Original Article
Correlation between obesity and bone health in Chinese children and adolescents

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Abstract: Background: Many researchers suggested that obesity and bone metabolism are correlative. Nevertheless, there are still obscured regarding the interactions among obesity and bone biologic markers, especially in children and adolescents. Methods: A cross-sectional study was performed in 4 groups of 95 children/adolescents. Serum adiponectin, ghrelin, osteopontin (OPN), and osteoprotegerin (OPG) were examined by enzyme-linked immunosorbent assay (ELISA). Blood lipid levels including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) were tested by enzymatic colorimetry. Results: Serum adiponectin level was lower in overweight group than that of lean group (P < 0.05). Ghrelin levels in overweight and obese groups significantly decreased compared with lean group (P < 0.05, respectively). Serum adiponectin and ghrelin levels inversely correlated with body mass index (BMI). Serum adiponectin reversely related with TG in all the subjects. For the female, serum adiponectin had a negative relation with TG, TC and LDL. Serum OPN had a positive relation with ghrelin level and negative relation with BMI in all subjects. Conclusions: Serum adiponectin and ghrelin levels were downregulated in obesity. BMI inversely correlated with the serum adiponectin and ghrelin levels. There were a significant negative correlation between adiponectin level and TG level. Serum OPN level had a significantly positive correlation with ghrelin and negative correlation with BMI. Ghrelin may play a fundamental role in the pathogenesis of obesity and related bone metabolic risk diseases through OPN in Chinese children and adolescents.

Keywords: Obesity, adiponectin, ghrelin, osteopontin, children and adolescents

Introduction

The amount of Chinese obese children and adolescents has a rapidly increased prevalence [1]. Childhood and adolescent obesity is a serious public problem and causes various metabolic complications [2-4]. At puberty, overweight and obesity has a detrimental effect on bone, which leads to increase the risks of fragility and fractures at present and future [5-7]. However, it's unclear that why body fat mass has a negative effect on bone remains. The further investigation on correlation between adipose tissue and bone should be performed [8].

It's known that body weight is a major determinant of bone density. The protective effect of fat mass on bone may be mediated by the peripheral signals of appetite regulation and energy homeostasis [9]. Adiponectin is a recently described and highly promising adipo-cyte-produced hormone and the receptor of adiponectin has recently been detected in the human bone-forming cells, which means that adiponectin may be a hormone linking bone and fat metabolism [10]. Besides, ghrelin plays a key role in the coordination of feeding behaviour and energy metabolism. Alamri BN et al. have reported that ghrelin increased fat mass by stimulating appetite and reducing fat use [11]. Moreover, circulating ghrelin level has an important effect on body fatness and decreased in human obesity [12, 13]. Remmel L et al. have reported that bone mineral density (BMD) was positively correlated with ghrelin in adolescent overweight boys [14].

Osteopontin (OPN) and osteoprotegerin (OPG) are two important bone biological markers in bone metabolism. OPN, a secreted phosphorylated glycoprotein, usually expressed in the mineralized extracellular matrix of bone or other

tissues and involved in many biological processes [15]. Recently, many studies have exhibited that OPN affected body weight control and glucose homeostasis [16-18]. OPG, a soluble decoy receptor disturbing the binding between receptor activator of NF-κB (RANK) and its ligand (RANKL) in osteoclasts, exerts an inhibitory function on differentiation, activation, and survival of osteoclasts [19-21]. Ayina Ayina CN et al. showed that OPG level remained negatively correlated to low-density lipoprotein (LDL) and positively correlated to high-density lipoprotein (HDL) in sub-Saharan African women, which are the markers of blood lipid levels [22].

Hence, the aim of this study was to examine the levels of serum adipokines, blood lipid levels, and bone biological markers, and to evaluate the association of obesity with bone biologic markers in Chinese children and adolescents.

Materials and methods

Subjects

A total of 95 healthy volunteers (range 8-18 years) who have attended orthodontic treatment at Peking University School and Hospital of Stomatology were included in the study. Subjects were systemically healthy without metabolic syndrome and excluded if they had the regular medication, secondary obesity or with abnormal liver function or exposure to smoking. Levels of alanine aminotransferase, serum total protein, serum albumin, and ratio of leukocytes to erythrocytes were within normal ranges, and subjects were negative for hepatitis B and C antigens. The research protocol was approved by Ethics Committee of Peking University School and Hospital of Stomatology and all parents of volunteers signed an informed consent documents to participate in this study.

