Low osteocalcin level is a risk factor for impaired glucose metabolism in a Chinese male population

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ABSTRACT
Aims/Introduction: This study was to assess the association between serum osteocalcin level and glucose metabolism in a Chinese male population.

Materials and Methods: We carried out a cross-sectional study with a cohort of participants from the Fangchenggang Area Male Health and Examination Survey. The cross-sectional study was carried out among 2,353 men, including 2,139 participants with normal glucose tolerance, 148 with impaired fasting glucose and 66 with type 2 diabetes. A subsample of 1,109 men with measurement of osteocalcin was observed in the cohort. After a 4-year follow-up period, 1,049 non-diabetic and 983 participants with normal glucose tolerance who submitted the available information were enrolled in the cohort. Participants were divided into group-H (≥23.33 ng/mL) and group-L (<23.33 ng/mL) by osteocalcin level.

Results: In the cross-sectional study, osteocalcin levels were highest in participants with normal glucose tolerance, followed by those with impaired fasting glucose and type 2 diabetes (P < 0.001). In partial correlation analysis adjusted for age, serum osteocalcin level was related to glucose level (r = −0.082, P < 0.001), insulin level (r = −0.079, P < 0.001) and insulin resistance (r = −0.065, P = 0.002). Compared with group-H, group-L was associated with an increased risk of type 2 diabetes (odds ratio 2.107, 95% confidence interval 1.123–3.955), impaired fasting glucose (odds ratio 2.106; 95% CI 1.528–2.902), and insulin resistance (odds ratio 1.359, 95% confidence interval 1.080–1.710) adjusted for age, education levels, cigarette smoking and lipid profiles. In the cohort study, the increased risk of impaired fasting glucose was significant in group-L vs group-H (3.3% vs 1.2%, P = 0.026).

Conclusions: Low serum osteocalcin level was a risk factor for impaired glucose metabolism and subsequent type 2 diabetes.

INTRODUCTION
Recently, more and more research has examined osteocalcin (OCN), which is reported to be central in the cross-talk between bone metabolism and glucose metabolism. OCN, a 49-amino acid bone matrix non-collagen protein, is secreted in the general circulation by osteoblastic cells. OCN consists of two forms, undercarboxylated osteocalcin (ucOCN) and carboxylated osteocalcin (cOCN). ucOCN is formed after the glutamic acid residues of OCN on the 17th, 21st and 24th sites are carboxylated by vitamin K-dependent carboxylase. OCN is typically considered a biochemical marker of bone turnover and bone formation, and is involved in several physiological functions, such as maintaining normal bone mineralization, suppressing abnormal hydroxyapatite formation and slowing down growth cartilage mineralization. However, a new function of OCN has been shown as a hormone regulating glucose metabolism. It is found to be responsible for some functions in...
type 2 diabetes, such as favoring β-cell proliferation, insulin expression and secretion, as well as sensitivity to insulin in peripheral tissues. Lack of the OCN gene in mice leads to insulin resistance and hyperglycemia. Furthermore, administration of recombinant OCN into wild-type mice enhances pancreatic β-cell proliferation and insulin secretion, and protects them from obesity and type 2 diabetes. Several cross-sectional studies on Caucasian and Asian populations have shown that total OCN level is significantly negatively correlated with fasting blood glucose, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR). A similar result was observed in a study on a Chinese population of 254 men and 246 women. That cross-sectional study showed that total OCN level is closely associated with glucose metabolism.

To date, the research investigating the relationship between OCN and glucose metabolism in the Chinese population has been carried out on a relatively small sample, and there is a lack of prospective study; thus, we carried out a cross-sectional study with a cohort in the Fangchenggang male population to further explore the relationship.

MATERIALS AND METHODS

Ethics statement

The study was approved by the medical ethics committee of Guangxi, China. All patients provided written informed consent.

