High serum Fetuin-B levels are associated with the presence of metabolic syndrome in women: a case-controlled study and interventional studies

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Research

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Abstract

Background: Previous studies have investigated circulating fetuin-B and its association with type 2 diabetes mellitus (T2DM). However, the variation and regulatory factors of serum fetuin-B levels in individuals with metabolic syndrome (MetS) have not been explained. This study evaluated circulating fetuin-B concentrations in newly diagnosed individuals with MetS and analyzed the impacts of blood glucose, insulin, and glucagon-like peptide-1 receptor agonists (GLP-1RA) treatment on circulating fetuin-B in vivo.

Methods: A total of 377 women (192 MetS and 185 healthy subjects) were recruited for cross-sectional study. The euglycemic-hyperinsulinemic clamp (EHC) and oral glucose tolerance test (OGTT) were implemented in healthy women and those with MetS. Serum fetuin-B were examined by an ELISA kit. For the GLP-1RA intervention experiment, twenty-four women with MetS were treated with Liraglutide for 6 months.

Results: Serum fetuin-B levels were markedly higher in women with MetS as compared to healthy women. Circulating fetuin-B reflected a positive correlation with body mass index, waist hip ratio (WHR), the percentage of fat in vivo (FAT%), triglyceride, fasting blood glucose, 2-hour blood glucose after glucose overload, glycosylated hemoglobin, fasting plasma insulin, 2-hour plasma insulin after glucose overload, homeostasis model assessment of insulin resistance (HOMA-IR), visceral adiposity index (VAI), and lipid accumulation product (LAP). Multivariate linear regression analyses demonstrated that triglyceride and WHR were independently related factors of serum fetuin-B. Serum fetuin-B levels were related to MetS by binary logistic regression analysis. In fact, serum fetuin-B was markedly elevated in healthy women after glucose loading and in MetS women during the EHC. After six months of GLP-1RA intervention, serum fetuin-B levels in MetS subjects decreased following improvement of metabolism and insulin sensitivity.

Conclusions: Serum fetuin-B levels were associated with MetS and its components and regulated by glucose and insulin. GLP-1RA treatment can reduce serum fetuin-B levels in women with MetS.

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Background

Metabolic syndrome (MetS) is a cluster of symptoms, including insulin resistance (IR), abdominal obesity, hypertension, and dyslipidemia(1, 2). Over the past few decades, MetS and obesity have developed into global epidemics due to high-fat diets and a sedentary lifestyle, causing a substantial economic burden for the health care system(3, 4). The risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) is increased by 2 or 5-fold in people with MetS, respectively(5, 6). Furthermore, MetS increases all-cause mortality from 1.30 - to 1.70-fold, and female patients show a higher incidence than male(7, 8). The pathogenesis of MetS and the way in which its components interact remain uncertain, and there are a lack
of consistent treatment recommendation. Therefore, for the optimal management of patients with MetS, it is critical to find a biomarker that can predict outcomes and therapeutic responses accurately.

Fetuin-like protein IRL685 (Fetuin-B) was first identified as the second member of the cystatin superfamily of cysteine protease inhibitors in 2000. It is 22% homologous with fetuin-A(9). Fetuin-B is encoded by the FETUB gene, which has a chromosomal localization of 3q27.3 with eight exons, where the region has been confirmed to be prone to MetS and diabetes(9). The expression of Fetuin-B was also found to increase in isolated hepatocytes from mice with liver steatosis, myotubes and IR hepatocytes. In addition, fetuin-B silencing improved glucose tolerance in obese mice but did not affect body weight(10). In a clinical study, serum fetuin-B was found to be strongly associated with triglyceride (TG) contents in the liver, abdominal obesity, IR, and early insulin secretion stimulated by glucose(11, 12). It has also been reported that the fetuin-B concentrations in patients with nonalcoholic fatty liver (NAFLD), T2DM, polycystic ovary syndrome (PCOS), and gestational diabetes mellitus (GDM) are significantly increased compared with healthy controls(13, 14, 15). Furthermore, fetuin-B is also considered to be related to cardiovascular disease (CVD) and chronic kidney disease (CKD)(16, 17). Therefore, previous studies have suggested that fetuin-B, as an adipokine or hepatokine, is closely related to glucose and lipid metabolism. However, the association of fetuin-B with MetS and its components is unclear at present.

In this study, we measured serum fetuin-B levels in healthy individuals and newly diagnosed MetS patients. To investigate the association of serum fetuin-B with MetS as well as IR, we performed multiple intervention experiments, including euglycemic- hyperinsulinemic clamp (EHC), oral glucose tolerance test (OGTT) and glucagon-like peptide-1 receptor agonists (GLP-1RA) treatment, in healthy subjects and patients with MetS.

