

# Association of nonalcoholic fatty liver disease with bone mineral density and serum osteocalcin levels in Korean men

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**Objective** Bone mineral density has been reported to negatively associate with nonalcoholic fatty liver disease. Osteocalcin, a bone formation marker and metabolic regulator, has been previously evaluated as the mediator between bone mineral density and nonalcoholic fatty liver disease. Herein, we aimed to investigate the correlations of nonalcoholic fatty liver disease with bone mineral density and serum osteocalcin levels in Korean men.

**Methods** A total of 859 men (249 and 610 men with and without nonalcoholic fatty liver disease, respectively) were recruited for this retrospective cross-sectional study. All participants underwent hepatic ultrasonography and dual energy X-ray absorptiometry. Anthropometric and biochemical data, including the serum osteocalcin levels and homeostasis model assessment of insulin resistance (HOMA-IR), were collected.

**Results** Nonalcoholic fatty liver disease negatively associated with right-hip bone mineral density (odds ratio, 0.797; 95% confidence interval, 0.645–0.984;  $P=0.035$ ) and serum osteocalcin (odds ratio, 0.948; 95% confidence interval, 0.910–0.988;  $P=0.011$ ) after adjusting for BMI and HOMA-IR. The mean right-hip bone mineral density was lower in men with versus without nonalcoholic fatty liver disease after adjusting for serum osteocalcin, BMI and HOMA-IR ( $0.11 \pm 0.06$  vs.  $0.29 \pm 0.04$ ;  $P=0.019$ ).

**Conclusion** Nonalcoholic fatty liver disease negatively associated with right-hip bone mineral density and serum osteocalcin in Korean men. General population-based prospective studies evaluating the causal relationship between bone metabolism and nonalcoholic fatty liver disease are needed, and the mechanism linking nonalcoholic fatty liver disease to bone mineral density beyond insulin resistance and osteocalcin should be evaluated in the future. *Eur J Gastroenterol Hepatol* 28:338–344  
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## Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as fatty infiltration of the liver proven by histology or radiology, in the absence of secondary causes of hepatitis such as alcoholic liver disease, drug-induced liver disease and viral hepatitis [1]. NAFLD is the most common chronic liver disease in developed countries; in Korea, the reported prevalence of NAFLD ranges between 16 and 33% [2]. NAFLD is associated with a risk of progression to cirrhosis and hepatocellular carcinoma [1]. Moreover, the effects of NAFLD are not only confined to the liver, but also expand to the extrahepatic organs [2]. The mechanism of this

influence of NAFLD on the extrahepatic internal organs has been demonstrated to be associated with insulin resistance and chronic inflammation, while the exact pathophysiology remains unclear [2].

Low bone mineral density (BMD) and osteoporosis are important contributors to osteoporotic fractures, which can consequently result in disability and secondary medical problems because of immobilization. Besides the traditional risk factors of osteoporotic fractures, such as older age, decreased BMI, current smoking and excessive alcohol consumption [3], insulin resistance and metabolic derangement have also been considered as factors associated with BMD [4–7]. Moreover, low BMD or osteoporotic fracture has been described not only in cholestatic liver disease and cirrhosis but also in noncirrhotic chronic liver diseases such as alcoholic liver disease and viral hepatitis [8–11]. NAFLD is the most common chronic liver disease associated with insulin resistance and metabolic derangement; thus, bone metabolism in patients with NAFLD needs to be investigated. Recently, low BMD in patients with NAFLD was reported [12–14]. However, there are currently only few studies in adult men [13–15].

