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Original article

Role of hyperglycaemia in the relationship between serum osteocalcin levels and relative skeletal muscle index

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SUMMARY

Background & aims: Studies have shown that osteocalcin is involved in energy metabolism and is sufficient to prevent age-related muscle loss. The present study investigated the association of serum osteocalcin levels with muscle mass and the influence of metabolic factors on this association in humans.
Methods: A total of 1742 middle-aged and elderly subjects (median age: 61.2 years; interquartile range: 56.9–65.3 years) were enrolled from Shanghai communities, including 775 men and 967 postmenopausal women. Serum osteocalcin levels were measured by an electrochemical immunoluminescence assay. An automatic bioelectric impedance analyser (BIA) was used to measure body compositions. Relative skeletal muscle index (SMI) was calculated using the BIA equation from Janssen et al.
Results: SMI was significantly higher in men than in postmenopausal women (37.30% [35.14%–39.63%] versus 27.72% [25.99%–29.66%], $p < 0.001$). Increasing SMI was associated with decreases in the frequency of overweight/obesity, central obesity, dyslipidaemia, elevated blood pressure, and hyperglycaemia (all $p < 0.001$). Serum osteocalcin levels were positively correlated with SMI in both men and women, regardless of treatment as a categorical or continuous variable (all $p < 0.001$). However, after accounting for confounding variables, the relationship remained only in men with hyperglycaemia (standardized $\beta = 0.068$, $p = 0.024$). Among men with isolated impaired glucose tolerance, the odds ratio of increased SMI was 2.861 in the fourth osteocalcin quartile compared with the lowest ($p = 0.046$). Multiple stepwise regression revealed that each standard deviation (SD) increase of serum osteocalcin levels resulted in an increase of 0.131 SD in SMI ($p = 0.024$).
Conclusion: Serum osteocalcin levels were positively related to SMI in men with hyperglycaemia, especially in those with isolated impaired glucose tolerance. No association was detected between serum osteocalcin levels and SMI in postmenopausal women.

1. Introduction

Sarcopenia is associated with adverse outcomes that seriously affect the quality of life in older people, such as fractures, disability, and frailty. Early-stage sarcopenia involves age-related skeletal muscle loss. Because skeletal muscle is the major site of insulin-mediated glucose utilization in the body, increasing evidence supports an association between a decline in muscle mass and metabolic risk factors. In both men and women, type 2 diabetes is associated with an increased risk of sarcopenia [1]. An increase in weight-adjusted skeletal muscle mass over time revealed a significant inverse relationship with the likelihood of developing metabolic syndrome [2]. Sarcopenic obesity was more closely associated with metabolic syndrome than obesity alone [3].

Abbreviations: BIA, bioelectric impedance analyser; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; Fat%, fat percentage; FM, fat mass; FFM, free-fat mass; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; LDL-C, low-density lipoprotein cholesterol; OR, odds ratios; SMI, skeletal muscle index; TC, total cholesterol; TG, triglyceride; W, waist circumference; 2hPG, 2-h plasma glucose.

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Osteocalcin, secreted by osteoblasts and osteocytes, is one of the most abundant non-collagenous proteins in bone [4]. Osteocalcin is post-translationally modified on three specific glutamate residues, in positions 17, 21, and 24, by γ-glutamyl carboxylase with vitamin K as a cofactor. Because γ-carboxylation of osteocalcin increases its affinity for hydroxyapatite crystals, most secreted osteocalcin is embedded in the bone matrix. However, the acidic environment generated during bone resorption processes decreases affinity for hydroxyapatite and therefore promotes its release into the circulation. Circulating osteocalcin exists in two forms, γ-carboxylated osteocalcin and under-carboxylated osteocalcin, the concentration of which in serum serves as a biochemical marker for bone formation. In addition, experimental work in knockout mice and cells delineated a role for osteocalcin, particularly its under-carboxylated form, in regulating glycolipid metabolism, stimulating insulin secretion, and enhancing insulin sensitivity [4].

In addition, recent studies have found that osteocalcin favours physiological functions that tend to decrease with age, such as testosterone synthesis, memory, and adaptation to exercise [5–7]. Animal studies have found that osteocalcin signalling in myofibres is necessary to maintain muscle mass in older mice, in part because it promotes protein synthesis in myotubes without affecting protein breakdown [8]. A human study found a positive correlation between serum osteocalcin levels and fat-free mass in healthy premenopausal women but not in healthy postmenopausal women [9]. However, fat-free mass does not directly indicate skeletal muscle mass. Data are still needed on the relationship between serum osteocalcin levels and skeletal muscle in humans.

Therefore, the goals of the present study were to explore the relationship between serum osteocalcin levels and relative skeletal muscle mass and to examine whether this relationship is affected by metabolic factors. All of the participants enrolled in the study were middle-aged and elderly individuals from Shanghai communities.