Anthropometric data

Anthropometric measurements were performed for all subjects. Height was measured to nearest 0.1 cm using a mobile height measure (YuanYan Medical Ltd., Jiangsu, China) and weight were measured to the nearest 0.1 kg using a digital weight (YuanYan Medical Ltd., Jiangsu, China) , respectively. Body mass index (BMI) was calculated as weight (kg) divided by

height squared (m^2). All measurements were obtained by the same experienced staff member. Anthropometric data were expressed as means ± SD.

According to the World Health Organization criteria for BMI [23], the BMI was standardized adjusted for age and sex. Lean, normal, overweight, and obese were defined by as BMI below the 10th, 10th to 85th, 85th to 95th, and above the 95th percentile, respectively. Accordingly, subjects were divided into 4 groups: lean (n = 18), normal (n = 9), overweight (n = 29) and obese (n = 39).

Laboratory analysis

Venous blood samples were collected from subjects after an overnight fast for 12 h. Serum was centrifuged for 5 min at 2600× g at 4°C and stored at -80°C until analysis. Serum levels of adiponectin, ghreiln, osteopontin (OPN) and osteoprotegerin (OPG) were measured with a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (eBioscience, San Diego, CA, USA). Serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), were all tested by use of commercial colorimetric kits (Biosino Biotechnology Company Ltd, Beijing, China) and the automatic analyzer HITACHI 7180 (HITACHI, Japan) as previously described [24]. The inter- and intra- assay coefficients of variation for all measured lipid parameters were less than 5%.

Statistical analysis

Statistical analysis involved use of SPSS v19.0 for Windows (SPSS Inc., Chicago, IL, USA). All values were reported as means ± SD. Normality of parameters was controlled by one sample Kolmogorov-Smirnov test ($P > 0.05$). Statistical comparisons between the groups were made using analysis of variance (ANOVA). LSD post-doc assay was used when the equal variance was assumed. Otherwise Dunnett's T3 test was used. Correlations were estimated by bivariate correlation analysis. Pearson product moment correlation coefficients were computed to evaluate the relationships between measured parameters. $P < 0.05$ was considered statistically significant.

Table 1. Anthropomorphic data in four groups in Chinese children and adolescents

Parameters	Lean	Normal	Overweight	Obese	*P*-value
Number of subjects	18	9	29	39	
Age (y)	14.56±3.21	14.50±1.93	13.88±2.49	14.13±3.26	0.912
Sex: Males (%)	(11/18) 61.11%	(6/9) 66.67%	(16/29) 55.17%	(27/39) 69.23%	0.685
Weight (kg)	45.72±11.43	58.22±7.76*	67.64±12.02*	80.99±15.38*,#,$	< 0.001
Height (cm)	161.06±12.91	160.22±8.18	163.55±11.18	166.03±12.46	0.380
Body mass index (BMI) (kg/m²)	16.34±1.39	20.91±2.72*	25.17±2.25*,#	28.69±3.29*,#,$	< 0.001
BMI percentile	4.25±2.36	62.27±24.73*	91.67±2.26*,#	97.57±1.16*,#,$	< 0.001

Values are means ± SD. *P < 0.05 compared with lean group. #P < 0.05 compared with normal group. $P < 0.05 compared with overweight group. One-way ANOVA followed by the LSD.

Table 2. Serum levels of adiponectin, ghreiln, bone biological markers, and blood lipid in Chinese children and adolescents

Parameters	Lean	Normal	Overweight	Obese
Adiponectin (µg/mL)	30.25±3.16	27.58±6.00	26.92±6.27*	28.07±5.22
Ghrelin (pg/mL)	829.5±488.6	742.7±308.8	608.22±279.7*	546.9±207.2*
Osteopontin (pg/mL)	41292.02±49961.91	33677.72±41748.30	34645.17±43813.34	35655.12±42999.24
Osteoprotegerin (pg/mL)	1694.88±787.97	1225.47±346.14	1436.77±659.58	1606.97±859.45
TC total (mmol/L)	3.83±0.56	3.56±0.41	4.09±0.43#	4.18±0.74*,##
TC female (mmol/L)	3.85±0.47	3.46±0.37	4.10±0.50#	4.12±0.58#
TC male (mmol/L)	3.81±0.64	3.76±0.49	4.09±0.39	4.19±0.79
TG total (mmol/L)	1.22±0.32	1.03±0.35	1.53±0.45#	1.71±0.71**,##
TG female (mmol/L)	1.18±0.22	0.94±0.33	1.45±0.43#	1.53±0.64#
TG male (mmol/L)	1.25±0.38	1.21±0.38	1.59±0.48	1.76±0.74*,#
HDL total (mmol/L)	1.44±0.27	1.28±0.18*	1.33±0.18	1.31±0.23*
HDL female (mmol/L)	1.46±0.18	1.34±0.13	1.28±0.16*	1.37±0.15
HDL male (mmol/L)	1.43±0.33	1.17±0.24	1.37±0.20	1.30±0.24
LDL total (mmol/L)	1.93±0.38	1.92±0.43	2.26±0.53*	2.38±0.62**,#
LDL female (mmol/L)	1.91±0.34	1.71±0.28	2.26±0.49#	2.24±0.49#
LDL male (mmol/L)	1.95±0.42	2.35±0.33	2.26±0.58	2.42±0.66*