**Methods**

**Human participants**

A total of 377 Chinese women (192 MetS, 185 healthy subjects; age, 38 ± 15 yr; BMI, 23.7 ± 4.2 kg/m²) were recruited from the outpatients attending the Department of Endocrinology at the Second Affiliated Hospital, Chongqing Medical University, as well as from the community or universities through advertisement or routine medical examination between December 2016 and December 2018. The diagnostic criteria of MetS are based on the diagnostic guideline of the United States National Cholesterol Education Program (NCEP) Expert Panel Adult Treatment Panel (ATP) III criteria(18). Participants are diagnosed for MetS with three or more following criteria: 1) central obesity ( Asian female: waist circumference ≥ 80cm, male ≥ 90cm); 2) hypertension ( ≥ 130/85 mmHg); 3) hyperglycemia (Fasting glucose ≥ 5.6mmol/L or T2DM); 4) elevated plasma TG ( ≥ 1.69mmol/L); 5) low level of high-density lipoprotein-cholesterol (Men: HDL-C < 1.03 mmol/L, Women: HDL-C < 1.29 mmol/L). Participants were divided into normal control (NGT), impaired glucose tolerance (IGT) or T2DM by the diagnostic criteria of the ADA (American Diabetes Association)(19). Exclusion criteria included heart, hepatic and renal failure, malignant tumors, type 1 diabetes, pregnancy, long-term use of steroids, acute infection, or other chronic metabolic diseases. In this study, all MetS participants were newly diagnosed without any drug treatment,
physical exercise, or diet control. The control subjects had no other disease history, hypertension, or diabetes family history, and the blood glucose was normal. This study was approved by the Human Research Ethics Committee of Chongqing Medical University and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

**Anthropometric examination and biochemical measurement**

After an overnight fast of at least 12 hours, anthropometric measurements and blood sample collections were performed by professionals in all participants. The details of body measurements [weight, height, waist circumference (WC), hip circumference (HC), blood pressure, the percentage of fat in vivo (FAT%) ] and biochemical varies including fasting (FBG), and 2h post-OGTT glucose (2h-BG), insulin, glycosylated hemoglobin (HbA1c), TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), free fatty acid (FFA) were measured as reported previously(20). A 75 g, 2 h OGTT by reference to the standard of ADA (19) was conducted in all the women.

**Measurements of serum fetuin-B**

Serum fetuin-B was determined by a commercial ELISA kit (RayBiotech, Inc. Norcross, GA, USA), following the manufacturer's instructions. The detection limit of fetuin-B Kit was 4.0 ng/mL, and the intra- and inter-assay coefficients of variation (CV) were 10 % and 12 %, respectively. The assay had high sensitivity and excellent specificity without obvious cross-reaction or interference.

**Euglycaemic-hyperinsulinaemic clamp (EHC)**

EHCs were performed in 16 patients with MetS and 27 healthy subjects, as previously reported(20). Regular human insulin (1mU/kg/min) was infused for 2h, and 20% glucose was infused to maintain blood glucose at the primary level. Blood glucose level was measured every 15min during the EHC to guide the glucose infusion rate (GIR). During the steady-state of the clamp, GIR was equal to glucose disposal rate (GRD) and related to body weight (M-value). Blood were collected at 0 min, 80 min, 100 min, and 120min, respectively, for determining serum fetuin-B and other parameters. Blood was centrifuged to separate serum and stored at -80 ºC for subsequent analysis.

**Interventional therapy of liraglutide (GLP-1RA)**

24 obese females with MetS participated in the GLP-1RA intervention study for six months. Inclusion criteria were age 18-35 years old and BMI 25-35 kg/m². Exclusion criteria included medullary thyroid carcinoma or family history of thyroid tumor, severe gastrointestinal diseases, pregnancy, and recent medication history. The initial dose of liraglutide was 0.6mg/d, increasing by 0.6mg/d every week until 1.8mg/d before breakfast. All participants signed informed consent for the side effects of liraglutide before treatment. Anthropometric and biochemical examination, the OGTT and EHC were performed in all subjects as detailed above at pre-therapy, week 12, and week 24, separately.

**Calculations**
BMI ($\text{kg/m}^2$) was calculated as weight (kg) divided by squared height (m$^2$). Waist-to-hip ratio (WHR) was calculated by WC (cm) / HC (cm). M-value was calculated by GIR/body weight, as previously described(20). Homeostasis model assessment of insulin resistance (HOMA-IR) = [fasting insulin (FLns, mU/L) × FBG (mmol/L)] / 22.5, and the cut-off value of IR was defined as HOMA-IR>3(21). Visceral adiposity index (VAI) (females)= [WC(cm)/(36.58 + BMI (kg/m$^2$) × 1.89)] × [TG (mmol/L)/0.81] × [1.52/HDL-C (mmol/L)]. Lipid accumulation product (LAP) (females)= [WC (cm) -58]×TG (mmol/L)(22,23).

