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Original Article

Correlation between osteocalcin and visceral fat area in overweight and obese male population

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Abstract: Objective: To investigate the correlation between serum level of osteocalcin (OC) and visceral fat area (VFA) in overweight and obese male subjects. Methods: A total of 70 men aged from 35 to 75 (mean 52.13±7.21) years were enrolled during January 2015 to December 2016, which were divided into control group (n=26), overweight group (n=20) and obese group (n=24) according to body mass index (BMI). Metabolic parameters, including waist circumference (WC), fasting insulin (FINS), fasting blood glucose (FBG), insulin resistance index, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and serum levels of OC, C reactive protein (CRP), adiponectin (APN) and visceral fat area (VFA), were evaluated before and after the treatment and the differences between groups were compared. The insulin resistance index was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) while visceral fat area (VFA) was quantified via magnetic resonance imaging. Results: OC had a decreasing trend in the control group (11.45±2.51 μg/L) and overweight group (6.73±2.31 μg/L) and obese group (4.25±1.29 μg/L) ($P<0.01$); among all the overweight and obese subjects, WC, HOMA-IR and VFA were independent factors of OC ($P<0.01$). Conclusion: With different levels of visceral fat content, the variation of OC in overweight and obese male subjects was closely related to VFA, indicating that OC might participate in the development of abdominal obesity.

Keywords: Metabolic parameters, obesity, visceral fat, osteocalcin

Introduction

Obesity is divided into peripheral obesity and abdominal obesity in accordance with the distribution of fat in the body. Abdominal fat is composed of abdominal fat (subcutaneous fat) and intra-abdominal fat (visceral fat), thereby it is also known as central obesity or visceral obesity. Especially, metabolic activity of visceral fat is higher than that of subcutaneous fat, which is closely related with the occurrence of hypertension and glycolipid metabolic disorders, and it accelerates the formation of arteriosclerosis and promotes cardiovascular diseases [1]. In the previous study, OC was considered as a marker of bone formation and bone turnover, additionally, it has been shown to be closely related to the secretion of insulin and adiponectin, and abdominal obesity [2-6]. For example, The Shanghai Six Hospital performed the abdominal visceral fat area quantitative determination via magnetic resonance imaging (MRI), confirming that the serum OC level and visceral fat area in Chinese male population were negatively correlated [3]. Here, this research is the first study to analyze the correlation between the serum OC level and visceral fat area of overweight and obese people in our province, suggesting that there exists interaction between bone and adipose tissue which affected the distribution of body fat. All of these findings were of great significance for the evaluation of the degree of insulin resistance of obesity, diabetes and other metabolic disorders and of great clinical value to assess the cardiovascular metabolic risk and to guide the treatment.

Materials and methods

Materials

Data collection: All subjects who had provided their informed consent prior to their inclusion in the study, and this study was approved by Chinese Ethics Committee and performed in accordance with the ethical standards. Seventy subjects aged from 35 to 75 years (52.13±7.21 years), who underwent physical examination and hospitalization, were selected from Clinic for Retired Veteran Cadres of Anhui Provincial Hospital Affiliated to Anhui Medical University from Jan. 2012 to Dec. 2016. Medical history and lifestyle of all the subjects were obtained. Among 70 subjects, 15 subjects had the history of hyperlipemia without a long period of using drugs; 12 subjects had history of high blood pressure, and the disease course was 2~35 months; totally 18 subjects were diagnosed with hyperlipemia, and the disease course was 0~7 years and none of them were long-term users of lipid-lowering drugs; eleven subjects represent myocardial ischemia with electrocardiograph. According to guidelines for the treatment of obesity in China, all subjects were divided into three groups as control group (18 ≤ body mass index (BMI) ≤ 24.9), overweight group (25 ≤ BMI ≤ 27.5) and obese group (BMI ≥ 27.5) and there were 26, 20, 24 subjects in the control, overweight and obesity groups, respectively.