Study design and population

The Fangchenggang Area Male Health and Examination Survey was a population-based prospective cohort study carried out in Guangxi among Chinese males aged 17–88 years. A total of 4,043 subjects were investigated at the baseline survey from September to December 2009. Subjects with the following conditions were excluded from the study: (i) current coronary heart disease, stroke, hyperthyroidism, hypothyroidism, rheumatoid arthritis, hypercortisolism, chronic and/or acute kidney disease and cancer; (ii) liver dysfunction (alanine transaminase >3 times upper limit of normal); and (iii) current treatment with relative drug (vitamin D, bisphosphonate, calcitriol, estrogen, tamoxifen, denosumab, parathyroid hormone etc.). Finally, 2,353 men completed the OCN measurements and were enrolled in the cross-sectional analysis. In the cohort, a relatively stable sample of 1,809 participants were included and followed up for 4 years, including 1,109 men with information on serum OCN. Finally, 1,049 non-diabetic and 983 participants with normal glucose tolerance (NGT) who submitted the available information entered the cohort. All participants provided written informed consent. Participants were divided into two groups according to the median OCN level, group-H (≥23.33 ng/mL) group-L (<23.33 ng/mL).

Baseline examination

A complete physical examination was carried out. Information on personal sociodemographic characteristics (age, educational levels etc.), lifestyle (cigarette smoking, alcohol consumption, physical activity), medical history and medication use were also obtained by in-person interviews. Education levels were categorized into three groups according to the number of years of education (0–6, 7–9 and ≥10 years). Cigarette smoking was defined as current smoker (still smoking or had smoked within the past 6 months), ex-smoker (quit smoking more than 6 months previously) and non-smoker (had never smoked). Frequency and total consumption of several forms of alcohol were recorded, including local spirits, branded spirits, wine, beer and so on. One drink was equal to 8 g of ethanol (260 mL of beer, 120 mL of wine and 30 mL of liquor etc.). Alcohol consumption was defined as more than one drink per week. Participants were prompted to report the frequency and duration of any physical activity with a minimum length of 10 consecutive minutes. Physical activity was considered as more than two times per week. Family history was considered as positive when at least one of the participant’s parents or siblings had suffered from type 2 diabetes. Weight and height were measured by trained interviewers in the morning, requiring participants to wear light clothing and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m²).

Laboratory examination

All blood samples were drawn after an overnight fast of more than 12 h, and transported frozen to the testing center of the Department of Clinical Laboratory at the First Affiliated Hospital of Guangxi Medical University in Nanning, China, within 2 h; there they were centrifuged for 15–25 min and stored at −80°C until analysis. Fasting plasma glucose, insulin and lipid profiles, including total cholesterol, high-density lipoprotein, low-density lipoprotein and triglycerides, were determined by a Dimension-RxL Chemistry Analyzer (Dade Behring, Newark, DE, USA) in the Department of Clinical Laboratory at the Fangchenggang First People’s Hospital. Serum OCN was analyzed using a Cobas 6000 system E601 (Elecys module) immunoassay analyzer (Roche Diagnostics, GmbH, Mannheim, Germany). The interassay coefficient of variation was 4.5%.

Definition of type 2 diabetes, impaired fasting glucose, NGT and insulin resistance

Type 2 diabetes was defined as the presence of one or more of the following: (i) fasting plasma glucose (FPG) level ≥7.0 mmol/L in two different measurements; (ii) use of antidiabetic medication; or (iii) self-reported or physician diagnosis. Impaired fasting glucose (IFG) was defined as glucose levels of 6.1–7.0 mmol/L. Subjects were defined as having normal fasting glucose when the glucose level was less than 6.1 mmol/L. Insulin resistance was estimated by calculating HOMA-IR. The formula was HOMA-IR = (fasting plasma glucose [mmol/L] × fasting insulin [mcU/mL])/22.5. Insulin resistance was considered as HOMA-IR greater than 2.6.
Statistical analysis
All statistical analyses were processed with SPSS 17.0 (SPSS, Chicago, IL, USA). Normal distributed data were expressed as the mean ± standard deviation, whereas variables with a skewed distribution were reported as the median (interquartile range 25–75%). Categorical variables were represented as frequency and percentage. The distribution of variables was analyzed by the Kolmogorov–Smirnov test. In the cross-sectional study, a one-way ANOVA was used to compare numerical variables among participants with type 2 diabetes, IFG and NGT. Numerical data were analyzed by a non-parametric test. Comparisons in categorical variables (cigarette smoking, alcohol consumption, family history, education levels) were analyzed by the χ²-test with continuity correction or Fisher’s exact test when required. Correlation coefficients between OCN level and BMI, lipid profiles, glucose level, HOMA-IR and insulin level were calculated by partial correlation controlling for age. Potential confounding factors, including age, BMI, lipid profiles, cigarette smoking and education levels, were included in the regression models. Logistic regression analysis was used to determine the risk for type 2 diabetes, IFG and insulin resistance between two groups (group-L and group-H) adjusted for potential confounding factors. Group-H acted as a reference, and the results were presented as odds ratios (OR) and 95% confidence interval (95% CI). In the cohort study, the incidences of type 2 diabetes and IFG were compared by the χ²-test. For all comparisons, values were considered to be significant when P < 0.05.