The pathophysiology linking bone metabolism and NAFLD is not clearly understood. Insulin resistance, chronic inflammation, 25-hydroxyvitamin D<sub>3</sub>, adipokines such as leptin and adiponectin, osteocalcin (OC) and osteoprotegerin from osteoblasts, and osteopontin have been considered as mediators of the interactions between the liver, adipose tissue and bone [16,17]. Among these, OC is an

*European Journal of Gastroenterology & Hepatology* 2016, 28:338–344

**Keywords:** bone density, insulin resistance, nonalcoholic fatty liver disease, osteocalcin

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Received 4 August 2015 Accepted 23 October 2015

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osteoblast-derived 49 amino acid-long noncollagen protein of the bone matrix. OC exists in two forms, carboxylated and undercarboxylated OC [18]. Undercarboxylated OC is the active form [18], and acts as an insulin secretagogue, an insulin sensitiser and a protector against fatty infiltration and inflammation of the adipose tissue, liver and skeletal muscle [17]. On the other hand, the role of carboxylated OC binding to hydroxyapatite of the bone matrix in bone formation and mineralization is unclear [19]. Of note, resorption of the bone matrix by osteoclasts results in the release and decarboxylation of bound carboxylated OC [18]. Thus, serum OC levels function as a marker of bone remodelling activity, irrespective of the predominance of formation or resorption. Several human epidemiological studies have demonstrated the relationship between serum OC levels and NAFLD, although the results are inconclusive [20–23].

Thus, with this in mind, our study aimed to investigate the correlations of NAFLD with BMD and serum OC levels in Korean men.

## Methods

Data were collected retrospectively from 2227 men aged 20–69 years who underwent hepatic ultrasonography, dual energy X-ray absorptiometry and serum OC measurement for health screening at Sungkyunkwan University Samsung Changwon hospital between January 2011 and December 2013.

The exclusion criteria were as follows: (a) absence of interview record ( $n=268$ ); (b) absence of information regarding alcohol consumption ( $n=117$ ); (c) absence of information regarding smoking ( $n=233$ ); (d) positive serologic hepatitis B surface antigen ( $n=108$ ); (e) positive serologic hepatitis C antibody ( $n=11$ ); (f) excessive alcohol consumption of greater than or equal to 210 g/week ( $n=633$ ); (g) history of malignancy, chronic use of medication, present illness or past history influencing the liver function or BMD ( $n=260$ ); (h) medication for diabetes, hypertension or dyslipidaemia ( $n=186$ ); and (i) metal implant in the right hip ( $n=2$ ) (Fig. 1).

Finally, 859 men were enrolled in this retrospective cross-sectional study. The patients were divided into the NAFLD ( $n=249$ ) and non-NAFLD groups ( $n=610$ ).

Weight, height, systolic blood pressure and diastolic blood pressure were measured. BMI was calculated by dividing the body weight (kg) by the square of height (m). The blood of each individual was sampled after overnight fasting. The aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, glucose, insulin, LDL cholesterol, triglyceride, HDL cholesterol, calcium and phosphorus levels were measured. The serum OC levels were determined by the electrochemiluminescence method using the Elecsys N-MID Osteocalcin kit (Roche Diagnostics, Mannheim, Germany) and Modular Analytics E170 (Roche Diagnostics).

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following equation:

$$\text{HOMA-IR} = \text{Glucose (mg/dl)} \times \text{insulin (mU/l)} / 305.$$

Data about smoking status and the amount of alcohol consumption (g/week) were collected from previously self-reported questionnaires. A current smoker was defined as

a subject who had smoked in the previous year from the date of completing the questionnaire.

BMD was measured by dual energy X-ray absorptiometry (Lunar Radiation Co., Madison, Wisconsin, USA). The right-hip BMD of each subject was determined and presented as the *T*-score.

The presence of fatty infiltration of the liver was diagnosed by experienced sonographers. Presence of NAFLD was defined as higher echogenicity of the liver parenchyma, as compared with that of the right kidney; bright echo in the liver parenchyma; and blurring of the intrahepatic architecture on the hepatic ultrasonography.

The protocol of our study was approved by the Institutional Review Board of Sungkyunkwan University Samsung Changwon hospital (2014-SCMC-076-00). The need for informed consent was waived by the Institutional Review Board as the study was retrospective in nature.

## Statistical analysis

PASW Statistics Window, version 18.0 (IBM Co., Armonk, New York, USA) was used for all analyses. Continuous variables were presented as mean and SD. Categorical variables were presented as number and proportion.