Exclusion criteria: Neither subjects with stress hyperglycemia, autoimmune diseases, thyroid dysfunction, acromegaly, hypercortisolism, liver or renal insufficiency, malignant tumor, osteoporosis, nutrition disorder, anemia, cataclasis within one year or continuous use of glucocorticoid, diuretic, niacin and indomethacin or taking medicines that affect the bone and the calcium metabolism (for example, Vitamin D, double phosphonate, calcitonin, estrogen, etc.) nor the persons who were given drugs with the effects on insulin-resistance such as angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists.

Methods

The anthropometric measurements were taken. BMI was calculated as weight divided by height squared (kg/m^2); WC was measured at midway between the lowest rib and the iliac crest in the standing position; BP was measured twice (5 min interval between the two measurements) with a mercury sphygmomanometer, and the average value was calculated and recorded. And venous blood was collected after overnight fasting for the following detections. FINS levels were measured with a commercial radioimmunoassay (Diagnostic Products, USA); level of FBG was measured using the standard glucose oxidase method; HOMA-IR was used to estimate insulin sensitivity and HOMA-IR = FINS (milliunits per liter) * FPG (millimoles per liter)/22.5. Moreover, fasting plasma lipid parameters, including TC, TG, HDL-C, LDL-C, were detected by an automatic enzymatic analyzer (Hitachi 7600ISE-020, Japan); CRP was assayed with ELISA kit (Thermo Scientific, Shanghai, China); OC and APN were assayed using a quantitative enzyme immunoassay kit (lot 1306041, 1306043, R&D systems, USA) by laboratory of Sengxiong Company in Shanghai. The bone density examination was performed by dual-energy X-ray absorptiometry. Osteoporosis was identified as T value ≤ -2.5 SD (the bone density is lower than the average peak bone density of young adults 2.5 standard deviation). Additionally, all subjects underwent abdominal MRI. Briefly, MRI scans were performed at the level of the umbilicus between L4 and L5 with the subjects in the supine position and Slice-O-Matic software (version 4.2) was used to generate graphical displays of the imaging data and to calculate the visceral fat area (VFA).

Statistical analysis

All statistical analyses were performed on SPSS 13.0 software (SPSS Inc., Chicago, USA). Measurement data expressed as mean ± SD, median and quartiles (1; 3). Data with normal distribution was assessed by independent sample t-test between two groups, and differences among multi-groups were compared using an one-way ANOVA, while data with non-normal distribution was performed with Kruskal-Wallis test. The relationships between two variables were assessed with Pearson correlation and rank correlation. Stepwise regression was used to explore the correlation of OC and VFA. $P<0.05$ was considered statistically significant.

Table 1. Clinical and biochemical parameters of obesity, overweight and control groups

Variables	Control	Overweight	Obesity	Ranges	P or Z
Number	26	20	24		
Age (y)	51.45±6.07	53.15±4.58	52.83±7.25	35~75	0.148
SBP (mmHg)	123±11	138±10	142±15	90~140	0.401
DBP (mmHg)	68±10	78±8	84±11	60~90	0.309
BMI (kg/m^2)	23.46±2.51	26.05±3.13*	28.49±3.14$^{*,▲}$	18.5~23.9	0.014
WC (cm)	84.4±3.67	88.6±5.7*	93.7±5.3$^{*,▲}$	<85 (M)	0.004
FINS (mU/L)	8.13 (5.25~11.35)	10.34 (7.45~13.16)	14.08 (8.95~16.03)$^{**,▲▲}$	5~20	<0.001
FBG (mmol/L)	4.23±0.74	4.98±0.82	6.04±1.02$^{*,▲}$	3.90~6.10	0.026
HOMA-IR	1.89 (0.83~2.15)	2.25 (1.08~2.45)*	3.76 (2.76~4.83)$^{**,▲}$		<0.001
TC (mmol/L)	3.56±0.34	4.83±1.29	5.04±1.03**	3.35~5.70	0.015
TG (mmol/L)	1.33±0.68	1.44±0.71	2.65±0.38*	0.30~1.80	0.041
HDL-C (mmol/L)	1.63±0.13	1.55±0.61	1.29±0.47*	0.74~2.09	0.038
LDL-C (mmol/L)	2.38±0.58	2.88±1.24	3.53±0.39$^{*,▲}$	1.00~4.40	0.003
CRP (mg/L)	0.965 (0.345~1.968)	1.341 (0.851~2.035)*	2.068 (0.945~3.701)$^{**,▲▲}$	0~8.00	<0.001
VFA (cm^2)	89.3±36.4	104.5±33.6**	120.6±39.8$^{**,▲▲}$		<0.001
APN (mg/L)	23.51±4.36	19.04±9.16*	13.23±5.82$^{**,▲▲}$	2.90~21.89	<0.001
OC (μg/L)	11.45±2.51	6.73±2.31*	4.25±1.29$^{**,▲▲}$	7.5~15.0	<0.001