RESULTS
We describe the general characteristics of the cross-sectional study participants in Table 1. In all, data are presented for 2,353 participants, divided into NGT (n = 2,139), IFG (n = 148) and type 2 diabetes (n = 66). The average age was 37.59 ± 10.936 years. Participants with type 2 diabetes had the lowest level of OCN, followed by those with IFG and NGT (18.58 ng/mL [15.75–22.59 ng/mL] vs 20.70 ng/mL [17.08–24.97 ng/mL] vs 23.6 ng/mL [19.24–29.23 ng/mL]; P = 0.001). Compared with participants with NGT, participants with IFG and type 2 diabetes were older, and showed higher insulin level, HOMA-IR, BMI, cholesterol, triglycerides, lower education level and were more likely to have smoked.

Figure 1 presents the distribution of OCN levels, and how the median level of OCN varied among participants with NGT, IFG and type 2 diabetes. Compared with participants with NGT, serum OCN level decreased significantly in those with IFG and type 2 diabetes.

Partial correlations analyses adjusted for age were carried out between OCN levels and selected factors (Table 2). In the

Table 1 | General characteristics of the participants in the cross-sectional study

|             | NGT (n = 2,139) | IFG (n = 148) | T2DM (n = 66) | P-value |
|-------------|-----------------|--------------|---------------|---------|
| Age (years) | 37 ± 11         | 44 ± 12      | 46 ± 11       | <0.001**|
| BMI (kg/m²) | 23.02 ± 3.19    | 24.28 ± 3.63 | 24.24 ± 3.61  | <0.001**|
| Osteocalcin (ng/mL) | 23.6 (19.24–29.23) | 20.70 (17.08–24.97) | 18.58 (15.75–22.59) | <0.001**|
| Insulin (uU/mL) | 6.26 (4.19–9.24) | 8.64 (5.31–15.11) | 8.76 (4.7–19.85) | <0.001**|
| HOMA-IR | 1.41 (0.94–2.15) | 2.47 (1.50–4.31) | 3.56 (1.98–7.78) | <0.001**|
| Total cholesterol (mmol/L) | 5.63 ± 1.00 | 6.10 ± 1.15 | 6.20 ± 1.31 | <0.001**|
| Triglyceride (mmol/L) | 1.10 (0.76–1.69) | 1.45 (0.90–2.63) | 1.96 (1.19–3.61) | <0.001**|
| HDL (mmol/L) | 1.40 ± 0.30 | 1.44 ± 0.38 | 1.67 ± 0.81 | 0.026* |
| LDL (mmol/L) | 2.93 ± 0.80 | 3.16 ± 0.88 | 3.11 ± 0.81 | 0.003* |
| Cigarette smoking, n (%) | | | | |
| Non-smoker | 997 (46.7%) | 63 (42.6%) | 29 (43.9%) | 0.03* |
| Ex-smoker | 72 (3.4%) | 6 (4.1%) | 8 (21.1%) | |
| Current smoker | 1,067 (50.0%) | 79 (53.4%) | 29 (43.9%) | |
| Alcohol consumption (yes), n (%) | 858 (40.2%) | 70 (47.3%) | 33 (50.0%) | 0.73 |
| Physical activity (yes), n (%) | 500 (23.4%) | 34 (23.0%) | 16 (24.2%) | 0.980 |
| Family history for type 2 diabetes (yes), n (%) | 79 (3.7%) | 7 (4.7%) | 6 (9.1%) | 0.073 |
| Education levels (years) | | | | |
| 0–6 | 52 (2.4%) | 13 (8.8%) | 10 (13.8%) | <0.001** |
| 7–10 | 1,359 (63.7%) | 101 (68.2%) | 41 (63.1%) | |
| >10 | 723 (33.9%) | 34 (23.0%) | 15 (23.1%) | |