The differences in the means of continuous variables between the NAFLD and non-NAFLD groups were compared using Student's *t*-test and the Mann–Whitney *U*-test. Differences in frequencies of categorical variables between the groups were compared by the  $\chi^2$ -test.

The correlations of the serum OC levels with other variables were evaluated using multiple backward linear regression analysis, including variables that showed significant correlations in the simple linear regression analysis. Before linear regression analysis, skewed variables were normalized by logarithmic transformation and variables with collinearity were determined as a variance proportion in excess of 0.6 in the model, with a conditional index greater than 30.

The relationships between right-hip BMD and other variables were analysed by multiple backward linear regression analysis, including variables that showed significant correlation in the simple linear regression analysis.

The associations of NAFLD with right-hip BMD and serum OC levels were determined by using multiple backward Wald binary logistic regression analysis, including variables that showed a significant correlation with serum OC or right-hip BMD in the multiple linear regression analysis.

The difference in the adjusted mean of the right-hip BMD (*T*-score) in subjects with versus without NAFLD was evaluated by the analysis of covariance. All *P* values were two tailed, and *P* less than 0.05 was considered statistically significant.

## Results

### Baseline demographic and clinical characteristics

Among the included 859 subjects (mean age,  $45 \pm 7$  years), 249 subjects had NAFLD (29%). The mean *T*-score of the right-hip BMD was  $0.24 \pm 0.94$ . The mean serum OC level was  $16.98 \pm 4.64$  ng/ml (Table 1).

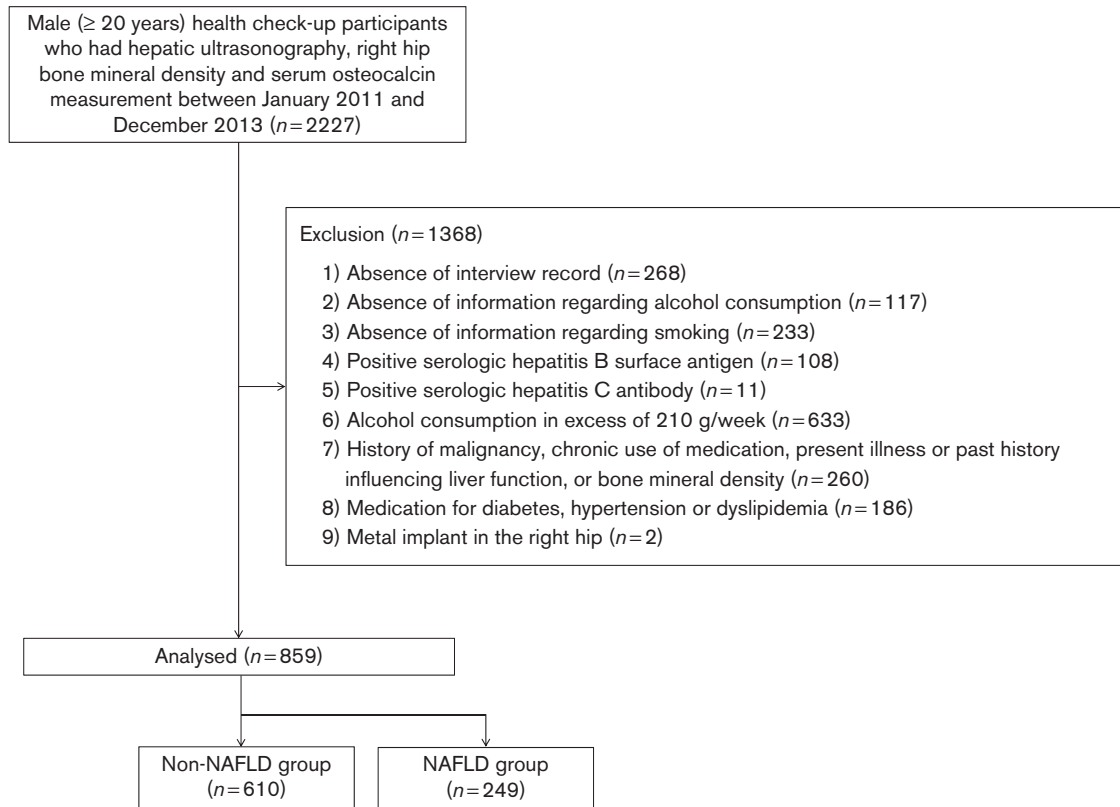


Fig. 1. Flow chart of the study. NAFLD, nonalcoholic fatty liver disease.

