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Serum level of fetuin B is associated with osteoporosis: a 4-year prospective study in China

Abstract

Purpose: As a novel hepatokine, fetuin B involves in various functions of energy metabolism. Recent advance reveals a complex interaction between bone and liver via the secretion of hepatokines. The association between serum fetuin B and osteoporosis was evaluated in a 4-year hospital-based prospective study of 1,370 Chinese postmenopausal women.

Methods: Bone mineral densities (BMDs) were obtained on femoral neck and lumbar spines by dual energy X-ray absorptiometry. Serum fetuin B level was tested by enzyme-linked immunosorbent assay.

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Results: Of the 1,370 participants in the baseline study (2012), 650 subjects were included in the 4-year follow-up study (2016). Serum fetuin B level presented higher in subjects with osteoporosis ($106.7 \pm 17.6 \mu\text{g/ml}$) than it in controls ($86.3 \pm 17.5 \mu\text{g/ml}$) ($P < 0.001$). Meanwhile, fetuin B positively correlated with triglycerides ($r = 0.227$, $P = 0.001$), femoral BMD ($r = -0.426$, $P < 0.001$) and lumbar BMD ($r = -0.332$, $P < 0.001$). At the 4-year follow-up, 116 subjects had developed osteoporosis. Serum fetuin B concentration was significantly higher in subjects who developed ($P < 0.001$). The osteoporosis incidence increased from Q1 9.9%, Q2 14.7%, and Q3 17.8% to Q4 30.2% (P for trend < 0.001), among the quartiles of baseline fetuin B. A higher fetuin B baseline level was linked to the incidence of osteoporosis (adjusted OR = 1.179, 95% CI [1.119 – 1.243], $P = 0.009$).

Conclusion: Serum fetuin B levels increased with the development of osteoporosis.

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Recent evidence suggests that the endocrine system plays an essential role in bone metabolism [1]. Proteins produced by adipocytes and liver may be involved in the pathophysiology of osteoporosis [2] and altered levels of adipokines and hepatokines have been reported in patients with osteoporosis [2-5].

Recent studies have suggested that fetuin, an endogenous inhibitor of the insulin receptor tyrosine kinase [6, 7], is involved in osteogenesis, mineralization and inflammation [8, 9]. The latest studies found elevated fetuin B levels in obese mice [10] and humans with steatosis [11, 12], which suggests a link between fetuin B and energy metabolism [11]. In one study, *in vitro* data indicated that fetuin B may lead to insulin resistance in myotubes and hepatocytes and rodent data indicated that the administration of fetuin B caused glucose intolerance rather than insulin resistance [11]. These data support a role for fetuin B in the metabolism of glucose and lipid [13, 14]. In addition, Jung et al. [15] reported that fetuin B was associated with the development of acute myocardial infarction.

More studies on cytokines have focused on the connections between these proteins and bone physiological function. Recent evidence links obesity, metabolic syndrome and diabetes to osteoporosis [16, 17]. This evidence reveals complex communication between the bone and the liver, which mutually regulate physical functions via the secretion of hepatokines, as indispensable modulators of bone remodelling [12, 18]. As a liver-derived molecule, fetuin B may be involved in the endocrine-bone axis. In this 4-year hospital-based cohort study of 1,370 Chinese postmenopausal women, the association between serum fetuin B and osteoporosis was evaluated.

Methods

Study design and patients

The hospital-based cohort study recruited the postmenopausal women who took the annual health examination at the Jintan Affiliated Hospital of Jiangsu University from January to December 2012. In 2016, the participants were invited back for re-assessment. Of the 1,370 subjects who participated in the initial study, 650 were eventually enrolled in the follow-up study. Subjects with the following medical conditions were excluded from the study: malignant tumor; severe cardiopulmonary disease; stroke; virus hepatitis; liver cirrhosis; jaundice; renal insufficiency; endocrine diseases; inflammatory rheumatism; and the use of estrogens or steroids. The research was approved by the Ethics Committee of the Jintan Affiliated

Hospital, Jiangsu University, in accordance with the Helsinki Declaration of 1964. Before participation, each participant gave written informed consent.