**Statistical analysis**

All analyses were performed with SPSS version 24.0 (SPSS, Chicago, IL). Data were expressed as means ± SD, SE, or median with interquartile range. Before analysis, the logarithmic conversion was completed in data of non-normal distribution. Student test, Nonparametric tests or ANOVA were used to compare the differences between groups. Simple and partial correlation analysis were conducted to explore the relationship between the variables and serum fetuin-B. The variables which had independent associations with serum fetuin-B levels were evaluated by multiple linear regression. Binary logistic regression analysis was performed to examine the association between fetuin-B and MetS. We used ROC curve to estimate the sensitivity or specificity of fetuin-B in predicting the presence of MetS. The change of fetuin-B level in MetS women was analyzed by the row mean score and Cochran Armitage trend test. When compared with the control group, $p < 0.05$ was considered to be significantly different.

**Results**

**Anthropometric and biochemical parameters and serum fetuin-B levels in healthy and MetS women**

In the current cross-sectional study, the distribution range of serum fetuin-B concentrations was from 1.08 to 10.30 mg/L for most healthy women (83.2%) (Fig. 1A). The main clinic features and metabolic parameters of the 377 participants (average age, 37.9 ± 15.6 years) were shown in Table 1. Compared with the healthy controls, MetS women had a significant increase in serum fetuin-B concentration (8.03 ± 3.75mg / L for MetS vs. 6.01 ± 3.94 mg/L for healthy women) ($p < 0.001$, Table 1 and Fig. 1B ). In MetS women, age, BMI, FAT%, WHR, blood pressure (BP), blood lipid (including TG, TC, LDL-C, and FFA), FBG, 2h-BG, FLns, 2h insulin after glucose overload (2h-Ins), HbA1c, HOMA-IR, VAI, LAP significantly raised, while HDL-C was lower compared with the healthy women ( all $p < 0.001$, Table 1). In addition, there was a statistically significant increase in serum fetuin-B levels of overweight/obese (OW/OB) women (n=161, BMI $\geq$ 24 kg/m$^2$) than the lean group (n=216, BMI < 24 kg/m$^2$; 7.52 ± 4.01 vs. 6.68 ± 3.91 mg/L, $p < 0.05$; Fig. 1C). To explore the association of serum fetuin-B with IR, we divided all subjects into IR (HOMA-IR > 3) and no-IR (HOMA-IR $\leq$ 3) group and found serum fetuin-B levels were markedly elevated in IR women than those in no-IR women (7.92 ± 4.04 vs. 6.33 ± 3.95 mg/L, $p < 0.01$; Fig. 1D).

**Association of fetuin-B with other variables**

Linear correlation analysis showed that there was a significant positive correlation between fetuin-B with obesity- and lipid-related parameters (BMI, WHR, fat, TG, lap, VAI), and glucose-related parameters
(HbA1c%, FBG, 2 h-BG, fIns, 2 h-INS, HOMA-IR) ($p < 0.05$ or $p < 0.01$) in all subjects (Table 2). We further adjusted age and WHR, and found that there was still a relationship between fetuin-B and TG, BMI, fat %, fIns, 2 h-INS, HOMA-IR, LAP as well as VAI, but no correlation between fetuin-B and HDL-C and FFA (Table 2). Moreover, we demonstrated that TG and WHR were independently related factors of serum fetuin-B (Fig. 1E). The multiple regression equation was $Y_{\text{fetuin-B}} = 4.381 + 0.674X_{\text{TG}} + 1.731X_{\text{WHR}} (R^2=0.063)$.

The relationship between serum fetuin-B and MetS

A logistic regression analysis was applied to the data, which demonstrated that serum fetuin-B were related to MetS ($OR, 1.150; 95\% CI, 1.086 - 1.217; p < 0.01$). This relationship persisted even after Age, BMI, FAT%, HbA1c%, insulin, TC, LDL, FFA, and possible confounding factors were controlled (Table 3). Meanwhile, a significant linear trend and independent correlation were found between serum fetuin-B and the MetS by the row mean score and the Cochran–Armitage trend test (Additional file 1: Table 1). Moreover, according to MetS components, we divided the mean levels of serum fetuin-B into six grades. Fig. 1F showed that with increase MetS components, serum fetuin-B increased progressively ($p$ for trend $< 0.05$). Individuals with 0, 1, 2, 3, 4 and 5 component of the MetS had increased serum fetuin-B levels of $5.75 \pm 3.49$, $6.03 \pm 4.00$, $6.29 \pm 4.38$, $7.75 \pm 3.39$, $8.08 \pm 4.05$ and $8.78 \pm 3.97$mg/L. Furthermore, we divided serum fetuin-B into three tertiles (tertile 1, $\leq 5.49$mg/L; tertile 2, 5.49-8.58mg/L; tertile 3, $> 8.58$mg/L). The odds ratio was calculated as an estimate of developing MetS. As shown in Fig. 1G, the risk of developing MetS in the tertile 2 and tertile 3 were obviously increased than tertile 1 ($OR, 2.07; 95\% CI, 1.25 - 3.43$ for tertile 2; $OR, 2.96; 95\% CI, 1.78 - 4.94$ for tertile 3; vs. tertile 1, all $p < 0.01$).