2. Materials and methods

2.1. Subjects

We recruited 1742 middle-aged and elderly men and postmenopausal women in Shanghai communities from October 2015 through July 2016. All participants underwent an examination comprising screening tests for the detection of obesity, dyslipidaemia, elevated blood pressure (BP), and hyperglycaemia. Every subject filled out a standardized questionnaire that provided information on current and previous illnesses and medications. Subjects were included if they met the following criteria: 1) age ≥40 years and 2) volunteered and able to articulate their own information regarding study participation. Exclusion criteria included current or recent cardiovascular disease, malignancy, liver or kidney dysfunction, hyper- or hypothyroidism or thyroid dysfunction, treatment with steroid hormones, and treatment with thyroxine or any treatment known to influence bone and calcium metabolism.

This study was conducted according to the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All participants provided written informed consent prior to participation.

2.2. Anthropometric measurements and skeletal muscle index

Body weight, height, and waist circumference (W) were measured by standard techniques [10]. Body mass index (BMI) was calculated as weight (kg) / height^2 (m^2). BP was measured three times at 3-min intervals by trained nurses with a mercury sphygmomanometer on the arm of the participants in a comfortable sitting position after at least 10 min of rest.

Fat percentage (fat%), total body fat mass (FM), and free-fat mass (FFM) were measured using an automatic bioelectric impedance analyser (BIA; TBF-418B; Tanita Corp., Tokyo, Japan). Subjects were required to hold the hand electrodes and stand barefoot on the foot electrodes. Body fat parameters and systemic bioelectrical impedance were obtained at the end of analysis. Skeletal muscle mass was calculated using the BIA equation of Janssen et al. [11]: skeletal muscle mass (kg) = [(height^2 / BIA-resistance × 0.401) + (gender × 3.825) + (age × −0.071)] + 5.102, where height is in cm; BIA-resistance is in ohms; for gender, men = 1 and women = 0; and age is in years. Absolute skeletal muscle mass (kg) was converted to percentage skeletal muscle mass (muscle mass/body mass × 100) and termed the skeletal muscle index (SMI).

2.3. Biochemical assessments

Fasting blood samples were collected to measure fasting plasma glucose (FPG), glycated haemoglobin A1c (HbA1c), fasting serum insulin, serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), and serum osteocalcin levels. In subjects without a clear history of diabetes, a 2-h plasma glucose (2hPG) blood sample was obtained after a 75-g oral glucose tolerance test; in diabetic patients, the sample was obtained after a carbohydrate tolerance test (100 g of steamed bread). These laboratory methods have been described elsewhere [10]. The homeostasis model assessment-insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = fasting serum insulin (mU/L) × FPG (mmol/L) / 22.5. Total serum osteocalcin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). The intra-assay and interassay coefficients of variation for serum osteocalcin levels were 1.2%–4.0% and 1.7%–6.5%, respectively [12].

2.4. Classification and definition

Participants with a BMI ≥ 25 kg/m^2 were assigned to the overweight/obese group [13]. Central obesity was defined as W ≥ 90 cm in men and W ≥ 85 cm in women [14]. Based on the 2016 Chinese Guidelines on Prevention and Treatment of Dyslipidaemia in Adults, dyslipidaemia was defined by the subject meeting any of the following criteria: (1) TC ≥ 5.2 mmol/L (200 mg/dL), (2) TG ≥ 1.7 mmol/L (150 mg/dL), (3) LDL-C ≥ 3.4 mmol/L (130 mg/dL), (4) non–HDL-C ≥ 4.1 mmol/L (160 mg/dL), or (5) HDL-C < 1.0 mmol/L (40 mg/dL) [15]. Elevated BP was defined as systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg or receipt of treatment for previously diagnosed hypertension [16]. Impaired glucose regulation and diabetes were diagnosed according to FPG and 2hPG values (impaired glucose regulation if 6.1 mmol/L ≤ FPG < 7.0 mmol/L and/or 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L; diabetes if FPG ≥ 7.0 mmol/L and/or 2hPG ≥ 11.1 mmol/L) [17]. Both impaired glucose regulation and diabetes were classified as hyperglycaemia. Patients with impaired glucose regulation were further divided into three groups for further analysis as follows: (1) participants with 6.1 mmol/L ≤ FPG < 7.0 mmol/L but 2hPG < 7.8 mmol/L were classified as isolated impaired fasting glucose, (2) participants with 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L but FPG < 6.1 mmol/L were classified as isolated impaired glucose tolerance, (3) participants with 6.1 mmol/L ≤ FPG < 7.0 mmol/L and 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L were classified as combined isolated impaired fasting glucose and isolated impaired glucose tolerance. Hypoglycaemic treatment refers to the use of insulin or

oral hypoglycaemic agents. Smoking was defined as ≥1 cigarette/day over the past 6 months [12]. Levels of physical activity were classified as light, moderate, or high according to the 2001 International Physical Activity Questionnaire [18].