Table 1. Baseline demographic and clinical characteristics of the study population

Parameters	Total ( $n=859$ )	Non-NAFLD group ( $n=610$ )	NAFLD group ( $n=249$ )	P value
Right-hip BMD (T-score)	$0.24 \pm 0.94$	$0.19 \pm 0.94$	$0.34 \pm 0.92$	0.038 <sup>a</sup>
Osteocalcin (ng/ml)	$16.98 \pm 4.64$	$17.36 \pm 4.68$	$16.07 \pm 4.42$	<0.001 <sup>a</sup>
Age (years)	$45 \pm 7$	$45 \pm 7$	$45 \pm 6$	0.342 <sup>a</sup>
Height (cm)	$171.3 \pm 5.3$	$172 \pm 5$	$171 \pm 6$	0.168 <sup>a</sup>
Weight (kg)	$70 \pm 12$	$68 \pm 13$	$75 \pm 9$	<0.001 <sup>a</sup>
BMI ( $\text{kg}/\text{m}^2$ )	$23.7 \pm 2.6$	$22.9 \pm 2.4$	$25.5 \pm 8.5$	<0.001 <sup>a</sup>
SBP (mmHg)	$121 \pm 15$	$119 \pm 15$	$126 \pm 15$	<0.001 <sup>a</sup>
DBP (mmHg)	$72 \pm 11$	$70 \pm 10$	$76 \pm 11$	<0.001 <sup>a</sup>
AST (IU/l)	$23 \pm 9$	$22 \pm 7$	$26 \pm 13$	<0.001 <sup>b</sup>
ALT (IU/l)	$24 \pm 15$	$20 \pm 10$	$33 \pm 19$	<0.001 <sup>b</sup>
Glucose (mg/dl)	$90 \pm 11$	$88 \pm 9$	$94 \pm 15$	<0.001 <sup>b</sup>
Insulin (mU/l)	$4.6 \pm 2.7$	$3.9 \pm 2.2$	$6.5 \pm 3.1$	<0.001 <sup>b</sup>
HOMA-IR	$1.4 \pm 0.9$	$1.1 \pm 0.7$	$2.0 \pm 1.1$	<0.001 <sup>b</sup>
LDL cholesterol (mg/dl)	$130 \pm 31$	$125 \pm 29$	$142 \pm 33$	<0.001 <sup>a</sup>
HDL cholesterol (mg/dl)	$55 \pm 13$	$57 \pm 13$	$49 \pm 11$	<0.001 <sup>b</sup>
Triglyceride (mg/dl)	$124 \pm 101$	$104 \pm 45$	$174 \pm 156$	<0.001 <sup>b</sup>
Calcium (mg/dl)	$9.4 \pm 0.4$	$9.4 \pm 0.4$	$9.5 \pm 0.4$	0.021 <sup>a</sup>
Phosphorus (mg/dl)	$3.3 \pm 0.4$	$3.3 \pm 0.4$	$3.3 \pm 0.4$	0.667 <sup>a</sup>
Amount of alcohol consumption (g/week)	$82 \pm 60$	$87 \pm 60$	$71 \pm 58$	0.001 <sup>a</sup>
Current smoker [n (%)]	358 (41.7)	252 (41.3)	106 (42.6)	0.734 <sup>c</sup>

The parameters are presented as the mean  $\pm$  SD for continuous variables and as  $n$  (%) for categorical variables.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure.