Receiver operating characteristic (ROC) curve analysis

We explored the predictive value of serum Fetuin-B on MetS by ROC curve. The results demonstrated that the areas under the ROC curves for MetS ($AUC_{\text{MetS}}$) were $0.64$ ($p < 0.01$) with a sensitivity of $91.1\%$ and specificity of $65.9\%$ (Fig. 2). The best cut-off value for the serum fetuin-B to predict MetS was $3.87$ mg/L.

Effects of hyperglycemia and hyperinsulinemia on serum Fetuin-B

To explore the impacts of glucose load on the circulating levels of fetuin-B, an OGTT was conducted in healthy women and those in MetS. As shown in Fig. 3A, serum fetuin-B in healthy subjects increased significantly after glucose challenges compared with the basal value, to a peak at 30 minutes (from $3.34 \pm 2.96$ to $10.52 \pm 5.35$mg/L) and retained to the end of the experiment (Fig. 3A). However, in patients with MetS, glucose load had not caused any significant changes in serum fetuin-B (Fig. 3A). In MetS women, the area under the curve for fetuin-B ($AUC_t$) was increased significantly relative to healthy women (Fig. 3A). Then, we performed an EHC in patients with MetS and healthy women to further explore the factors affecting the secretion of serum fetuin-B (Fig. 3B). In response to hyperinsulinemia, serum fetuin-B significantly increased in MetS individuals, whereas there was no change in healthy women (Fig. 3C). During the stable-state of the EHC, serum fetuin-B in MetS women was significantly increased as compared to the baseline($6.03 \pm 4.15$ vs. $9.74 \pm 4.45$mg/L, $p < 0.01$, Fig. 3D). Meanwhile, the women with MetS had a lower M-values than those of healthy controls ($4.47 \pm 1.88$ vs. $10.23 \pm 2.79$ mg/kg/min; $p$
<0.01, Fig. 3C). The above results indicate that patients with MetS had an IR, and the secretion of serum fetuin-B in vivo might be regulated by circulating insulin levels.

**Effects of GLP-1RA intervention on serum Fetuin-B concentration**

Twenty-four patients with MetS participated in the GLP-1RA intervention study for six months (Fig. 4A). The main clinic features and metabolic parameters for pre- and post-treatment were shown in Table 4. After three months of GLP-1RA treatment, the markers of lipid metabolism and obesity (BMI, FAT%, TG, TC, HDL-C, LDL-C, and LAP) and the parameters of glucose metabolism and IR (HbA1c, FIns, HOMA-IR) were significantly ameliorated compared with those in pre-treatment in these patients with MetS ($p < 0.01$ or $p < 0.05$). Furthermore, after treatment for six months, FBG, 2h-BG, VAI also declined significantly than those of pre-treatment ($p < 0.01$). Importantly, serum fetuin-B exhibited a noticeable decline from $10.67 \pm 4.87$ at pre-treatment to $8.90 \pm 3.45$ for post-treatment 3 months, and finally to $7.38 \pm 2.74$ mg/L for post-treatment 6 months (Fig. 4B). Meanwhile, we found that blood glucose at 120min and the area under the curve for glucose (AUCg, $16.68 \pm 2.79$ vs. $19.46 \pm 4.74$ mmol/h/L, $p < 0.05$) during the OGTT were significantly lower than that before GLP-1RA intervention (Fig. 4C). When compared with pre-treatment, the M-values during the EHC were significantly increased at both 3 and 6 months post-treatment ($4.39 \pm 1.30$ and $4.66 \pm 1.53$ vs. $3.29 \pm 0.82$; all $p < 0.01$; Fig. 4D). Therefore, these data further confirm that fetuin-B levels decreased in vivo with the improvement of IR.

