European Review for Medical and Pharmacological Sciences 2018; 22: 1457-1462

Analysis of the correlation between non-alcoholic fatty liver disease and bone metabolism indicators in healthy middle-aged men

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Abstract. – OBJECTIVE: To investigate the relationship between bone metabolic indicators and non-alcoholic fatty liver disease (NAFLD) in healthy middle-aged men.

PATIENTS AND METHODS: The bone metabolic indicators of 232 healthy middle-age men with NAFLD (NAFLD group) and 308 healthy controls without fatty liver (Control group) were measured, including non-collagenous osteocalcin, the procollagen type 1 N-terminal propeptide (P1NP), beta-C-terminal telopeptide of type I collagen (β-CTX). The Student's t-test was used to analyze the differences in the bone metabolic indicators, age, clinical data, biochemical indicators, and the indicators of glucose and lipid metabolism between the two groups. The correlation of fatty liver-related indicators was detected using the logistic regression analysis.

RESULTS: The body mass index (BMI), diastolic blood pressure, and heart rate in NAFLD group were significantly higher than those in Control group. Among the indicators of glucose and lipid metabolism in NAFLD group, the levels of blood glucose [fasting plasma glucose, postprandial blood glucose and hemoglobin A1c (HbA1c)] were significantly higher than those in Control group. In addition, the insulin resistance and secretion indexes were also significantly higher than those in Control group. The levels of lipid metabolic indicators such as triglyceride were higher, but high-density lipoprotein cholesterol was lower than that in Control group. From logistic regression analysis, the BMI, Homeostasis model assessment (HOMA)-β, HOMA-IR, HbA1c and P1NP were positively associated with the occurrence of NAFLD.

CONCLUSIONS: The bone metabolic indicator P1NP might be a potential predicator for the diagnosis of NAFLD in clinical application.

Key Words:
Non-alcoholic fatty liver, Non-collagen osteocalcin, Type I collagen Amino-extended peptide, Ð-collagen special sequence.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by intrahepatic triglyceride deposition, which can develop from simple steatosis to steatohepatitis and even to cirrhosis. In the epidemiological studies, the prevalence of NAFLD is 10-35% worldwide[1], and it has become the leading cause of chronic liver disease. In China, with the development of the economy and the varied lifestyles, it has been reported that the prevalence of NAFLD is also up to 15-31%[2-4]. In addition to lipid metabolic disorders, patients with NAFLD are accompanied by obesity, hyperglycemia, hypertension, high uric acid, and high microalbuminuria. It is considered that the insulin resistance is the common pathogenesis of these diseases presented as the liver metabolic syndrome.

Recently, the relationship between bone metabolism and insulin resistance becomes a hot topic in research. Exceptional to be a conventional calcium and phosphorus storage organ, several scholars have proven bone involved in the glucose and energy metabolism to be an endocrine organ. One important evidence of this argument is that the osteoblast-specific secreted non-collagenous osteocalcin (OCN) has been shown to be involved in the regulation of insulin secretion, improvement of insulin sensitivity, decrement of blood glucose and triglyceride, weight loss, increment of energy consuming and so on[5]. OCN is closely related to glucose and lipid metabolism as well as the body fat distribution, and is recognized to be an important predictor of insulin resistance and abdominal obesity[6-9].

In addition to OCN, the bone metabolic indicators such as the bone formation factor procollagen type 1 N-terminal propeptide (P1NP) and bone

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turnover indicator Beta-C-terminal telopeptide of type I collagen (β-CTX) were also detected. It was believed that the OCN was not the only key molecule involved in the signaling pathway of glycolipid and bone metabolism. However, there is no study on P1NP and β-CTX involved in the energy metabolism and body fat distribution.

Therefore, the correlation between various bone metabolic indicators and the occurrence of fatty liver was investigated in this work via studying the NAFLD in healthy middle-aged men, by which the association between bone metabolism and the development of fatty liver could be further explored.