<sup>a</sup>Student's *t*-test.

<sup>b</sup>Mann-Whitney *U*-test.

<sup>c</sup> $\chi^2$ -test.

### Correlations of the serum OC levels with other variables

The serum OC levels were inversely correlated with the right-hip BMD [ $\beta = -0.009$ ; 95% confidence interval (CI),  $-0.018$  to  $0.000$ ;  $P=0.040$ ], age ( $\beta = -0.304$ ; 95% CI,  $-0.408$  to  $-1.190$ ;  $P < 0.001$ ), BMI ( $\beta = -0.005$ ; 95% CI,

$-0.008$  to  $0.002$ ;  $P=0.002$ ) and systolic blood pressure ( $\beta = -0.160$ ; 95% CI,  $-0.315$  to  $-0.005$ ;  $P=0.043$ ), and positively correlated with the serum calcium ( $\beta = 0.645$ ; 95% CI,  $0.196$ – $1.093$ ;  $P=0.005$ ) and phosphorus levels ( $\beta = 0.229$ ; 95% CI,  $0.083$ – $0.374$ ;  $P=0.002$ ) (Table 2).

**Table 2.** Correlation between serum osteocalcin levels and other variables

	Univariable analysis		Multivariable analysis <sup>a</sup>	
	$\beta$ (95% CI)	<i>P</i> value	$\beta$ (95% CI)	<i>P</i> value
Right-hip BMD ( <i>T</i> -score)	-0.014 (-0.222 to -0.005)	0.001	-0.009 (-0.018 to 0.000)	0.040
Age <sup>b</sup> (years)	-0.336 (-0.452 to -0.220)	<0.001	-0.304 (-0.408 to -1.190)	<0.001
BMI (kg/m <sup>2</sup> )	-0.008 (-0.011 to -0.005)	<0.001	-0.005 (-0.008 to -0.002)	0.002
SBP <sup>b</sup> (mmHg)	-0.289 (-0.437 to -0.141)	<0.001	-0.160 (-0.315 to -0.005)	0.043
DBP <sup>b,c</sup> (mmHg)	-0.281 (-0.402 to -0.160)	<0.001	-	-
AST <sup>b,d</sup> (IU/l)	-0.077 (-0.135 to -0.020)	0.008	-	-
ALT <sup>b</sup> (IU/l)	-0.057 (-0.094 to -0.020)	0.002	-	-
Glucose <sup>b</sup> (mg/dl)	-0.250 (-0.410 to -0.089)	0.002	-	-
HOMA-IR <sup>b</sup>	-0.011 (-0.037 to 0.016)	0.439	-	-
LDL cholesterol <sup>b</sup> (mg/dl)	-0.044 (-0.119 to 0.031)	0.247	-	-
HDL cholesterol <sup>b</sup> (mg/dl)	0.044 (-0.034 to 0.123)	0.269	-	-
Triglyceride <sup>b</sup> (mg/dl)	-0.055 (-0.088 to -0.021)	0.001	-	-
Calcium <sup>b</sup> (mg/dl)	0.843 (0.392-1.293)	<0.001	0.645 (0.196-1.093)	0.005
Phosphorus <sup>b</sup> (mg/dl)	0.323 (0.176-0.470)	<0.001	0.229 (0.083-0.374)	0.002
Amount of alcohol consumption <sup>b</sup> (g/week)	-0.016 (-0.036 to 0.005)	0.141	-	-
Current smoker	0.005 (-0.011 to 0.021)	0.537	-	-

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; CI, confidence interval; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure.

<sup>a</sup>Variables inserted into the multiple backward linear regression analysis were as follows: right-hip BMD, age, BMI, SBP, ALT, glucose, triglyceride, calcium, and phosphorus.

<sup>b</sup>Variables with skewed distribution were normalized by logarithmic transformation before analysis.

<sup>c</sup>DBP was not included in the multivariable analysis since the variance proportions of SBP and DBP were 0.77 and 0.60, respectively, in the model, of which the condition index was 340 in the multicollinearity analysis.