**Discussion**

Fetuin-B is mainly expressed in liver and white adipose tissue (WAT), placenta and heart. It's thought to be a hepatokine or/and adipokine. This protein was strongly associated with energy metabolism(9). Several case-control and cross-sectional studies have reported that circulating fetuin-B concentration was obviously elevated in patients with NAFLD, T2DM, GDM, and PCOS(13,14,15). However, most of these studies were preliminary explorations and did not adequately demonstrate the association of fetuin-B with IR in humans nor use state-of-the-art methodology. It is well known that IR, hyperlipidemia, and chronic inflammation are the core components of MetS, but there are few reports available about the relationship between fetuin-B and MetS in humans. In the current study, we observe that serum fetuin-B concentrations are significantly increased in women with MetS as compared with controls. In addition, fetuin-B is positively related to lipid-related parameters and glucose metabolic parameters. Further, analysis found that serum fetuin-B showed a linear trend and was independently associated with MetS. The risk of developing MetS was increased with the increase of serum fetuin-B levels. Therefore, our results suggest that fetuin-B is related to the occurrence and development of MetS through its impact on glucose and lipid metabolism. However, the cause of increased fetuin-B in MetS individuals has not been discovered. We consider that increased serum fetuin-B in MetS individuals might be due to increased metabolic stress, including hyperinsulinemia, and dyslipidosis, etc. These disorders stimulate the release and secretion of fetuin-B. However, to address this issue, further research was needed. Recently, Meex and his colleagues found that the silencing of fetuin-B improved glucose tolerance but did not affect body weight in obese mice. In human research, they found that serum fetuin-B was positively associated with fasting insulin and
HOMA-IR but did not correlate with markers of obesity, inflammation, and blood fat(10). Another study in women with GDM also reported that serum fetuin-B showed no association with obesity, hypertension, and dyslipidemia(15). In addition, Qu et al. found that serum fetuin-B in T2DM patients had a significant positive association with TG but did not relate to other lipid profiles or BMI(12). However, we found that serum fetuin-B levels were obviously elevated in overweight/obese women than those in the lean category, and was associated with BMI, LAP, and VAI. TG and WHR were independently related factors of serum fetuin-B. These results suggest that circulating fetuin-B is associated with obesity. However, the cause of the discrepancy with previous studies is not known but may be due to the higher BMI in the MetS population in this study. It is also possible that the pharmacotherapy and other confounding factors in previous studies such as gender and the physical activity of individuals might contribute to the heterogeneity of the study cohort. Therefore, the association of fetuin-B and obesity remains ambiguous and needs to be investigated in the future.

To evaluate whether blood glucose and insulin affect the secretion of fetuin-B, an OGTT experiment was carried out to observe the change of circulating fetuin-B levels in vivo. During the OGTT, we found a significant increase in circulating fetuin-B levels in normal controls, but no change in MetS individuals. These results suggest that hyperglycemia and/or hyperinsulinemia promoted the secretion of fetuin-B in normal controls.

The EHC technique is the gold standard for evaluating IR in both humans and animals. This method has been widely used to investigate the effects of an intervention and hyperinsulinemia. During the EHC, under the condition of hyperinsulinemia and euglycemia, the circulating fetuin-B levels did not change in healthy individuals. Surprisingly, there was a significant increase in serum fetuin-B levels in MetS individuals. Therefore, we conclude that hyperglycemia, not hyperinsulinemia, was the main factor affecting circulating fetuin-B in healthy individuals, suggesting that fetuin-B is related to glucose metabolism. In MetS individuals, elevated insulin concentration increased circulating fetuin-B levels, while hyperglycemia inhibited fetuin-B release. Therefore, the serum fetuin-B level did not change during the OGTT under the conditions of hyperglycemia and hyperinsulinemia. However, the cause of this phenomenon is unknown. We speculate that this may be related to long-term metabolic disorders and IR.

Liraglutide is a GLP-1RA and is used in the therapy of T2DM and obesity, which has beneficial effects on a variety of metabolic parameters and is one of the preferred drugs for improving IR(24,25). In previous studies, we found that liraglutide could promote the secretion of some adipokines in vivo, such as adiponectin and vesfatin(26,27). In this study, we found that GLP-1RA treatment for six months led to a significant decrease in circulating fetuin-B levels, which was accompanied by ameliorated glucose metabolism and IR as indicated by increased M-values. Therefore, it is possible that the chronic hyperinsulinemia related to IR results in the increase of fetuin-B levels. This indicates that the effect of GLP-1RA on fetuin-B levels is at least partially mediated by GLP-1RA induced change of insulin levels, which is secondary to the role of GLP-1RA in enhancing insulin sensitivity. These results further suggest that fetuin-B is associated with IR and MetS, and demonstrate a beneficial role of GLP-1RA in regulating the secretion and release of fetuin-B in vivo. Based on the above results, it is apparent that fetuin-B
concentration tends to be lower in states of insulin sensitivity and increased in states of IR. However, the fact that fetuin-B decreased to a certain extent after six months of GLP-1RA treatment indicate that fetuin-B is unlikely to be the only reason for GLP-1RA induced insulin sensitivity.

Our research has some limitations that might impact its results: 1) this study is a cross-sectional design, which dose not prove causal relationships; 2) this study has relatively small samples, especially in the intervention experiments. Therefore, our data may be affected by some outliers; 3) our research cohort is composed of Chinese women, which should be carefully applied to other ethnic populations; 4) as a secretory protein, fetuin-B may be secreted in a pulsed manner. The data collected at one time cannot fully reflect its actual situation in vivo. In addition, we did not observe the change of fetuin-B levels in MetS patients dynamically for a long period of time. However, the main strengths of this study are that we chose women of different ages. Therefore, the influence of gender and age was avoided. Importantly, MetS patients were newly diagnosed without lifestyle intervention and drug treatment, avoiding the interference of disease course and other confounding factors. More importantly, in the current study, we performed a variety of intervention experiments, including the EHC, to evaluate the association of fetuin-B with metabolism and IR. Thus, this study still provides sufficient evidence for the association of Fetuin-B with MetS.