Note: data were expressed as mean ± SD, median and quartiles (1; 3). *P<0.05, compared with control group; **P<0.01, compared with control group; $^{▲}$P<0.05, compared with overweight group; $^{▲▲}$P<0.01, compared with overweight group. Ranges, the normal ranges for the variables.

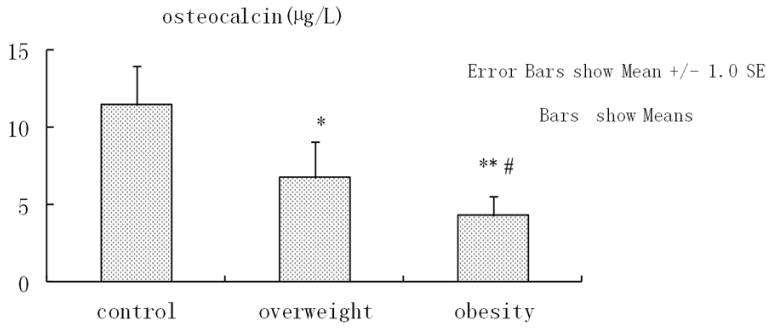

Figure 1. Levels of serum osteocalcin in groups of control, overweight and obesity. *P<0.05, compared with control group; **P<0.01, compared with control group; $^{\#}$P<0.01, compared with overweight group.

Results

Clinical and biochemical characteristics

The metabolic indexes of subjects in three groups are listed in **Table 1**. As expected, increased levels of BMI, FINS, WC, HOMA-IR, LDL-C and CRP and decreased level of APN were detected in obesity group compared with the other two groups (P<0.05). Compared with control group, levels of FBG, TG, TC were obviously higher in obesity group (P<0.05), while HDL-C was significantly lower in obesity group (P<0.05). There was no significant difference of age, diastolic blood pressure (DBP), systolic blood pressure (SBP) among three groups (P>0.05).

Levels of OC and VFA

The decreasing trend of the OC level could be observed in the obesity group, overweight group, and control group sequentially (P<0.05, **Figure 1**; **Table 1**). In addition, increased level of VFA was observed in obesity group compared with the control and overweight groups (P<0.01, **Figure 2**; **Table 1**).

Correlations between OC and VFA

As shown in **Table 2**, the OC level was significantly correlated with WC (r=-0.532, P=0.000), FINS (r=-0.416, P=0.000), HOMA-IR (r=-0.509, P=0.000), FBG (r=-0.297, P=0.037), APN (r= 0.302, P=0.034), CRP (r=-0.475, P=0.000), and VFA (r=-0.325, P=0.016) in overweight and obesity groups. However, the OC level was only obviously correlated with WC (r=-0.609, P=0.000) in control group. Meanwhile, multiple

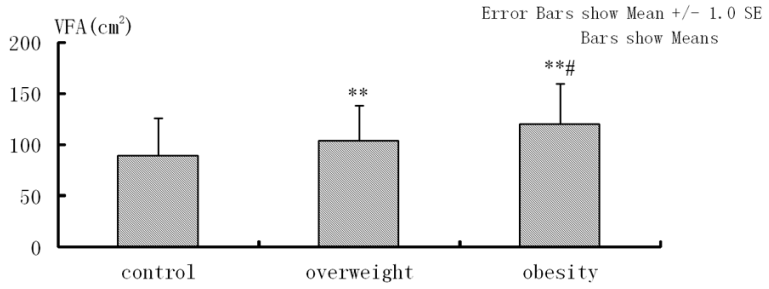

Figure 2. Levels of VFA in groups of control, overweight and obesity. **P<0.01, compared with control group; #P<0.01, compared with overweight group.