<sup>d</sup>AST was not included in the multivariable analysis since the variance proportions of AST and ALT were 0.86 and 0.69, respectively, in the model, with a condition index of 58 in the multicollinearity analysis.

### Correlations of right-hip BMD (*T*-score) with other variables

The right-hip BMD was inversely correlated with age ( $\beta = -1.122$ ; 95% CI,  $-2.027$  to  $-0.217$ ;  $P = 0.015$ ) and HOMA-IR ( $\beta = -0.430$ ; 95% CI,  $-0.663$  to  $-0.197$ ;  $P < 0.001$ ), and positively correlated with BMI ( $\beta = 0.145$ ; 95% CI,  $0.119$ - $0.171$ ;  $P < 0.001$ ). No independent correlation between the right-hip BMD and serum OC level was found ( $\beta = -0.441$ ; 95% CI,  $-0.962$  to  $0.081$ ;  $P = 0.097$ ) (Table 3).

### Associations of NAFLD with right-hip BMD and serum OC levels

NAFLD was negatively associated with the right-hip BMD [odds ratio (OR), 0.797; 95% CI, 0.645-0.984;  $P = 0.035$ ]

and serum OC levels (OR, 0.948; 95% CI, 0.910-0.988;  $P = 0.011$ ), and positively associated with BMI (OR, 1.460; 95% CI, 1.336-1.594;  $P < 0.001$ ) and HOMA-IR (OR, 2.508; 95% CI, 1.992-3.159;  $P < 0.001$ ) (Table 4).

### Differences in the mean serum OC levels and *T*-scores of right-hip BMD in subjects with versus without NAFLD

The mean serum OC levels were lower in men with versus without NAFLD ( $16.07 \pm 4.42$  vs.  $17.36 \pm 4.68$  ng/ml;  $P < 0.001$ ). The mean *T*-score of right-hip BMD was higher in men with versus without NAFLD ( $0.34 \pm 0.92$  vs.  $0.19 \pm 0.94$ ;  $P = 0.038$ ). Finally, the mean right-hip BMD was lower in men with NAFLD than in those without NAFLD after

**Table 3.** Correlations between the *T*-score of right-hip BMD and other variables

	Univariable analysis		Multivariable analysis <sup>a</sup>	
	$\beta$ (95% CI)	<i>P</i> value	$\beta$ (95% CI)	<i>P</i> value
Osteocalcin <sup>b</sup> (ng/ml)	-0.872 (-1.402 to -0.343)	0.001	-0.441 (-0.962 to 0.081)	0.097
Age <sup>b</sup> (years)	-1.025 (-1.961 to -0.088)	0.032	-1.122 (-2.027 to -0.217)	0.015
BMI (kg/m <sup>2</sup> )	0.124 (0.102-0.146)	<0.001	0.145 (0.119-0.171)	<0.001
SBP <sup>b</sup> (mmHg)	1.953 (0.775-3.132)	0.001	-	-
DBP <sup>b</sup> (mmHg)	0.695 (-0.279 to 1.669)	0.162	-	-
AST <sup>b</sup> (IU/l)	0.011 (-0.447 to 0.469)	0.963	-	-
ALT <sup>b</sup> (IU/l)	0.054 (-0.241 to 0.348)	0.720	-	-
Glucose <sup>b</sup> (mg/dl)	0.923 (-0.354 to 2.199)	0.156	-	-
HOMA-IR <sup>b</sup>	0.209 (-0.004 to 0.422)	0.055	-0.430 (-0.663 to -0.197)	<0.001
LDL cholesterol <sup>b</sup> (mg/dl)	0.487 (-0.150 to 1.080)	0.107	-	-
HDL cholesterol <sup>b</sup> (mg/dl)	-0.922 (-1.543 to -0.301)	0.004	-	-
Triglyceride <sup>b</sup> (mg/dl)	0.221 (-0.046 to 0.487)	0.104	-	-
Calcium <sup>b</sup> (mg/dl)	-0.794 (-4.396 to 2.809)	0.666	-	-
Phosphorus <sup>b</sup> (mg/dl)	-0.001 (-1.180 to 1.179)	0.999	-	-
Amount of alcohol consumption <sup>b</sup> (g/week)	0.146 (-0.018 to 0.311)	0.081	-	-
Current smoker	0.072 (-0.055 to 0.199)	0.267	-	-

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; CI, confidence interval; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure.