2.5. Statistical analysis

Continuous data that was normally distributed was reported as means ± standard deviation (SD), while skewed data was reported as median with interquartile range. Categorical variables were reported as counts with percentages. Subjects were divided into four groups according to gender-specific quartiles of SMI levels: in men, quartile 1, ≤35.13%; quartile 2, 35.14%–37.29%; quartile 3, 37.30%–39.62%; quartile 4, ≥39.63%; and in women, quartile 1, ≤25.97%; quartile 2, 25.98%–27.71%; quartile 3, 27.72%–29.65%; quartile 4, ≥29.66%. The gender-specific cutoff points of serum osteocalcin levels quartiles were as following: in men, quartile 1, ≤13.45 ng/mL; quartile 2, 13.46–16.70 ng/mL; quartile 3, 16.71–20.36 ng/mL; quartile 4, ≥20.37 ng/mL; and in women, quartile 1, ≤17.17 ng/mL; quartile 2, 17.18–21.10 ng/mL; quartile 3, 21.11–26.00 ng/mL; quartile 4, ≥26.01 ng/mL. Chi-square test was used to compare the prevalence of metabolic disorders among SMI and osteocalcin quartiles. SMI and serum osteocalcin levels were normalized by logarithmic transformation. Linear regression was conducted to examine the relationship between SMI and serum osteocalcin levels, both in the overall data set and in subgroups defined by different metabolic disorders. We further conducted univariate and multiple linear regression analysis to investigate the relationship between serum osteocalcin levels and SMI in men with hyperglycaemia. To test whether glucose metabolism status influenced the relationship between SMI and serum osteocalcin levels, ordinal logistic regression was performed to assess odds ratios (ORs) and corresponding 95% confidence intervals (CIs). SMI and serum osteocalcin levels were standardized to a mean of 0 and SD of 1 (based on the study sample distribution) before multiple linear regression analysis in men with isolated impaired glucose tolerance. All tests were two-tailed, with $p < 0.05$ considered to indicate statistical significance. Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of the study participants

In total, 1742 subjects were enrolled in this study (median age: 61.2 [56.9–65.3] years), including 775 men and 967 postmenopausal women. Table 1 lists clinical and laboratory characteristics. Overall, the median BMI was 23.60 (21.70–25.70) kg/m^2; 51.5% of subjects had moderate levels of physical activity, and 30.8% had high levels. The median serum osteocalcin levels were 19.04 (15.37–23.76) ng/mL. Men had significantly lower serum osteocalcin levels than women (16.71 [13.46–20.37] versus 21.11 [17.19–26.02] ng/mL, $p < 0.001$). In terms of body composition, men were more likely to have a lower percentage of fat than women (20.30% [17.10%–23.50%] versus 32.60% [28.80%–36.40%], $p < 0.001$). Compared with men, women had less skeletal muscle mass (16.43 [15.15–17.68] versus 26.04 [24.37–27.70] kg, $p < 0.001$). SMI levels were significantly higher in men than in women (37.30% [35.14%–39.63%] versus 27.72% [25.99%–29.66%], $p < 0.001$).

Among the entire study population, the prevalence of overweight/obesity, central obesity, dyslipidaemia, elevated BP, and hyperglycaemia were 32.9%, 38.2%, 73.2%, 65.7%, and 58.3%, respectively. As shown in Fig. 1, incremental SMI quartiles were associated with a decrease in the frequency of overweight/obesity, central obesity, dyslipidaemia, elevated BP, and hyperglycaemia (all $p < 0.001$). In addition, increasing serum osteocalcin quartiles were associated with reduced frequency of overweight/obesity, central obesity, and hyperglycaemia (all $p < 0.001$). However, dyslipidaemia and elevated BP did not vary with osteocalcin quartiles ($p = 0.646$; $p = 0.309$).

3.2. Association between SMI and serum osteocalcin levels

Figure 2 shows that in men and women, SMI levels displayed an increasing trend from serum osteocalcin levels quartile 1 to quartile 4 ($p = 0.001$; $p < 0.001$). As shown in Table 2, serum osteocalcin levels, either as categorical variable or continuous variable, were a positive predictor of SMI (all $p < 0.001$). However, these relationships disappeared in both men and women after we corrected for age and BMI (standardized $\beta = 0.045$, $p = 0.070$; standardized $\beta = -0.004$, $p = 0.876$).

Table 3 lists the age and BMI-adjusted associations between SMI and serum osteocalcin levels in subgroups of metabolic disorders. Among men, the positive association between SMI and serum osteocalcin levels remained significant in the hyperglycaemia group (standardized $\beta = 0.065$, $p = 0.032$), but not for subgroups of overweight/obesity, central obesity, dyslipidaemia, or elevated BP (all $p > 0.05$). In women, there was no correlation between serum osteocalcin levels and SMI in any subgroup (all $p > 0.05$). Table 4 provides univariate regression analysis with SMI designated as the dependent variable, age, smoking, physical activity, BMI, and other metabolic parameters designated as the independent variables. The analysis demonstrated that physical activity, elevated BP, BMI, HOMA-IR, HDL-C, LDL-C, and serum osteocalcin levels were correlated with SMI ($p = 0.034$–<0.001). The multivariable regression analysis demonstrated that serum osteocalcin levels were independent determinants of SMI (standardized $\beta = 0.068$, $p = 0.024$) in addition to physical activity, BMI, HOMA-IR, and LDL-C. There were no interactions between the variables in the multivariate model ($p = 0.587$).