Patients and Methods

Subject Enrolled and Exclusion Criteria

Subjects were enrolled from Department of Geriatrics in Nanjing Drum Tower Hospital since January 2014 to December 2016, according to the criteria of diagnosis and treatment for NAFLD from the fatty liver and alcoholic liver disease group of the Liver Diseases Section of the Chinese Medical Association in 2010[10]. The abdominal ultrasonography was used to diagnose NAFLD. To exclude the influences on the bone metabolism by the hormone of perimenopausal women, a total of 540 middle-aged men were selected and divided into NAFLD group [232 patients with NAFLD, aged (50.52±4.87) years old] and Control group [308 healthy people with no fatty liver during the same period, aged (50.35±5.18) years old]. These subjects had no history of alcohol consumption or less than 140 g alcohol consumption weekly. Exclusion criteria: patients with viral, alcoholic, autoimmune, orhereditary liver disease, patients with renal insufficiency, cancer, rheumatic or rheumatoid disease, or prolonged bed rest, patients with other endocrine diseases, such as thyroid dysfunction, parathyroid function abnormal and adrenal cortical dysfunction, patients taking drugs that affect bone metabolism, such as bisphosphonates, vitamin D (Vit D), calcium and derivatives, sex hormones, glucocorticoids, diuretics, etc., and patients who had fractures in recent one year or had severe smoking, drinking and coffee habits. This study was approved by the Ethics Committee of Drum Tower Clinical Medical College of Nanjing Medical University. Signed written informed consents were obtained from all participants before the study.

Parameters Measurement

The epidemiological data of patients were collected by a qualified medical staff. The data included lifestyle (smoking and drinking history), history of hypertension, diabetes mellitus, fracture, and surgery. The parameters of height, weight, waist circumference, heart rate, and blood pressure were measured. No cap, coat, and shoe were worn when the height and weight were measured. The waist circumference was measured from the lower edge of the rib to the midpoint of the iliac crest connection. The blood pressure at right brachial artery was detected. Exercises were avoided for at least 30 minutes before measuring blood pressure. Body mass index (BMI) = weight (kg)/height (m^2).

Venous blood was collected from all subjects after 8 hours of fasting to determine the liver function by measuring the parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transpeptidase (GGT), total bilirubin (TB), direct bilirubin (DB) and albumin (ALb), to determine the renal function by measuring the parameters including urea nitrogen (BUN), creatinine (CR) and uric acid (UA), to determine blood lipid levels by measuring the parameters including triglyceride (TG), total cholesterol (TC), low density lipids (LDL-C) and high-density lipoprotein cholesterol (HDL-C), to determine the thyroid function by measuring the parameters including thyroid stimulating hormone (TSH), free triiodothyronine (FT3), serum free thyroxine (FT4), and to determine the pancreatic islet function by measuring the parameters including fasting plasma glucose (FPG), fasting insulin (FINS), fasting C-peptide (FCP), hemoglobin A1c (HbA1c) and venous blood pressure measured by 2-hour postprandial blood glucose (PPG). Homeostasis model assessment (HOMA)-IR = FPG × FINS/22.5 (FPG unit: mmol/L, FINS unit: U/mL), which was used to assess the insulin resistance. HOMA-β was used to assess islet function, HOMA-β = 20 × FINS / (FPG-3.5)% (FPG unit: mmol/L, FINS unit: U/mL). The bone metabolic indicator was determined by serum calcium, alkaline phosphatase (AKP), OCN, P1NP, β-CTX and Vit D.

Statistical Analysis

The data were analyzed by statistical product and service solutions (SPSS) 23.0 software (IBM, Armonk, NY, USA). The continuous variables were presented as mean ± standard deviation (SD), and Student's *t*-test was used to compare

Table I. Comparison of clinical data between two groups.

Groups	Cases	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Heart rate (Times/min)	Abdominal circumference (cm)	BMI (kg/m^2)
NAFLD	232	128.72±67.21	81.76±9.89*	73.99±8.64*	94.35±8.62	26.33±2.68**
Control	308	121.44±14.08	78.93±10.57	72.16±7.75	95.49±65.51	24.74±2.71

Student's *t*-test, *p<0.05 and **p<0.01.

two groups. The counting data were analyzed by x^2-test. The correlation between two variables was analyzed through logistic correlation analysis. p<0.05 represented that the difference was statistically significant.

Results

There was no significant difference in age between the two groups (p>0.05). There were significant differences in diastolic blood pressure, HR, urinary albumin, ALT, GGT, FPG, PPG, HbA1c, FINS, FCP, HOMA-IR, HOMA-β, UA, TG, and HDL-C (Table I, II, and III). Among the bone metabolic indicators, only OCN and P1NP were statistically different between the two groups (p<0.01). However, there were no statistically significant differences in calcium, Vit D, and CTX between the two groups (p>0.05) (Table IV).