Conclusions

In summary, our data demonstrates that circulating fetuin-B concentrations were elevated in MetS women, and were related to glucose metabolism and IR. Using multiple interventions, including the EHC, OGTT, and GLP-1RA treatment, we further found that circulating fetuin-B was regulated by blood glucose, insulin, and GLP-1RA in vivo. Therefore, we conclude that fetuin-B might be a biomarker for screening MetS.

Abbreviations

T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome; OGTT, oral glucose tolerance test; EHC, euglycemic-hyperinsulinemic clamp; GLP-1RA, glucagon-like peptide-1 receptor agonists; BMI, body mass index; WHR, waist hip ratio; FAT%, the percentage of fat in vivo; TG, triglyceride; FBG, fasting blood glucose; 2h-BG, 2-h blood glucose after glucose overload; FIns, fasting plasma insulin; 2h-Ins, 2-h plasma insulin after glucose overload; HOMA-IR, homeostasis model assessment of insulin resistance; LAP, lipid accumulation product; VAI, visceral adiposity index

Declarations

Ethics approval and consent to participate: This study was approved by the Human Research Ethics Committee of Chongqing Medical University and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for publication: Not applicable.
**Availability of data and materials:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Competing interests:** The authors declare that they have no competing interests.

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**Authors' contributions:** S.X., Y.R., M.Y., W.D. and S.G. researched and analyzed data. B.Z. and H.L. reviewed and edited the manuscript. L.L. and G.Y. wrote and edited the manuscript. L.L. is the guarantor of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Tables

Table 1 Main clinical features and circulating fetuin-B concentrations in MetS and control subjects

Values are given as mean ± SD or median (Inter quartile Range). Abbreviations: BMI, body mass index; FAT%, the percentage of fat in vivo; WHR, waist hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acid; FBG, fasting blood glucose; 2h-BG, 2-h blood glucose after glucose overload; FIns, fasting plasma insulin; 2h-Ins, 2-h plasma insulin after glucose overload; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; LAP, lipid accumulation product; VAI, visceral adiposity index. †, Log transformed before analysis; ‡, Nonparametric tests;