3.3. Influence of glucose metabolism status on relationship between SMI and serum osteocalcin levels

Figure 3 shows the ordinal logistic regression for SMI divided by glucose metabolism status in men. After adjustment for BMI, physical activity, and hypoglycaemic therapy, the second and fourth serum osteocalcin quartiles had higher OR for increased SMI in men with impaired glucose regulation compared with the lowest quartile (OR = 2.305, 95% CI 1.163–4.572, $p = 0.017$; OR = 2.259, 95% CI 1.127–4.522, $p = 0.021$); however, the OR of the third serum osteocalcin quartile was not significant (OR = 1.958, 95% CI 0.962–3.987, $p = 0.064$). In diabetic patients, there were no significant differences in the ORs for increased SMI from the second to fourth serum osteocalcin quartiles compared with the lowest ($p = 0.412$; $p = 0.317$; $p = 0.357$). Further analysis was performed in the impaired glucose regulation population. The results revealed that in men with isolated impaired glucose tolerance, the OR of increased SMI was 2.861 in the highest serum osteocalcin levels quartile compared with the lowest (95% CI 1.021–8.021, $p = 0.046$). In men with combined impaired fasting glucose and impaired glucose tolerance, the ORs of SMI tended to increase with the rise in serum osteocalcin levels, but this did not reach statistical significance ($p = 0.219$; $p = 0.423$; $p = 0.136$). In men with isolated impaired fasting glucose, the ORs of increased SMI were not significant in any other serum osteocalcin levels quartiles compared with the lowest ($p = 0.300$; $p = 0.370$; $p = 0.853$).

Table 1
Clinical Characteristics of the study subjects.

Variables	Total (n = 1742)	Men (n = 775)	Women (n = 967)
Age (years)	61.20 (56.86–65.32)	61.92 (56.40–66.32)	60.80 (57.00–64.48)*
BMI (kg/m^2)	23.60 (21.70–25.70)	23.90 (22.20–25.80)	23.30 (21.40–25.60)**
W (cm)	84.00 (78.00–91.00)	88.00 (82.00–94.00)	81.20 (76.00–88.00)**
Total body fat percentage (%)	26.70 (20.70–33.40)	20.30 (17.10–23.50)	32.60 (28.80–36.40)**
Total body fat mass (kg)	16.90 (13.00–21.10)	14.10 (11.10–17.70)	19.00 (15.50–23.30)**
Total lean body mass (kg)	43.80 (39.30–54.70)	55.40 (52.20–59.10)	39.60 (37.50–42.00)**
Skeletal muscle mass (kg)	19.03 (16.21–25.65)	26.04 (24.37–27.70)	16.43 (15.15–17.68)**
SMI (%)	31.46 (27.43–36.96)	37.30 (35.14–39.63)	27.72 (25.99–29.66)**
SBP (mmHg)	132.00 (121.00–145.00)	134.00 (124.00–147.00)	129.00 (118.00–142.00)**
DBP (mmHg)	78.00 (71.00–85.00)	80.00 (74.00–87.00)	76.00 (69.00–82.00)**
FPG (mmol/L)	5.84 (5.44–6.44)	5.88 (5.47–6.60)	5.80 (5.42–6.32)**
2hPG (mmol/L)	7.67 (6.17–9.88)	7.81 (6.18–9.96)	7.54 (6.15–9.79)
HbA1c (%)	5.7 (5.5–6.1)	5.7 (5.4–6.1)	5.8 (5.5–6.1)**
Fasting serum insulin (mU/L)	9.00 (6.40–12.81)	8.57 (6.01–12.30)	9.50 (6.76–13.13)**
HOMA-IR	2.43 (1.66–3.61)	2.30 (1.58–3.59)	2.53 (1.73–3.67)*
TC (mmol/L)	5.38 (4.76–6.07)	5.11 (4.54–5.72)	5.59 (5.02–6.30)**
TG (mmol/L)	1.45 (1.02–2.08)	1.50 (1.05–2.21)	1.42 (1.00–1.99)**
HDL-C (mmol/L)	1.37 (1.16–1.64)	1.23 (1.06–1.43)	1.49 (1.28–1.74)**
LDL-C (mmol/L)	3.32 ± 0.83	3.16 ± 0.78	3.44 ± 0.86**
CRP (mg/L)	0.96 (0.44–1.79)	0.88 (0.39–1.65)	1.02 (0.51–1.90)**
Osteocalcin (ng/mL)	19.04 (15.37–23.76)	16.71 (13.46–20.37)	21.11 (17.19–26.02)**
Smoking status, n (%)	372 (21.4)	362 (46.7)	10 (1.00)**
Physical activity			
Light, n (%)	308 (17.7)	137 (17.7)	171 (17.7)
Moderate, n (%)	897 (51.5)	388 (50.1)	509 (52.6)
High, n (%)	537 (30.8)	250 (32.2)	287 (29.7)
Overweight/Obesity, n (%)	573 (32.9)	270 (34.8)	303 (31.3)
Central obesity, n (%)	666 (38.2)	322 (41.5)	344 (35.6)*
Dyslipidaemia, n (%)	1276 (73.2)	527 (68.0)	749 (77.5)**
Elevated BP, n (%)	1145 (65.7)	565 (72.9)	580 (60.0)**
Hyperglycaemia, n (%)	1015 (58.3)	478 (61.7)	537 (55.5)*
Impaired glucose regulation, n (%)	570 (32.7)	262 (33.8)	308 (31.9)*
Diabetes, n (%)	445 (25.5)	216 (27.5)	229 (23.7)*
Hypoglycaemic therapy, n (%)	160 (9.2)	79 (10.2)	81 (8.4)