Table II. Comparisons of biochemical indicators between two groups.

	Group NAFLD	Control
No.	232	308
ALT (U/L)	33.75±17.87**	27.38±22.79
AST (U/L)	23.36±8.75	21.95±11.06
GGT (U/L)	52.65±37.71**	40.78±28.90
TB (μmol/L)	13.57±5.03	13.54±4.40
DB (μmol/L)	3.64±1.46	3.60±1.30
ALB (g/L)	42.73±2.48	42.42±2.43
BUN (mmol/L)	5.25±1.10	5.23±1.24
CR (μmol/L)	72.28±10.98	72.55±12.03
UA (μmol/L)	411.46±75.22**	376.18±79.78
Urinary microalbumin (mg/L)	22.33±61.48**	10.11±18.41
TSH (mIU/L)	2.33±1.17	2.29±1.30
FT3 (pmol/L)	5.12±0.60	5.03±0.56
CRP (mg/L)	3.73±2.75	3.94±3.51
AST (U/L)	23.36±8.75	21.95±11.06
GGT (U/L)	52.65±37.71**	40.78±28.90
TB (μmol/L)	13.57±5.03	13.54±4.40
DB (μmol/L)	3.64±1.46	3.60±1.30
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Student's *t*-test, *p<0.05, **p<0.01.

Logistic regression analysis was used to analyze the association of the following indicators with the occurrence of fatty liver, including age, abdominal circumference, BMI, blood glucose, HbA1c, HOMA-IR, HOMA-β, OCN, P1NP, β-CTX, and Vit D. The results showed that BMI, HOMA-β, HOMA-IR, and HbA1c were associated with the occurrence of fatty liver [r(BMI)=0.015, r(HOMA-β)=0.039, r(HOMA-IR)=0, and r(HbA1c)=0.004, respectively]. It was found for the first time that among the bone metabolic indicators, only P1NP (r=0.032), CTX (r=0.140), OCN (r=0.179) and Vit D (r=0.302) were positively correlated with the occurrence of fatty liver.

Discussion

It was found that BMI, diastolic blood pressure, and heart rate in NAFLD group were significantly higher than those in Control group, which were consistent with results of previous reports. Among the indicators of glucose and lipid metabolism, the levels of blood glucose such as FPG, PPG, and HbA1c were significantly higher than those of the Control group. In addition, the insulin resistance index, insulin secretion index and TG were also significantly higher than those in Control group. The levels of lipid metabolic indicators like HDL-C were significantly lower than those in Control group. Therefore, this study proved that patients with NAFLD were more likely to have complications like hyperglycemia, insulin resistance, higher TG, and lower HDL-C.

The levels of bone metabolic indicators such as OCN and P1NP in NAFLD group were significant-

Table III. Comparisons of glucose and lipid metabolic indicators between two groups.

	Group NAFLD	Control
FPG (mmol/L)	5.67±1.30**	5.10±0.81
PPG (mmol/L)	7.44±2.90**	6.38±2.21
HbA1c (%)	5.80±0.77**	5.46±0.46
F-INS (U/mL)	12.96±6.34**	7.79±4.47
F-CP (mmol/L)	1072.00±338.91**	755.65±242.03
HOMA-IR	3.32±1.94**	1.80±1.23
HOMA-β	144.51±81.34**	112.75±80.24
TG (mmol/L)	2.40±1.56**	1.62±1.10
TC (mmol/L)	4.93±0.99	4.83±0.91
LDL-C (mmol/L)	2.73±0.83	2.68±0.71
HDL-C (mmol/L)	1.05±0.25**	1.21±0.32

Student's *t*-test, *p<0.05, **p<0.01.