Examinations

All participants received BMD scans of their femoral neck and lumbar spines (L2-L4) using dual energy X-ray absorptiometry (Lunar Prodigy, General Electric Medical Systems, USA). A T score of less than -2.5 was diagnosed as osteoporosis. Blood samples were obtained and frozen for the further evaluation of serum fetuin B levels by using enzyme-linked immunosorbent assay (ELISA) (catalogue no. SEB860Hu; USCN Life Science, China). The intra- and inter-assay variations of serum fetuin B measurement were 6.7% and 11.5%, respectively. Routine blood chemistry analyses and anthropometric exams were performed in our hospital, as reported previously [19]. Menopause was defined as the absence of menstruation for ≥ 1 year and was confirmed by a serum follicle-stimulating hormone assay.

Statistical methods

The Student's unpaired *t* test and χ^2 test were used to compare the parameters between two groups. The χ^2 test was used for testing the difference of osteoporosis within the quartiles of serum Fetuin B. Pair-sample *t* test was used for comparison between baseline and follow-up. To assess the association between serum fetuin B at baseline and incidence of osteoporosis, the adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated, with a multivariable logistic regression model. Pearson/Spearman bivariate correlation analyses were performed between fetuin B and the anthropometric/biomedical parameters. All statistical analyses were performed by SPSS (version 21.0, SPSS, USA). Power of sample size was calculated using G*Power (version 3.1, Heinrich-Heine-Universität, Germany) [20]. A two-sided *P* < 0.05 was considered statistically significant.

Results

Of the 1,370 participants in the original baseline study (2012), 650 subjects were included in the 4-year follow-up (2016) (Figure 1). The osteoporosis incidence rate at this 4-year follow-up was 17.8% (116/650). The annual incidence rate was 4.5%.

Table 1 indicates the baseline characteristics of the participants in 2012. Serum fetuin B level was significantly higher in patients with osteoporosis (106.7 ± 17.6 $\mu\text{g/ml}$) than it in controls (86.3 ± 17.5 $\mu\text{g/ml}$) (*P* < 0.001, Figure 2A).

TABLE 1. Baseline characteristics of subjects with non-Osteoporosis and Osteoporosis in 2012

Parameter	Non-osteoporosis	Osteoporosis	<i>P</i> value
No. of subjects	1,107	263	-
Age (years)	68.8 ± 11.0	69.1 ± 11.3	0.692
Diabetes, n	343	78	0.710
Hypertension, n	399	108	0.136
Bone fracture, n	33	47	< 0.001
BMI (kg/m ²)	22.0 ± 3.5	24.1 ± 4.6	< 0.001
Fetuin B (µg/ml)	86.3 ± 17.5	106.7 ± 17.6	< 0.001
FBMD (g/cm ²)	0.79 ± 0.07	0.64 ± 0.04	< 0.001
LBMD (g/cm ²)	1.02 ± 0.12	0.79 ± 0.07	< 0.001
TG (mmol/L)	1.58 ± 0.31	1.67 ± 0.23	< 0.001
TC (mmol/L)	6.13 ± 0.47	6.33 ± 0.47	< 0.001
HDL-C (mmol/L)	1.52 ± 0.33	1.48 ± 0.30	0.073
LDL-C (mmol/L)	3.09 ± 0.25	3.04 ± 0.24	0.003
FPG (mmol/L)	5.85 ± 1.11	5.95 ± 1.16	0.193
Insulin (mU/L)	18.4 ± 4.1	16.8 ± 4.8	< 0.001
HOMA-IR	4.92 ± 1.83	4.61 ± 2.02	0.016

Data are mean ± standard deviation for continuous variables. *P* values indicated the comparisons between controls and patients. BMI, body mass index; FPG, fasting plasma glucose; FBMD: femoral bone mineral density; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; LBMD: lumbar bone mineral density; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

In all the postmenopausal women, serum fetuin B level was positively correlated with triglycerides (TG) ($r = 0.227$, $P = 0.001$) but negatively correlated with femoral BMD ($r = -0.426$, $P < 0.001$) and lumbar BMD ($r = -0.332$, $P < 0.001$) (Table 2).

At the 4-year follow-up, 650 postmenopausal women who were without osteoporosis at the baseline study completed the re-evaluation: a total of 116 of the subjects had developed osteoporosis, while 534 subjects had not.

As shown in Figure 2B, the fetuin B concentration was significantly higher in subjects who developed osteoporosis ($n = 116$, 114.9 ± 22.7 µg/ml) compared with subjects who did not develop ($n = 534$, 81.4 ± 22.5 µg/ml) ($P < 0.001$). Comparison between baseline and 4-year follow-up fetuin B levels revealed a difference in subjects who developed osteoporosis ($P < 0.001$, Figure 2C).