Data was expressed as mean ± standard deviation for normally distributed variables or the median (interquartile range) for skewed-distribution variables.
*$p < 0.05$ men versus women, **$p < 0.01$ men versus women.
Abbreviation: BMI: body mass index; W: waist circumference; SMI: skeletal muscle mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; 2hPG: 2-h plasma glucose; HbA1c: glycated haemoglobin A1c; HOMA-IR: homoeostasis model assessment-insulin resistance index; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein; BP: blood pressure.

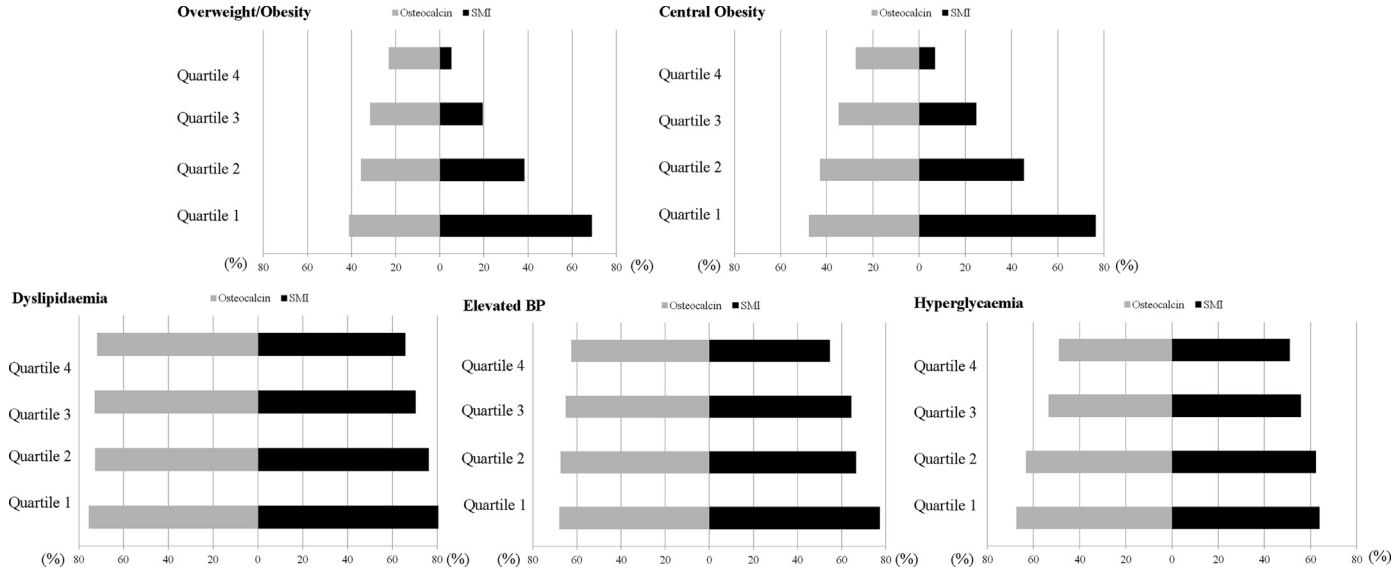

Fig. 1. Prevalence of metabolic disorders by sex-specific quartiles of serum osteocalcin levels and SMI.

Table 5 shows the relationship between standardized SMI and serum osteocalcin levels in the multivariate model among men with isolated impaired glucose tolerance. Analysis revealed that the fourth serum osteocalcin levels quartile resulted in an increase of 0.262 SD in SMI compared with the lowest quartile ($p = 0.027$). Similarly, each SD increase in serum osteocalcin levels resulted in an increase of 0.131 SD in SMI ($p = 0.024$).

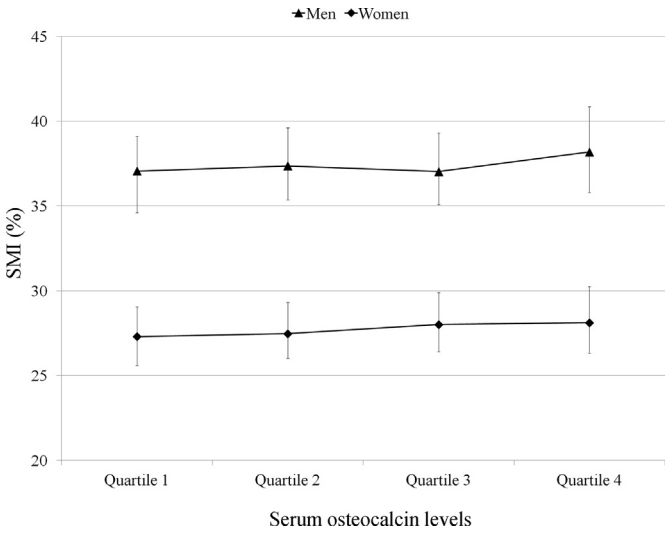

Fig. 2. Distribution of SMIs according to serum osteocalcin quartile group ($p = 0.001$ for men, $p < 0.001$ for postmenopausal women).