Values are means ± SD. *P < 0.05, **P < 0.01 compared with lean group. #P < 0.05, ##P < 0.01 compared with normal group. One-way ANOVA followed by the LSD. Blood lipids levels in accordance with the different genders in four groups.

Results

Demographic data

There were no significant differences in age, gender, height among the lean, normal, overweight, and obese group. However, the four groups were significantly different regarding weight (45.72 *vs.* 58.22 *vs.* 67.64 *vs.* 80.99 kg), BMI (16.34 *vs.* 20.91 *vs.* 25.17 *vs.* 28.69 kg/m²), and BMI percentile (4.25 *vs.* 62.27 *vs.* 91.67 *vs.* 91.57) among lean, normal, overweight, and obese group (*P* < 0.001, respectively) (**Table 1**).

Association of adiponectin or ghrelin with BMI

Serum levels of adiponectin and ghrelin in four groups were summarized in **Table 2**. Serum adi-
nopectin level in overweight group was lower than that of lean group (*P* < 0.05). Serum levels of ghrelin in overweight and obese group were significantly lower than that in lean group (*P* < 0.05, respectively). Interestingly, the same tendency was also showed among the four groups of male, but not in the female groups (Data not shown). No correlation exhibited between serum level of adiponectin and ghrelin.

Of note, serum levels of adiponectin and BMI showed a negative correlation (r = -0.256, *P* < 0.05) (**Figure 1A**), while ghrelin and BMI also exhibited a negative correlation (r = -0.341, *P* < 0.01) (**Figure 1B**). According to the statistics by gender, the male group maintained a similar correlation (r = -0.314, *P* < 0.05 for adiponectin and BMI; r = -0.3, *P* < 0.05 for ghrelin and BMI).
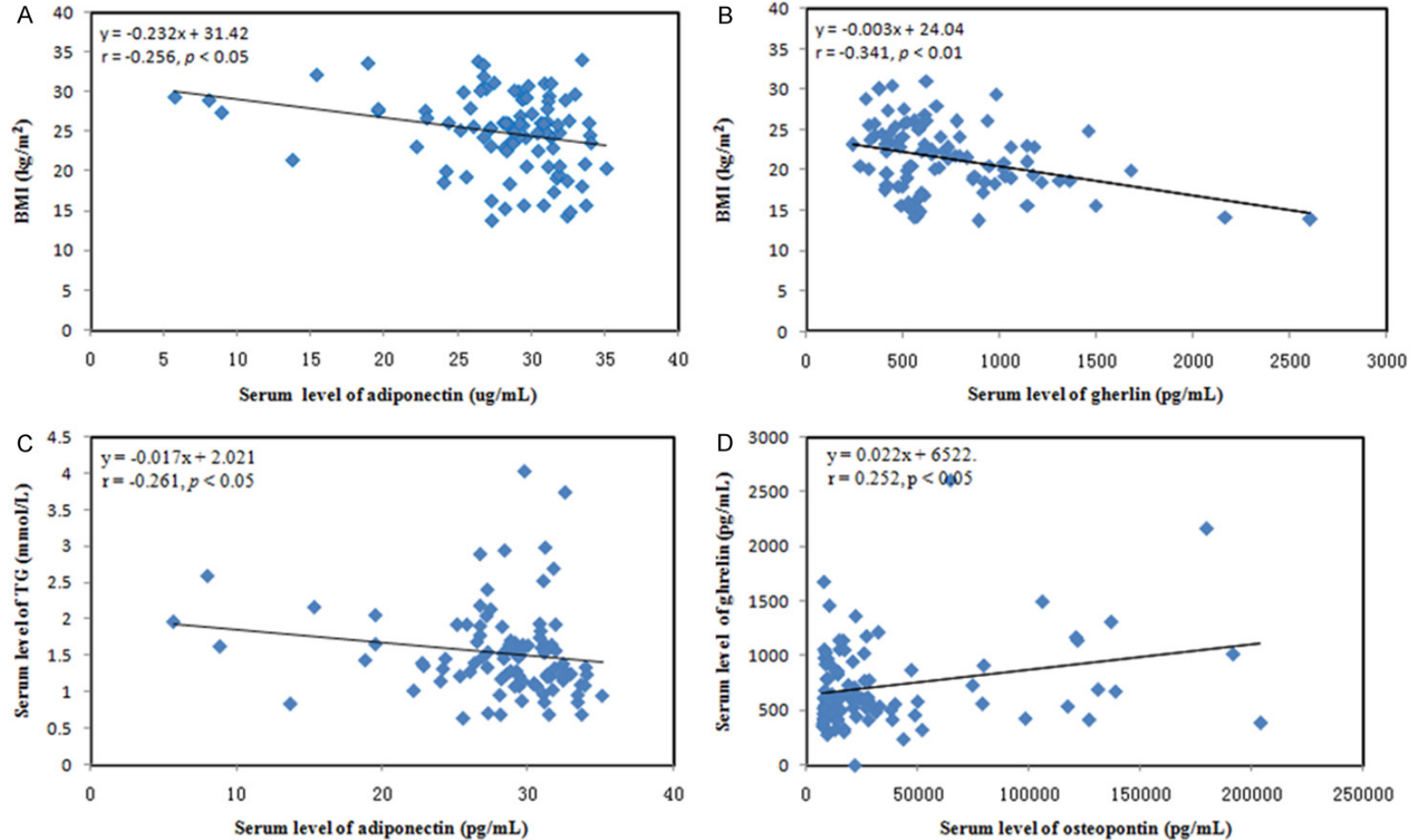
Figure 1. Correlation on BMI and adiponectin (A), BMI and ghrelin (B), TG and adiponectin (C), ghrelin and osteopontin (D) in Chinese children and adolescents. (A) Shows significant negative correlations between BMI and serum level of adiponectin, r = -0.256, $P < 0.05$. (B) Shows significant positive correlations between BMI and serum level of ghrelin, r = -0.341, $P < 0.01$. (C) Shows significant negative correlations between serum levels of TG and adiponectin, r = -0.261, $P < 0.05$. (D) Shows significant positive correlations between serum levels of ghrelin and osteopontin, r = 0.252, $P < 0.05$.