Data are presented as mean ± standard deviation or n (%). Osteocalcin, insulin and homeostasis model assessment of insulin resistance (HOMA-IR); the data were shown as median and interquartile range. The percentage may not sum to 100 because of rounding, *P < 0.05, **P < 0.001 (one-way ANOVA and non-parametric test was used for numerical data, while the χ²-test with continuity correction or Fisher’s exact test was used for categorical variable). BMI, body mass index; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus.
whole study population, serum OCN level was negatively correlated with glucose level \((r = -0.082, \ P < 0.001)\), insulin level \((r = -0.079, \ P < 0.01)\), low-density lipoprotein \((r = -0.094, \ P < 0.001)\), total cholesterol \((r = -0.105, \ P < 0.001)\), triglycerides \((r = -0.140, \ P < 0.001)\), BMI \((r = -0.258, \ P < 0.001)\) and HOMA-IR \((r = -0.065, \ P = 0.002)\). OCN level, however, did not show a significant correlation with high-density lipoprotein \((P = 0.282)\).

Logistic regression analysis was carried out using the presence of type 2 diabetes, IFG and insulin resistance as dependent variables, and Group-H as a reference (Table 3). As the results (model 3) show, participants with low OCN level were significantly associated with type 2 diabetes \((OR 2.107, 95\% \ CI 1.123–3.955)\), IFG \((OR 1.752, 95\% \ CI 1.258–2.439)\) and insulin resistance \((OR 1.359, 95\% \ CI 1.080–1.710)\). After further adjustment for BMI (model 4), OR decreased slightly and remained significant for type 2 diabetes \((OR 1.988, 95\% \ CI 1.052–3.75)\) and IFG \((OR 1.626, 95\% \ CI 1.162–2.274)\), but not for insulin resistance \((OR 1.034, 95\% \ CI 0.807–1.326)\).

After the 4-year follow up, no difference was observed between the two groups (group-L 1.9%, group-H 0.8%; \(P = 0.117)\). In contrast, low OCN level was associated with increased incidence of IFG (group-L 3.3%, group-H 1.2%; \(P = 0.026)\).

**DISCUSSION**

Ever growing research explores the role of OCN in impaired glucose metabolism, and whether serum OCN level is independently associated with glucose metabolism is still under debate.

In the current study, we assessed the association between OCN level and glucose metabolism by carrying out a cross-sectional study within a cohort. As the results show, in the cross-sectional study, we found a significantly higher OCN level in participants with NGT compared with those with IFG and type 2 diabetes. Furthermore, OCN level is negatively correlated with glucose level, insulin level and HOMA-IR.

Participants with a low OCN level showed an increased risk for type 2 diabetes, IFG and insulin resistance compared with those with a high OCN level. In the cohort, we also found increased incidences of type 2 diabetes and IFG in participants with a low OCN level. These results confirm the relationship that low OCN level is a risk factor for impaired glucose metabolism, in line with previous studies\(^{12,13}\).

The mechanisms associated with OCN and glucose metabolism are explained in several ways. Animal experiments show
that OCN mediates Gprc6a to promote the secretion of gut glucagon-like peptide-1, which mediates the stimulatory effect of OCN on insulin secretion. Another researcher showed that OCN increases CyclinD2 and Cdk4 expression, two genes necessary for β-cell proliferation in vivo, with a dose response similar to the one observed for insulin expression. Previous studies also suggest that OCN increases insulin sensitivity in the liver, muscle and adipose tissue by upregulation of adiponectin gene expression, which mediates through two adiponectin receptors (AdipoR1 and 2) to enhance insulin sensitivity and maintain a functional β-cell mass. As the active form of OCN, the interaction of ucOCN with glucose metabolism has also been widely discussed. In animal models, the role of ucOCN has been shown to be a positive modulator of insulin secretion. In human studies, a significant association of higher osteocalcin level with a lower risk of diabetes is observed. These observations, including ours, show that total OCN is associated with glucose metabolism.