4. Discussion

The present study revealed that before accounting for age and BMI, serum osteocalcin levels were positively correlated with SMI in middle-aged and elderly individuals. After stratifying the data set by metabolic factors, the positive relationship between adjusted serum osteocalcin levels and SMI remained significant only in hyperglycaemic men, especially those with isolated impaired glucose tolerance.

Skeletal muscle is the most abundant insulin-sensitive tissue and plays a crucial role in the maintenance of systemic glucose metabolism. Therefore, the loss of skeletal muscle mass can have a negative impact on insulin sensitivity [19]. In addition, adipose tissue expansion contributes to reduced insulin signalling and action in skeletal muscle through increased inflammatory adipokines and increased reactive oxygen species [20]. A study of Korean elderly people reported that HOMA-IR was negatively correlated with appendicular skeletal muscle mass/weight after adjusting for sex and age. After adjusting for confounding factors, this ratio could be used as a strong predictor of metabolic syndrome [21]. Consistent with the aforementioned studies, we further revealed that a decline in SMI had a close correlation with increased body fat and other metabolic or cardiovascular risks. Meanwhile, another study suggested that the development of insulin resistance might further exacerbate skeletal muscle loss [22]. Accumulating evidence shows a pathological, bidirectional association between sarcopenia and metabolic syndrome [20,22].

Osteocalcin regulates glucose metabolism by inducing islet beta-cell proliferation, increasing insulin secretion, and improving insulin sensitivity in mice [23,24]. Animal studies have shown that treating mice with intraperitoneal injections of low-dose osteocalcin can significantly restore insulin sensitivity [23]. Human studies have found that serum osteocalcin levels were negatively associated with HOMA-IR, hyperglycaemia, nonalcoholic fatty liver disease, and metabolic syndrome [10,12,25–27]. In addition to the close relationships among osteocalcin and metabolic diseases, osteocalcin has also received increasing attention because

Table 2
Sex-specific relationships between serum osteocalcin levels and SMI.

	Men (n = 775)			Women (n = 967)		
	Standardized β	t	p	Standardized β	t	p
Quartiles of osteocalcin						
Quartile 1	reference			reference		
Quartile 2	0.056	1.289	0.198	0.037	0.948	0.344
Quartile 3	0.006	0.147	0.883	0.116	2.958	0.003
Quartile 4	0.158	3.627	<0.001	0.154	3.932	<0.001
p for trend	0.002			<0.001		
Continuous osteocalcin						
Unadjusted	0.140	3.932	<0.001	0.150	4.701	<0.001
Age and BMI-adjusted	0.045	1.812	0.070	−0.004	−0.156	0.876

Abbreviation: SMI: skeletal muscle mass index; BMI: body mass index.

Table 3
Age and BMI-adjusted association between serum osteocalcin levels and SMI in subgroups of metabolic disorders.

	Men (n = 775)				Women (n = 967)			
	n	standardized β	t	p	n	standardized β	t	p
Overweight/Obesity								
No	505	0.052	1.491	0.136	664	0.004	0.118	0.906
Yes	270	0.044	0.797	0.426	303	−0.032	−0.607	0.544
Central obesity								
No	453	0.031	0.817	0.414	623	0.009	0.273	0.785
Yes	322	0.067	1.397	0.163	344	−0.050	−0.985	0.326
Dyslipidaemia								
No	248	0.025	0.568	0.570	218	−0.014	−0.260	0.795
Yes	527	0.048	1.563	0.119	749	−0.001	−0.030	0.976
Elevated BP								
No	210	0.016	0.316	0.752	387	−0.010	−0.253	0.801
Yes	565	0.054	1.823	0.069	580	−0.001	−0.031	0.976
Hyperglycaemia								
No	297	0.007	0.157	0.876	430	0.028	0.727	0.468
Yes	478	0.065	2.151	0.032	537	−0.023	−0.679	0.497

Abbreviation: BMI: body mass index; SMI: skeletal muscle mass index; BP: blood pressure.

Table 4
Multivariate regression analysis on SMI in men with hyperglycaemia.