No correlation between adiponectin/ghrelin with BMI was shown in the female group.

Association of adiponectin or ghrelin with blood lipid markers

Serum TC level of obese group was higher than lean and normal subjects ($P < 0.05$, $P < 0.01$, respectively). Meanwhile, TC levels in overweight group increased significantly compared with normal group ($P < 0.05$). Consistent with serum TC levels, TG levels of overweight and obese group had a significant increase compared with that of normal group ($P < 0.05$ and $P < 0.01$, respectively). And then, TG levels in obese group were higher than those of lean groups ($P < 0.01$). Moreover, the obese group had lower HDL cholesterol levels and higher LDL cholesterol levels compared with those of lean group ($P < 0.05$ and $P < 0.01$, respectively). Besides, the obese group had a significantly increased LDL levels compared with those of normal group ($P < 0.05$). The overweight group had more LDL in the serum than that of lean group ($P < 0.05$). Meanwhile, the HDL levels in normal group significantly decreased compared with the lean group ($P < 0.05$) (**Table 2**).

The effects of gender on blood lipid markers were also showed in **Table 2**. For female, both the overweight and obese groups had a significant increase on TC, TG, and LDL compared with the normal group ($P < 0.05$, respectively). Besides, the HDL levels of the overweight group significantly decreased compared with those of lean group ($P < 0.05$). However, for male, the obese group had a significantly increased TG levels compared with the lean group and the normal group ($P < 0.05$, respectively), and a significantly increased LDL than that of lean group ($P < 0.05$).

Notably, TG levels had an inverse correlation with serum adiponectin for all subjects (r = -0.261, $P < 0.05$, **Figure 1C**) and girls only (r = -0.392, $P < 0.05$). Moreover, serum adiponectin in female also inversely related with LDL (r = -0.428, $P < 0.05$). There were no relationship between ghrelin and TC, TG, HDL and LDL.

Associations of adiponectin, ghrelin and BMI with OPG and OPN

Serum levels of OPN and OPG did not differ significantly between four groups (data not shown).