Table 3 | Logistic regression calculate the odds ratios for type 2 diabetes mellitus, impaired fasting glucose and insulin resistance in the cross-sectional study

|                     | Group-L (n = 1,177) | Group-H (n = 1,173) |
|---------------------|---------------------|---------------------|
|                     | <23.33 ng/mL        | ≥23.33 ng/mL        |
| T2DM                |                     |                     |
| Unadjusted          | 1                   | 3.497 (1.955–6.255)** |
| Model 1             | 1                   | 2.562 (1.416–4.653)*  |
| Model 2             | 1                   | 2.759 (1.495–5.089)*  |
| Model 3             | 1                   | 2.107 (1.123–3.955)*  |
| Model 4             | 1                   | 1.988 (1.052–3.758)*  |
| IFG                 |                     |                     |
| Unadjusted          | 1                   | 2.657 (1.954–3.613)** |
| Model 1             | 1                   | 2.000 (1.457–2.746)** |
| Model 2             | 1                   | 2.106 (1.528–2.902)** |
| Model 3             | 1                   | 1.752 (1.258–2.439)*  |
| Model 4             | 1                   | 1.626 (1.162–2.274)*  |
| Insulin resistance  |                     |                     |
| Unadjusted          | 1                   | 1.754 (1.426–2.159)** |
| Model 1             | 1                   | 1.702 (1.372–2.112)** |
| Model 2             | 1                   | 1.673 (1.346–2.078)** |
| Model 3             | 1                   | 1.359 (1.080–1.710)*  |
| Model 4             | 1                   | 1.034 (0.807–1.326)   |

Data were analyzed by odds ratios and 95% confidence intervals. *P < 0.05, **P < 0.001 (logistic regression was used to calculate the odds ratios for type 2 diabetes mellitus [T2DM], impaired fasting glucose [IFG] and insulin resistance). Model 1 was adjusted for age. Model 2 was further adjusted for cigarette smoking and education level. Model 3 was further adjusted for total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein. Model 4 was further adjusted for body mass index. Group-H, osteocalcin level ≥23.33 ng/mL; group-L, osteocalcin level <23.33 ng/mL.
To our knowledge, studies on the association between OCN and glucose metabolism in the Chinese population are limited; furthermore, the sample sizes included in studies are relatively small. In an attempt to fill this gap, we carried out a cross-sectional study with our cohort. In this way, we can confirm not only the cross-talk between OCN and glucose metabolism in a cross-sectional study, but also test the role of OCN in impaired glucose metabolism after follow up.

Of course, limitations also exist. First, the study population only included male participants who visited hospital for a check-up. Therefore, we do not know whether the association between OCN and glucose metabolism exists in the female population. Furthermore, we do not use the oral glucose tolerance test or glycated hemoglobin for the diagnosis of diabetes. Thus, the incidence of diabetes might be underestimated. Third, we describe the association between obesity and osteocalcin only according to BMI: more measurements are required in further research. What’s more, we do not detect undercarboxylated osteocalcin, which might provide more insight to explain the mechanism. Despite these limitations, we were also able to provide initial evidence for a relationship between OCN and glucose metabolism.

The present study explored the association between OCN level and glucose metabolism. In a cross-sectional study, the present results showed that decreased OCN serum level is significantly associated with increased fasting plasma glucose, insulin level and HOMA-IR, and related to a high prevalence of type 2 diabetes, IFG and insulin resistance. Furthermore, in the cohort, participants with a decreased OCN level showed an increased risk of IFG after the 4-year follow up. These observations suggest that low OCN level is a risk factor for impaired glucose metabolism. However, the underlying mechanism is still not identified, and further studies are necessary to clarify this point.

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DISCLOSURE

The authors declare no conflict of interest.

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