Independent variables	Men with hyperglycaemia (n = 478)					
	Univariate			Multivariate		
	standardized β	t	p	standardized β	t	p
Age	−0.025	−0.536	0.592	–	–	–
Smoking	0.027	0.582	0.561	–	–	–
Physical activity	0.097	2.122	0.034	0.070	2.325	0.020
Elevated BP	−0.237	−5.325	<0.001	−0.008	−0.245	0.806
BMI	−0.745	−24.394	<0.001	−0.702	−19.324	<0.001
FPG	0.019	0.424	0.672	–	–	–
2hPG	−0.036	−0.793	0.428	–	–	–
HOMA-IR	−0.342	−7.940	<0.001	−0.094	−2.580	0.010
TC	−0.088	−1.917	0.056	–	–	–
TG	−0.037	−0.815	0.415	–	–	–
HDL-C	0.204	4.546	<0.001	−0.062	−1.886	0.060
LDL-C	−0.164	−3.638	<0.001	−0.083	−2.759	0.006
CRP	−0.070	−1.540	0.124	–	–	–
Osteocalcin	0.117	2.570	0.010	0.068	2.257	0.024
Hypoglycaemic therapy	−0.031	−0.684	0.494	–	–	–

Multivariate model included variables that showed a significant association ($p < 0.05$) in the univariate analysis.
Abbreviation: SMI: skeletal muscle mass index; BP: blood pressure; BMI: body mass index; FPG: fasting plasma glucose; 2hPG: 2-h plasma glucose; HOMA-IR: homoeostasis model assessment-insulin resistance index; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein.

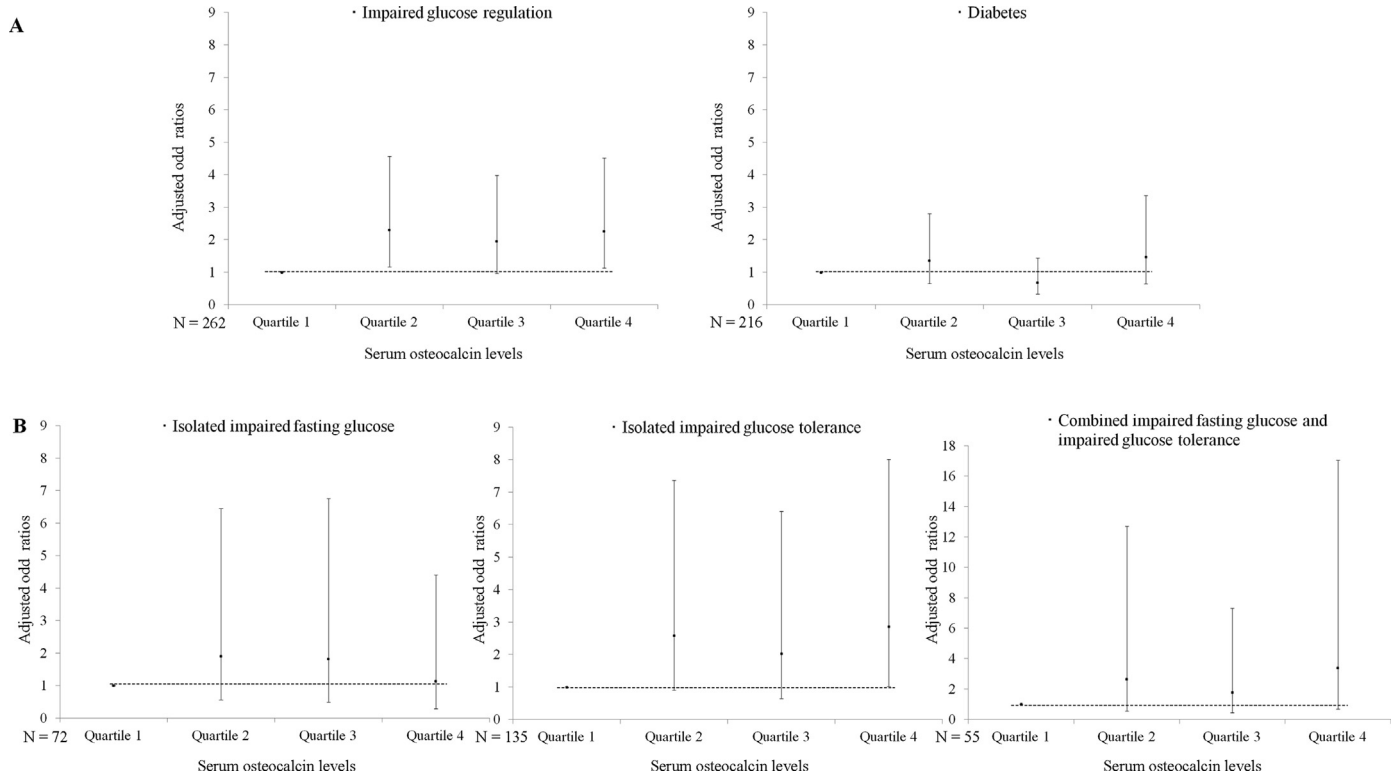

Fig. 3. Adjusted odds ratios of increased SMI by serum osteocalcin quartile (A) in impaired glucose regulation and diabetes, respectively, and (B) in impaired glucose regulation divided by isolated impaired fasting glucose, isolated impaired glucose tolerance, and combined isolated impaired fasting glucose and isolated impaired glucose tolerance. Odds ratios were adjusted for BMI, physical activity, and hypoglycaemic therapy.

of its relationship with skeletal muscle mass. Animal studies have found that exogenous osteocalcin is sufficient to restore 15-month-old mice to the exercise capacity of 3-month-old mice [6]. Treatment with exogenous osteocalcin for 28 days was sufficient to increase muscle mass of 9-month-old wild-type mice [8]. Osteocalcin could promote protein synthesis in myotubes without affecting protein breakdown [8]. However, the relationship between serum osteocalcin levels and skeletal muscle mass in humans remained unclear.