In Figure 3, when stratified by quartile of baseline serum fetuin B levels, subjects in the Q1 of fetuin B had an incidence

rate of 9.9% at the 4-year follow-up. The incidence rates of osteoporosis increased to 30.2% by Q4 (P for trend < 0.001). Furthermore, a higher serum level of fetuin B in the baseline study was associated with the incidence of osteoporosis at the 4-year follow-up (adjusted OR = 1.179, 95% CI [1.119 – 1.243], $P = 0.009$), after adjustment, as shown in Table 3.

Discussion

The connection between the endocrine system and osteoporosis has led to speculation that specific liver-derived proteins are involved in the regulation of bone metabolism [21]. A recent study has suggested that hepatokines may play a role in liver-bone axis mediation [22]. As a liver-derived protein, fetuin B might also play a role in the endocrine–bone axis.

This study is the first to report the role of serum fetuin B in the development of osteoporosis. In the 2012 hospital-based

FIGURE 1. Flow chart of the study population

TABLE 2. Correlations of serum Fetuin B with various parameters among all participants at baseline in 2012

Parameter	<i>r</i> value	<i>P</i> value
Age	-0.002	0.973
BMI	-0.104	0.141
FBMD	-0.426	< 0.001
LBMD*	-0.332	< 0.001
FPG*	-0.004	0.951
Insulin	-0.084	0.239
HOMA-IR*	-0.041	0.564
TG*	0.227	0.001
TC	0.121	0.088
HDL-C*	-0.053	0.458
LDL-C	0.008	0.910

*Spearman correlation analysis; BMI, body mass index; FPG, fasting plasma glucose; FBMD: femoral bone mineral density; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; LBMD: lumbar bone mineral density; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

FIGURE 2. Comparisons of serum fetuin B levels: A) baseline in 2012: control vs. osteoporosis ($P < 0.001$); B) follow-up in 2016: not developed osteoporosis in 4 years vs. developed osteoporosis in 4 years ($P < 0.001$); and C) subjects developed osteoporosis in 4 years: baseline levels vs. follow-up levels ($P < 0.001$).

TABLE 3. Baseline serum fetuin B predictive of development of Osteoporosis at 4 years

Adjustment	Odds ratio	95% confidence interval	P value
Model 1	1.165	1.144 – 1.186	< 0.001
Model 2	1.165	1.144 – 1.185	< 0.001
Model 3	1.171	1.122 – 1.222	0.004
Model 4	1.179	1.119 – 1.243	0.009

Model 1: unadjusted; model 2: adjusted for age; model 3: model 2 + adjusted for FBMD and LBMD; model 4: model 3 + adjusted for TG, diabetes, hypertension, BMI, and bone fracture; BMI, body mass index; FBMD: femoral bone mineral density; LBMD: lumbar bone mineral density; TG, triglycerides.

cohort study, we previously reported that serum levels of fetuin B were higher in postmenopausal women with osteoporosis. Serum fetuin B level was positively correlated with TG, providing evidence for the relationship between fetuin B and obesity and dyslipidemia, as was reported previously [12]. On the other hand, both femoral and lumbar BMDs correlated negatively with the serum fetuin B levels. At the 4-year follow-up, serum fetuin B levels increased further with the development of osteoporosis. Thus, our findings indicate a role of fetuin B in the energy metabolism and pathophysiology of osteoporosis, which might relate with the complex liver-bone axis.

Some limitations merit comment. Firstly, this hospital-based longitudinal study enrolled subjects at one urban medical center. Thus, there might be potential selection bias, and the findings should be interpreted with caution. Furthermore, the 4-year follow-up had a high dropout rate (41.3%, 457/1,107). Half of the follow-up subjects (43.3%, 198/457) were lost due to medical reasons, including malignant tumor, cardiopulmonary disease and stroke, which may reflect the population enrolled (older urban postmenopausal women). Lastly, the levels of vitamin D and other cytokines were not measured.

This hospital-based prospective cohort study, conducted in the postmenopausal women, revealed that serum fetuin B levels increased as the development of osteoporosis. More population-based studies in the future are needed to explore the underlying mechanism of fetuin B in the pathophysiology of osteoporosis.

FIGURE 3. Incidence rate of osteoporosis in the 4-year follow-up by quartile of fetuin B levels at baseline (P for trend < 0.001).

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