Table 2 Correlation analysis of variables associated with circulating fetuin-B levels in study population
| Variable                | Controls (n = 185) | MetS (n = 192) | p    |
|------------------------|-------------------|----------------|------|
| Age (years)‡           | 33.4 ± 13.1       | 42.2 ± 16.5    | < 0.001 |
| BMI (kg/m²)            | 20.9 ± 2.7        | 26.3 ± 3.6     | < 0.001 |
| FAT (%)                | 26.8 ± 5.3        | 37.3 ± 6.1     | < 0.001 |
| WHR‡                   | 0.80 ± 0.07       | 0.98 ± 0.34    | < 0.001 |
| SBP (mmHg)             | 112.0 ± 13.3      | 129.4 ± 18.9   | < 0.001 |
| DBP (mmHg)             | 73.3 ± 10.2       | 81.2 ± 12.1    | < 0.001 |
| TC (mmol/L)            | 4.18 ± 1.01       | 4.79 ± 1.15    | < 0.001 |
| TG (mmol/L)†           | 1.02 ± 0.58       | 2.27 ± 1.23    | < 0.001 |
| HDL-C (mmol/L)†        | 1.35 ± 0.36       | 1.18 ± 0.34    | < 0.001 |
| LDL-C (mmol/L)         | 2.37 ± 0.82       | 2.86 ± 0.88    | < 0.001 |
| FFA (µmol/L)           | 0.51 ± 0.23       | 0.63 ± 0.27    | < 0.001 |
| HbA1c (%)‡             | 5.2 ± 0.3         | 6.2 ± 1.5      | < 0.001 |
| FBG (mmol/L)‡          | 4.73 ± 0.52       | 6.55 ± 2.19    | < 0.001 |
| 2h-BG (mmol/L)†        | 5.57 (4.85 - 6.44)| 9.46 (7.55 - 12.02)| < 0.001 |
| FIns (mU/L)†           | 6.79 (5.70 - 8.28)| 17.94 (11.46 - 27.51)| < 0.001 |
| 2h-Ins (mU/L)†         | 41.09 (26.13 - 60.29)| 124.10 (67.76 - 221.90)| < 0.001 |
| HOMA-IR†               | 1.43 (1.16 - 1.78)| 5.13 (3.42 - 7.51)| < 0.001 |
| LAP†                   | 11.50 (5.36 - 20.71)| 65.84 (48.29 - 89.02)| < 0.001 |
| VAI†                   | 1.23 (0.85 - 1.74)| 3.55 (2.58 - 4.81)| < 0.001 |
| Fetuin-B (mg/L)        | 6.01 ± 3.94       | 8.03 ± 3.75    | < 0.001 |
| Variable | Model 1 | | Model 2 | | Model 3 |
|----------|---------|---------|---------|---------|---------|
|          | $r$     | $p$     | $r$     | $p$     | $B$     | $P$     |
| Age (years) ‡ | 0.094 | 0.070 | - | - | - | - |
| WHR‡ | 0.211 | $< 0.001$ | - | - | 1.731 | $< 0.05$ |
| BMI (kg/m²) | 0.154 | $< 0.01$ | 0.144 | $< 0.05$ | - | - |
| FAT (%) | 0.209 | $< 0.001$ | 0.191 | $< 0.01$ | - | - |
| SBP (mmHg) | 0.096 | 0.062 | 0.053 | 0.356 | - | - |
| DBP (mmHg) | 0.073 | 0.156 | 0.052 | 0.357 | - | - |
| TC (mmol/L) | 0.070 | 0.180 | 0.077 | 0.176 | - | - |
| TG (mmol/L) † | 0.237 | $< 0.001$ | 0.206 | $< 0.001$ | 0.674 | $< 0.001$ |
| HDL-C (mmol/L) † | -0.098 | 0.057 | -0.104 | 0.066 | - | - |
| LDL-C (mmol/L) | 0.070 | 0.177 | 0.088 | 0.120 | - | - |
| FFA (µmol/L) | -0.024 | 0.675 | -0.045 | 0.430 | - | - |
| HbA1c (%) ‡ | 0.146 | $< 0.01$ | 0.062 | 0.278 | - | - |
| FBG (mmol/L) ‡ | 0.180 | $< 0.001$ | 0.068 | 0.229 | - | - |
| 2h-BG (mmol/L) † | 0.136 | $< 0.01$ | 0.111 | 0.051 | - | - |
| FIns (mU/L) † | 0.188 | $< 0.001$ | 0.178 | $< 0.01$ | - | - |
| 2h-Ins (mU/L) † | 0.140 | $< 0.01$ | 0.123 | $< 0.05$ | - | - |
| HOMA-IR † | 0.196 | $< 0.001$ | 0.178 | $< 0.01$ | - | - |
| LAP † | 0.263 | $< 0.001$ | 0.223 | $< 0.001$ | - | - |
| VAI † | 0.257 | $< 0.001$ | 0.221 | $< 0.001$ | - | - |

Model 1: unadjusted simple linear regression analysis, Model 2: adjusted age and WHR, partial linear regression analysis, Model 3: Multiple linear stepwise regression analysis, values included for analysis were TG, WHR, BMI, HOMA-IR. †, Log transformed before analysis; ‡, Spearman correlation tests;
**Table 3** Association of circulating fetuin-B levels with MetS in fully adjusted models

| Model adjust | MetS   |
|--------------|--------|
| OR           | 95%CI  | P    |
| Age          | 1.144  | 1.080 - 1.212 | < 0.001 |
| Age, BMI     | 1.160  | 1.071 - 1.258 | < 0.001 |
| Age, BMI, FAT% | 1.136 | 1.045 - 1.236 | < 0.01  |
| Age, BMI, FAT%, HbA1c | 1.133 | 1.034 - 1.242 | < 0.01  |
| Age, BMI, FAT%, HbA1c, Fins | 1.142 | 1.023 - 1.274 | < 0.05  |
| Age, BMI, FAT%, HbA1c, Fins, 2h-Ins | 1.178 | 1.042 - 1.333 | < 0.01  |
| Age, BMI, FAT%, HbA1c, Fins, 2h-Ins, TC, LDL-C, FFA | 1.217 | 1.058 - 1.401 | < 0.01  |

Results of binary logistic regression analysis are presented 95%CI, confidence interval; Abbreviations: BMI, body mass index; FAT%, the percentage of fat *in vivo*; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acid; TG, triglyceride; HbA1c, Glycosylated hemoglobin; FIns, fasting plasma insulin; 2h-Ins, 2-h plasma insulin after glucose overload; OR, odds ratio.