ly lower than those in Control group. There were no significant differences in the levels of Vit D and β-CTX between this two groups. The logistic regression analysis showed that P1NP was positively correlated with the occurrence of fatty liver. Bone matrix is composed of collagen and non-collagen proteins. OCN is a specific non-collagen bone protein synthesized and secreted by the non-proliferative osteoblast. Most OCNs are deposited in the bone matrix, but the concentrations of OCN in serum can reflect the rate of osteogenesis[11]. Similarly, many researches have demonstrated the correlation between OCN and fatty liver, and this study disclosed the same results. For example, Aller et al[12] has divided 69 NAFLD patients into two groups: high OCN group and low OCN group according to the median of OCN (11.34 ng/mL). They found that the occurrence of hepatic fibrosis is significantly lower in high OCN group (9.4%) rather than in low OCN group (22.9%) with p<0.05. However, after adjusting age, sex, body fat, BMI, and insulin levels, there is no significant correlation between OCN and the occurrence of hepatic fibrosis. Two big-sample Chinese researches also showed that the levels of OCN in serum are significantly lower in male patients with NAFLD than those in normal participants[13,14]. However, no significant correlation can be found between OCN and NAFLD after adjusting age and multiple metabolic factors. Some clinical studies have shown that there is a clear negative correlation between OCN and the levels of blood glucose, HbA1c, and insulin resistance. The correlation was present in both normal glucose tolerance, pre-diabetes, and diabetic patients and was independent of gender, age, BMI, ethnicity and other factors[15-17]. In an animal study, it is confirmed that the appropriate concentrations of OCN vector implanted in the mice can increase insulin secretion and decrease the level of blood glucose[18]. Therefore, there is quite a clear association between OCN and glucose metabolism, regulation of insulin secretion, and improvement of insulin resistance. In addition to our findings, a number of studies have revealed that the level of OCN is significantly lower in fatty liver patients. However, after adjusting the factors affecting glucose metabolism, such as the level of blood glucose and islet function, the correlation between the level of OCN and the occurrence of fatty liver is not significant. Thus, the effect of OCN does not directly regulate the fat deposition in liver. Instead, it conducts regulation through improving glucose metabolism, insulin secretion, and resistance.

P1NP secreted by osteoblasts is the only collagen in bone tissues. It is the most abundant collagen in the body, accounting for 90% of the organic content of bone. The increased serum level of P1NP reflects the elevation of bone turnover[19]. β-CTX is a specific product of type I collagen degradation, which can reflect the activity of osteoclasts and the rate of bone resorption[20]. The levels of P1NP in normal middle-aged men are not significantly different[21,22]. To exclude the interference of age, perimenopausal and hormone levels with bone metabolic indicators, only healthy middle-aged men were enrolled in this study. Therefore, the difference between these two groups can objectively reflect the correlation between the levels of P1NP and the occurrence of fatty liver. The condition is different for OCN, and there are few studies on the correlation among P1NP, β-CTX and blood glucose, lipids and energy metabolism.

Table IV. Comparisons of bone metabolism related indicators between two groups.

Group	No.	Serum calcium (mmol/L)	AKP (U/L)	OCN (ng/mL)	P1NP (ng/ml)	CTX-β (ng/mL)	VitD (ng/mL)
NAFLD	232	2.42±0.14	66.74±17.20	20.23±7.26**	38.47±12.97**	0.42±0.17	17.57±8.40
Control	308	2.43±0.14	66.97±15.95	22.71±7.67	42.75±15.41	0.45±0.18	17.10±8.58

Student's *t*-test, *p<0.05 and **p<0.01.

Iglesias et al[23] found that there were no statistically significant differences in the levels of P1NP and β-CTX among the obese people divided into normal glucose tolerance group, pre-diabetes, and diabetic group. Further line-of-line analysis showed that the level of P1NP was negatively correlated with the level of HDL-C pre-diabetes and positively correlated with HbA1c in obese people with pre-diabetes. However, after adjustment in the multiple linear regression analysis, and there was no significant correlation of P1NP and β-CTX withHbA1c, HOMA-IR and HOMA-β. Zhou et al[24] showed that the level of OCN is associated with the level of HDL-C in men, and is associated with the level of TG in postmenopausal women. The study indicated that in middle-aged men, the level of P1NP in NAFLD group was significantly lower than that in Control group, and even after adjusting the age, BMI, the level of blood glucose and pancreatic islet function, there was still a significant association between the level of P1NPand the occurrence of fatty liver.

Conclusions

Therefore, it was speculated that unlike OCN, the correlation between the level of P1NP and the occurrence of fatty liver was independent of blood glucose and might be directly involved in lipid metabolism. The results of this study only present the conclusion of the clinical research, and its specific mechanism requires further research and discussion.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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