Table 2. Correlation analysis of osteocalcin and various metabolic parameters

Variable	Control group			Overweight and obesity groups		
	Estimate	*P*-value	Test	Estimate	*P*-value	Test
Age	0.065	0.763	Pearson	0.094	0.627	Pearson
SBP	-0.077	0.689	Spearman	-0.174	0.201	Spearman
DBP	-0.248	0.298	Spearman	-0.254	0.102	Spearman
BMI	-0.325	0.067	Pearson	-0.185	0.189	Spearman
WC	-0.609	0.000	Spearman	-0.532	0.000	Spearman
FINS	0.126	0.308	Spearman	-0.416	0.001	Spearman
FBG	-0.079	0.843	Pearson	-0.297	0.037	Pearson
HOMA-IR	0.168	0.466	Spearman	-0.509	0.000	Spearman
TC	0.064	0.783	Pearson	-0.213	0.204	Spearman
TG	0.084	0.803	Pearson	-0.276	0.053	Pearson
HDL-C	0.279	0.184	Spearman	0.169	0.204	Spearman
LDL-C	-0.053	0.669	Spearman	-0.198	0.118	Spearman
CRP	0.284	0.219	Spearman	-0.475	0.000	Spearman
APN	0.079	0.619	Pearson	0.302	0.034	Pearson
VFA	0.078	0.806	Spearman	-0.325	0.016	Spearman

Table 3. Multiple stepwise regression analysis showing variables independently associated with osteocalcin in overweight and obesity group

Independent variables	β	SE	Standardized β	*P*
HOMA-IR	-0.296	0.065	-0.859	0.005
WC	-0.318	0.073	-0.416	0.000
VFA	-0.234	0.121	-0.497	0.019

Note: variables included in overweight and obesity groups are as followings: age, BMI, WC, VFA, SBP, DBP, FPG, FINS, HOMA-IR, TC, TG, HDL-C, LDL-C, CRP, and APN.

linear stepwise regression analysis demonstrated that WC (standardized β=-0.416, P=0.000), HOMA-IR (standardized β=-0.859, P=0.005), and VFA (standardized β=-0.497, P=0.019) were independently and inversely associated with serum OC level in overweight and obesity subjects (**Table 3**).

Discussion

There are lots of indicators for the evaluation of body fat, such as BMI, WC and etc. Although these indicators are simple and cheap to get, a number of studies in China and abroad indicate that these indicators do not accurately reflect the correlation of the extent of human obesity and disease, cannot distinguish between subcutaneous fat and visceral fat either, suggesting such indicators lack enough accuracy to evaluate the fat distribution [7]. With the development of medical imaging technology, a variety of techniques, such as CT, magnetic resonance and others, can be used to more precisely measure the distribution of body fat [8, 9]. Especially, magnetic resonance, which does not have the weakness of radiation of CT, can not only access the character of tissue with clear magnetic resonance values, but also accurately calculate the area of the fat distribution by computer software. What's more, the error of the magnetic resonance result is smaller. All of these advantages make it apply as the gold standard for the determination of fat distribution [7]. In this study, we quantified the area of visceral fat by MRI, and investigated whether the fasting serum levels of OC in obese and overweight male subjects and normal people were related to the visceral fat area, and whether there were interaction between skeleton and adipose tissue, to explore a novel approach for the understanding of the mechanism of energy metabolism and to provide the theoretical basis for the future development of OC and its analogues and its application in the treatment of obese subjects.