Of note, there was a significant positive correlation between serum ghrelin levels and OPN levels for all subjects (r = 0.252, $P < 0.05$, **Figure 1D**). Serum OPN had a negative relation with BMI in all subjects (r = -0.135, $P < 0.05$) and male only (r = -0.326, $P < 0.05$).

Discussion

Many researchers suggested that obesity and bone metabolism are correlative [7, 25-27]. Dimitri P *et al.* have exhibited that there were an inversely correlation between total body fat mass and total body, lumbar, and ultradistal radius BMC in 103 children, which require more targeted interventions to increase bone mass in obese adolescence [26]. Nevertheless, there are still obscured regarding the interactions among obesity and bone biologic markers, especially in children and adolescents. In this study, we demonstrated for the first time the relationship among obesity, adipokines, blood lipid levels, and biomarkers of bone metabolism directly in a population of Chinese children and adolescents. And the research exhibited evidences that BMI of all the children and adolescents had a significant negative correlation with the serum adiponectin level and ghrelin level. Moreover, there was a significant negative correlation between adiponectin level and TG level in serum. Of note, serum OPN level had a significant positive correlation with ghrelin and negative correlation with BMI.

In this study, we exhibited that the serum ghrelin level in obese group was lowest in Chinese children and adolescents. Moreover, there was a significant negative correlation between ghrelin level and BMI in Chinese children and adolescents. Many studies have showed that circulating ghrelin levels are decreased in human obesity compared with human of normal body weight [13, 28, 29]. Su C et al. also provided the evidence that serum ghrelin level correlated negatively with BMI among Chinese adults [30], which was consistent with our conclusions. Briggs DI et al. demonstrated the mechanisms that diet induced obesity (DIO) suppressed the neuroendocrine ghrelin system and induced ghrelin resistance through the down-regulation and dysfunction of NPY/AGRP neurons in mice [31]. Meanwhile, it was reported that serum adiponectin was negatively correlated with BMI and TG in the Japanese population [32]. The same results were also exhibit-

ed by our research of Chinese children and adolescents.

As expected, the blood lipid levels (TC, TG, HDL, LDL) in the overweight/obese group significantly increased compared with those of lean/normal group, which was same as previous study [33]. Interestingly, the percentage of overweight/obese group was over 70% in the all children and adolescents of our study, which was much higher than the 21.4% in Beijing, China assessed by Shan XY et al. in 2004 [34]. Three reasons may be explained the difference. Firstly, the overweight/obese children and adolescents increased dramatically as living standard improved quickly, especially in Beijing. Moreover, the subjects besides our study was the volunteers of 8-18 years, which was the period of more learning and less movement at school in China; however, the range of age was 2-18 years in Shan XY's research, which included more subjects with wider range of age. At last, orthodontic treatment costs a lot, which meant that the subjects may be from the family in good economic conditions. Hence, it may be reasonable for the significant high percentage of overweight/obese group in our study.

Previous research concluded that OPN was highly up-regulated in adipose tissue in human and murine obesity [35] and had been recently shown to be functionally involved in the pathogenesis of obesity-induced adipose tissue inflammation and associated insulin resistance in mice [36]. Moreover, OPN levels in serum and adipose tissue were both increased in obesity [37]. Our results exhibited that serum OPN level had the ascending tendency along with increased BMI among normal, overweight, and obese group, which was consistent with above study. Moreover, serum OPN level had a positive relation with serum ghrelin level and negative relation with BMI in all subjects and boys only. Considering that there was a negative correlation between serum ghrelin level and BMI, our research also provided evidence that serum OPN levels were up-regulated in obesity and maybe associated with expression of ghrelin.

OPG is consistently identified as one of critical regulators of osteoclastogenesis. Dimitri, P. et al. deemed that serum OPG level has no association with adiponectin level in serum,

although OPG level may play a fundamental role in the failure of obese children to accrue bone mass appropriately [7]. Moreover, Tenta, R. et al. also had showed that there were no correlation between OPG level and adiponectin, and serum OPG level was negative correlation with serum leptin level [38]. The further investigations should be performed for the correlation between OPG and other adipokines.

Serum adiponectin and ghrelin level decreased in obesity. BMI had a significant negative correlation with the serum adiponectin and ghrelin level. There was a significant negative correlation between adiponectin level and TG level. Serum OPN level had a significant positive correlation with ghrelin and negative correlation with BMI. Ghrelin level maybe play a fundamental role in the pathogenesis of obesity and related bone metabolic risk diseases through OPN in Chinese children and adolescents.

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Disclosure of conflict of interest

None.

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