**Table 4** Main clinical and metabolic features pre- and post-treatment with GLP-1RA in MetS women
| Variable          | Baseline         | Post-treatment 3 months | Post-treatment 6 months |
|-------------------|------------------|-------------------------|-------------------------|
| BMI (kg/m²)       | 29.4 ± 2.9       | 27.1 ± 2.6**            | 26.7 ± 2.9**            |
| FAT (%)           | 42.9 ± 8.3       | 37.2 ± 3.7**            | 37.1 ± 4.5**            |
| WHR               | 0.90 ± 0.05      | 0.89 ± 0.05             | 0.89 ± 0.06             |
| SBP (mmHg)        | 118.2 ± 9.9      | 113.5 ± 10.9            | 115.4 ± 12.1            |
| DBP (mmHg)        | 76.0 ± 10.2      | 77.2 ± 9.8              | 77.4 ± 8.6              |
| TC (mmol/L)       | 4.85 ± 0.91      | 4.19 ± 0.74**           | 4.20 ± 0.76**           |
| TG (mmol/L)       | 2.31 ± 0.82      | 1.79 ± 0.63*            | 1.48 ± 0.69**           |
| HDL-C (mmol/L)    | 1.06 ± 0.17      | 0.99 ± 0.18*            | 1.19 ± 0.71             |
| LDL-C (mmol/L)    | 3.08 ± 0.80      | 2.63 ± 0.67*            | 2.53 ± 0.73*            |
| FFAs (µmol/L)     | 0.54 ± 0.21      | 0.49 ± 0.17             | 0.47 ± 0.47             |
| FBG (mmol/L)      | 5.63 ± 0.88      | 5.37 ± 0.43             | 5.26 ± 0.37*            |
| 2h-BG (mmol/L)    | 9.56 ± 2.90      | 8.58 ± 2.45             | 7.39 ± 1.90**           |
| HbA1c (%)         | 5.7 ± 0.5        | 5.4 ± 0.3*              | 5.3 ± 0.3**             |
| FIns (mU/L)       | 29.05 (21.52 - 39.88) | 19.01 (12.92 - 30.55)** | 16.75 (12.38 - 28.11)** |
| 2h-Ins (mU/L)     | 203.45 (157.93 - 348.98) | 219.25 (115.03 - 385.78) | 151.85 (80.63 - 250.10) |
| HOMA-IR           | 7.31 (4.83 - 10.77) | 4.67 (3.05 - 7.12)**    | 4.18 (2.90 - 6.53)**    |
| LAP               | 74.24 (50.99 - 107.27) | 50.98 (40.29 - 60.74)** | 43.23 (22.76 - 63.05)** |
| VAI               | 3.77 (3.13 - 5.47) | 3.51 (2.39 - 4.68)**    | 2.51 (1.43 - 3.84)**    |
| M-values          | 3.29 ± 0.82      | 4.39 ± 1.30**           | 4.66 ± 1.53**           |
| AUCg              | 19.46 ± 4.74     | 17.52 ± 3.82            | 16.68 ± 2.79*           |
| Fetuin-B (mg/L)   | 10.67 ± 4.87     | 8.90 ± 3.45             | 7.38 ± 2.74*            |

Values are given as mean ± SD or median (Inter quartile Range). AUCg, the area under the curve for glucose. *p < 0.05, **p < 0.01 vs. Baseline; ▲p < 0.05, ▲▲p < 0.01 vs. post-treatment 3 months.

**Figures**
Figure 1

Serum fetuin-B levels in the study population. (A) Distribution of serum fetuin-B in 185 healthy women. (B) Circulating fetuin-B levels in MetS and healthy subjects. (C) Circulating fetuin-B levels according to BMI (lean: BMI < 24 kg/m²; overweight/obese: BMI ≥ 24 kg/m²). (D) Circulating fetuin-B levels, according to HOMA-IR (IR: HOMA-IR > 3; non-IR: HOMA-IR ≤ 3). (E) All factors and stepwise multiple regression analyses of the serum fetuin-B and MetS in study individuals. (F) Serum fetuin-B levels in relation to the number of MetS components. (G) The odds ratio of having MetS in different tertiles of serum fetuin-B (tertile 1, ≤ 5.49 mg/L; tertile 2, 5.49-8.58 mg/L; tertile 3, > 8.58 mg/L) Data were means ± SME. * p < 0.05 or ** p < 0.01 vs. Controls, lean, no-IR or tertile 1.
Figure 2

ROC curve analyses for the prediction of MetS according to circulating fetuin-B levels
Figure 3

Circulating fetuin-B levels in interventional studies. (A) Time course of changes in circulating fetuin-B levels in healthy and MetS subjects during the OGTT and the area under the curve for serum fetuin-B (AUCf). (B) EHC protocol. (C) Time course of circulating fetuin-B changes and the M-values in healthy and MetS subjects during the EHC. (D) Cumulative fetuin-B levels during the EHC. Data were means ± SME. * p < 0.05 or ** p < 0.01 vs. Control or baseline.
**Figure 4**

Effects of GLP-1RA treatment on serum fetuin-B and insulin sensitivity in MetS women. (A) Liraglutide treatment protocol. (B) Serum fetuin-B levels in MetS subjects after GLP-1RA treatment. (C) Blood glucose levels and the area under the curve for Blood glucose (AUCg) in MetS subjects during the OGTT after GLP-1RA treatment. (D) Changes of M-value in MetS subjects during the EHC after GLP-1RA treatment. Data were means ± SME. * p < 0.05 or ** p < 0.01 vs. Baseline.

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