The strength of this study is that we conducted a subgroup analysis of the effect of metabolic disorders on the relationship between serum osteocalcin levels and SMI; moreover, men with intermediate hyperglycaemia were further divided into three impaired glucose statuses. This study found an independent relationship between serum osteocalcin levels and SMI adjusted for BMI and other confounding factors in men with hyperglycaemia, particularly in those with isolated impaired glucose tolerance. The explanation for the close relationship between serum osteocalcin

Table 5
Association of serum osteocalcin levels on per 1.0 SD increase SMI in isolated impaired glucose tolerance.

Variables	Men (n = 135)		
	standardized β	t	*p*
Quartiles of serum osteocalcin			
Quartile 1	reference		
Quartile 2	0.162	1.399	0.164
Quartile 3	0.121	1.093	0.277
Quartile 4	0.262	2.243	0.027
p for trend	0.043		
Continuous osteocalcin (multivariate model)			
Per 1.0 SD increase osteocalcin	0.131	2.287	0.024

Multivariate model included physical activity, elevated BP, BMI, HOMA-IR, HDL-C, LDL-C, and osteocalcin.
Abbreviation: SD: standard deviation; SMI: skeletal muscle mass index; 2hPG: 2-h plasma glucose; BMI: body mass index; W: waist circumference; BP: blood pressure; FPG: fasting plasma glucose; HOMA-IR: homoeostasis model assessment-insulin resistance index; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein.

levels and SMI is that impaired glucose tolerance is characterized by insulin resistance in peripheral tissues such as muscle. Serum osteocalcin levels had a protective effect on maintaining muscle mass in men with impaired glucose tolerance, which was likely to be mediated by improving insulin resistance. In the overall data set of men and postmenopausal women, serum osteocalcin levels were not associated with SMI after adjusting for age and BMI. Therefore, we hypothesized that the association between serum osteocalcin levels and relative skeletal muscle mass in men was possibly affected by hyperglycaemia and insulin resistance. However, we found no evidence of an association between serum osteocalcin levels and SMI in diabetic patients, 36.0% of whom were receiving hypoglycaemic treatment. This result suggests that normalization of blood glucose may attenuate the influence of serum osteocalcin levels on SMI. Isolated impaired glucose tolerance is characterized by insulin resistance in peripheral tissues such as muscle. The present study demonstrated that serum osteocalcin levels were significantly correlated with SMI in isolated impaired glucose tolerance. A previous study found that among 504 healthy Chinese women with an average BMI of approximately 22 kg/m^2, serum levels of total osteocalcin were positively associated with FFM in premenopausal women but not in postmenopausal women [9].

We found no relationship between serum osteocalcin levels and SMI in postmenopausal women. This gender difference has not been fully elucidated, but possible explanations may include the effects of sex hormones on skeletal muscle. Testosterone is positively related to muscle size and strength in men, while oestrogen primarily affects FM and has a neutral or negative effect on muscle mass [28]. Women generally have less skeletal muscle mass than men, and this reduced skeletal muscle tissue may weaken the influence of osteocalcin. In addition, cross-talk has been observed among signals derived from both skeletal muscle and adipose tissue [29]. A study involving 6021 Koreans found that sarcopenia was not associated with a metabolically unhealthy phenotype in obese subjects, indicating that skeletal muscle mass had a limited direct biological effect in obese subjects [30]. Women tend to have more body fat than men, and whether this contributes to the observed gender differences is unknown.

One limitation of our study was that skeletal muscle mass was measured via BIA. Magnetic resonance imaging is the gold standard for the measurement of skeletal muscle mass. However, the correlation between muscle mass as predicted using BIA and muscle mass measured using magnetic resonance imaging was 0.93, and the standard error of the estimate for predicting skeletal muscle mass from BIA was 9%, supporting our use of BIA. We did not differentiate serum osteocalcin levels with respect to γ-carboxylated status; nevertheless, total osteocalcin levels were associated with energy metabolism, similar to its under-carboxylated form [31]. In addition, the bioavailability of metabolically active under-carboxylated osteocalcin was dependent on circulating vitamin K levels. However, vitamin K was not measured in this study. Our study was not designed to test for a causal relationship between skeletal muscle mass and serum osteocalcin levels, and further formalized and large-scale prospective designs are needed to clarify this issue.

In conclusion, a positive relationship between serum osteocalcin levels and SMI was detected in men with hyperglycaemia, particularly in those with isolated impaired glucose tolerance. No relationship was detected between serum osteocalcin levels and SMI in postmenopausal women.

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Statement of authorship

Yuqian Bao conceived and designed the experiments. Yiting Xu, Xiaojing Ma, Yun Shen, Chengchen Gu, and Junling Tang performed the experiments. Yiting Xu and Xiaojing Ma performed the statistical analysis and wrote the paper.

Conflict of interest

The authors have no conflict of interest to disclose.

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