human, lower osteocalcin level had been shown to be associated with lower testosterone in different energy metabolism diseases [11, 19]. However, some studies didn't agree with our study. For example, a study in men from infertile couples maintained that osteocalcin was not a strong determinant of serum testosterone and sperm count

A	T		B	OC	
	r	P		r	P
Age	-0.354	0.012 *	Age	-0.458	0.001 *
Current smoking	0.175	0.225	Current smoking	0.125	0.389
Current drinking	-0.093	0.521	Current drinking	0.032	0.828
Duration	-0.278	0.052	Duration	-0.208	0.148
BMI	-0.057	0.728	BMI	-0.13	0.424
TC	-0.105	0.489	TC	0.014	0.924
OC	0.486	<0.001 *	T	0.486	<0.001 *
CTX	0.228	0.123	CTX	0.285	0.052
PTH	-0.241	0.120	PTH	-0.102	0.514
25(OH)VitD	0.120	0.507	25 (OH)VitD	0.122	0.500
FT3	0.59	<0.001 *	FT3	0.464	0.003 *
FT4	0.575	<0.001 *	FT4	0.401	0.013 *
TSH	-0.203	0.229	TSH	-1.141	0.262
LH	0.089	0.595	LH	-0.026	0.877
FSH	-0.273	0.108	FSH	-0.272	0.109

* * *: The association has statistical significance. OC: Osteocalcin; T: testosterone

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

Table 3 Multiple linear regression analysis between testosterone and osteocalcin as well as other parameters: Except for osteocalcin, other parameters also involved in different Models were as follow. In the liner regression analysis, testosterone was only positively associated with Osteocalcin in male hyperthyroidism patients and other parameters have no statistical significance in Model 1–4).

	R ²	P
Model1	0.428	0.002
Model2	0.587	<0.001
Model3	0.409	0.015
Model4	0.454	0.008

Model 1: Age, BMI, Current smoking, Current drinking and osteocalcin

Model 2: Model1 + Duration of disease, FT3, FT4, TSH

Model 3: Model2 + FSH, LH

Model 4: Model3 + CTX

[20]. Another study conducted in 614 older male Dutch population (65–88 years) indicated that serum osteocalcin was negatively associated with free and bioavailable testosterone and positively with luteinizing hormone levels [21]. The inconsistency might be related to different ethnic groups and different study population such as the different age, diseases and other reasons. More specifically, the opinions about higher testosterone levels in patients with hyperthyroidism were controversial. Some suggested that the reason was the increased synthesis of sex hormone binding globulin (SHBG) in the liver cells of hyperthyroidism patients [22], which makes the clearance rate of testosterone slow down. Some indicated that the reason was the increased gonadotropin concentration like LH and FSH, for the unreduced function of hypothalamic-pituitary-gonadal axis in patients with hyperthyroidism [23], which was not consistent with our study. In addition, some demonstrated that the reason was the enhanced degradation rate of cholesterol to steroid hormone, which was promoted by thyroid hormone, since steroids could convert into testosterone in adrenal [24]. Lastly, other studies considered the reason was that the thyroid hormone directly acted on the ovary, which increased the secretion of testosterone by ovaries.

Another explanation for the above clinical result was that testosterone affected osteocalcin. Testosterone elevation could promote bone formation and remodeling in senile [25]. Under this process, the OC secretion of osteoblasts would correspondingly increase. In the meantime, as our study was a cross section

study, we were not able to exclude that there were other parameters existed that could both influence testosterone and osteocalcin, such as the parameter FT3 and FT4. High testosterone and high total OC were all reported in hyperthyroidism patients. The respective relationship of testosterone or total OC with FT3 or FT4 was indeed observed in our study. However, when total OC, FT3 or FT4 were all considered in the multi-linear regression analysis, we found testosterone has no linear association with FT3 or FT4 in the exist of total OC, so the third consideration could not also be supported in the population we studied.

The underlying mechanism about how osteocalcin affects testosterone was unclear in human but relatively clear in animal studies. The mechanism was that osteocalcin, in parallel to and independent of the hypothalamus-pituitary-testis axis, via a pancreas-bone-testis axis, promotes testosterone biosynthesis and thus regulates male reproductive functions [26,27]. In animal studies, Osteocalcin binds to its receptor Gprc6a on Leydig cells, which favors cAMP production and then induce the activation of the transcription factor CREB (cAMP response element binding). CREB triggers the expression of several genes, like-Cyp11a, StAR, 3b-HSD andCyp17, encoding the enzymes that are essential to testosterone biosynthesis [28]. Thus osteocalcin has been increasingly considered to play a critical role in the cross-talk between bone and male fertility. But the undercarboxylated form of osteocalcin (ucOC) might be the main factor acting on Leydig cells as an upstream regulator of reproductive function by favoring testosterone biosynthesis in mice [4], since osteocalcin consisted of both the carboxylated and undercarboxylated form [26] and only ucOC was active in metabolism. However, studies which analyzed this association in human or in male hyperthyroidism patients under the condition of high bone turnover or with high serum level of osteocalcin were limited. Therefore, further prospective studies are needed to verify whether osteocalcin level plays a primary role in the reproductive function of male.

Some limitations still exist in our study. First, the sample size is relatively small. Since some of these discrepancies may due to the underlying differences in individual study population, measurement method and adjustment for potential confounders. Second, serum total osteocalcin is composed of uncarboxylated and carboxylated forms. There have already been animal studies reporting uncarboxylated osteocalcin (ucOC) appears to be the active form and the form of affecting on testosterone [18,27].

However, it is said total OC and ucOC are often with a certain proportion. And several recent clinical studies have demonstrated that not only undercarboxylated but also total osteocalcin were associated with energy metabolism and testosterone as well [11]. Since lack of an automated assay to examine the undercarboxylated form, our study only measured serum total osteocalcin. Thirdly, we'd better introduce sex hormone binding globulin (SHBG) as an adjust parameter for testosterone, for that testosterone in blood composes of free testosterone (1–4%) and bound testosterone, and the latter loosely combine with SHBG. In our future study, we will surely measure free testosterone, SHBG and undercarboxylated osteocalcin so as to get a more scientific conclusion. Lastly, we should further emphasize the nature of our cross-sectional study and thus no inferences of causality can be made.

Conclusion

In conclusion, the present study suggests that testosterone was positively correlated with serum osteocalcin in male hyperthyroidism patients, which indirectly supported the topic that serum osteocalcin participated in the regulation of sex hormone.

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