OC is one kind of noncollagenous protein produced by the specific secretion of osteoblasts in the differentiation stage, which can promote the calcification of osteoclast and the form of

osteoblasts to participate in skeletal development. Lee et al. [10] confirmed that OC could stimulate the expression and secretion of insulin of β cells, promote the proliferation of islet β cells, increase energy release, up-regulate the expression of APN in the adipose tissue and so on, to increase the sensitivity of insulin and to adjust the content of fat by knocking-out or over-expressing OC gene in mice, infusing exogenous and recombinant OC genes in mice, observing the effect of OC on tissues and cells in vitro and etc. In the clinical study, our previous research findings [11] indicated that fasting serum levels of OC in type 2 diabetes mellitus group and impaired glucose tolerance group were obviously lower than that in normal glucose tolerance group ($P<0.01$). OC was negatively correlated with HOMA-IR, FBG, 2 hour glucose level, and CRP, but it was positively correlated with APN, suggesting that OC might be involved in insulin resistance, and further participate in the regulation of glycolipid metabolism. In this study, the results showed that the serum level of OC was significantly different among varied states of fat distribution, moreover, OC was inversely correlated with the risk factors of abdominal obesity such as FINS, FBG, HOMA-IR, CRP, though it was positively associated with APN, indicating that OC might be involved in insulin resistance, which were consistent with previous studies [12, 13].

Some studies have shown that the serum OC level was associated with obesity in population [11, 14], but the functions of the adipose tissues in different parts of human body were different, and visceral fat accumulation is an important risk factor for cardiovascular disease [15]. Therefore, here, we attempted to investigate the relationship between serum level of OC and abnormal distribution of body fat. In this study, we found that there was a negative correlation between OC and WC, VFA in overweight and obese male subjects. Previous study [13] also has shown that the expressions of TG gene and perilipin (lipolytic) gene in OC knockout mice were significantly decreased. Meanwhile, Shanghai obesity research found that the serum level of OC and the level of high density lipoprotein cholesterol in males were independently and positively correlated [2, 3, 16]. Nevertheless, the relationship between human OC and blood lipids as well as their regulatory mechanism of is still unclear, yet to be further studied. Unfortunately, this study did not find the relationship between OC and blood lipids, which may be the result of the small sample size. In addition, multiple stepwise regression analysis further found that OC was independently associated with the risk factors of abdominal obesity including WC, VFA, and HOMA-IR. What's more, OC may be a protective factor for VFA. Previous study of Kanazawa et al. [17] performed a similar study among 180 males with type 2 diabetes and 109 postmenopausal females with type 2 diabetes. The results suggested a negative correlation between serum OC level and abdominal fat area and a positive correlation between serum OC concentration and serum APN level. In addition, a study with 214 postmenopausal women in South Korea also confirmed that serum level of OC was negatively correlated with VFA [18]. Thereby, only male subjects involved in this study, considering that the female hormones might have an impact on the OC level.

Besides, this study found that OC not only associated with abdominal obesity risk factors, but also closely related with APN. APN is an endogenous biologically active polypeptide secreted by adipocytes, which can bind to APN receptor 1 and APN receptor 2 to regulate fat metabolism and to participate in bone metabolism, then to affect the synthesis of OC accordingly [19]. Some studies have confirmed that the APN level in obese people was lower than that in the normal population, and the decrease of serum APN level in people with abdominal obesity was more remarkable, indicating that the decline of APN and abdominal obesity are closely related [20]. Moreover, OC can increase the sensitivity of insulin by inducing the expression of APN in adipocytes and reducing the binds between APN and its receptors in skeletal muscle, adipose tissue and liver. In addition, by promoting the consumption and burning of fat in muscle tissue and liver, OC can decrease the levels of free fatty acid and TG to reduce weight [21].

Conclusion

In conclusion, compared with the control group, serum OC levels were significantly lower in the

overweight and obese groups, meanwhile, OC which was negatively correlated with VFA, was independent of other metabolic disorders, indicating that men with low OC level were more likely to occur abdominal obesity. Additionally, there may exist interaction between bone and energy metabolism and abdominal obesity. Therefore, our findings suggested that high levels of OC may be beneficial for abdominal obesity, providing new insights into the researches of mechanisms of energy metabolism and cardiovascular diseases.

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Disclosure of conflict of interest

None.

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