<sup>a</sup>Variables inserted into the multiple backward linear regression analysis were as follows: osteocalcin, age, BMI, SBP, HOMA-IR, HDL cholesterol, and amount of alcohol consumption.

<sup>b</sup>Variables with skewed distribution were normalized by logarithmic transformation before analysis.



**Table 4.** Associations of NAFLD with right-hip BMD and osteocalcin levels

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Right-hip BMD (T-score)	1.181 (1.009–1.383)	0.038	0.797 (0.645–0.984)	0.035
Osteocalcin (ng/ml)	0.938 (0.906–0.971)	<0.001	0.948 (0.910–0.988)	0.011
BMI (kg/m <sup>2</sup> )	1.584 (1.467–1.710)	<0.001	1.460 (1.336–1.594)	<0.001
HOMA-IR	3.538 (2.844–4.401)	<0.001	2.508 (1.992–3.159)	<0.001
Age (years)	1.011 (0.989–1.033)	0.341	–	–
SBP (mmHg)	1.031 (1.021–1.042)	<0.001	–	–
Calcium (mg/dl)	1.576 (1.069–2.324)	0.022	–	–
Phosphorus (mg/dl)	0.923 (0.641–1.329)	0.667	–	–

Variables inserted into the backward Wald binary logistic regression analysis were as follows: right-hip BMD, osteocalcin, BMI, HOMA-IR, age, SBP, calcium, and phosphorus.

BMD, bone mineral density; CI, confidence interval; HOMA-IR; homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; SBP, systolic blood pressure.

adjusting for the serum OC levels, BMI and HOMA-IR ( $0.11 \pm 0.06$  vs.  $0.29 \pm 0.04$ ;  $P = 0.019$ ) (Table 5).

## Discussion

In this study, the serum OC levels predominantly associated with factors related to bone metabolism, such as BMD and serum calcium and phosphorus levels, rather than those related to glucose and energy metabolism. This result was inconsistent with some previous reports. In animal and in-vitro models, obvious correlations between OC and metabolic derangements have been demonstrated. In one study, OC-deficient mice fed a high fat diet were demonstrated to show glucose intolerance and insulin resistance, whereas administration of recombinant OC improved these metabolic derangements [24,25]. Moreover, human epidemiologic studies have demonstrated that the serum OC levels correlate with glucose intolerance, insulin resistance, obesity, and metabolic syndrome [26–28]. We speculate that the highly selective subjects in this study contributed to the finding of predominant associations between the serum OC levels and bone metabolism factors. The subjects were all recruited from health screening participants of the working population in small-to-large businesses, and we excluded subjects with medication for diabetes, dyslipidaemia or hypertension. Thus, the baseline demographic indicated that the subjects of our study tended to have relatively few metabolic derangements. This might have augmented the role of OC as a marker of bone formation, while attenuating its role in energy and glucose metabolism.

In this study, insulin resistance was found to be a negative determinant of bone metabolism, besides the traditional determinants of bone metabolism such as BMI and age. It has been previously reported that insulin resistance is negatively associated with BMD in women with type 2 diabetes, and a higher BMD, but increased risk of hip fracture in patients with type 2 diabetes has also been demonstrated [4,7,29,30]. Several animal models and in-vitro studies support this negative association between BMD and insulin resistance. For example, high fat diet-induced insulin resistance impairs osteoblast proliferation and survival resulting in osteoporosis [31], and cytokines such as C-reactive protein and interleukin-6, which induce chronic inflammation, have been shown to result in low BMD in insulin resistant states [32]. Moreover, low insulin-like growth factor-1 and high fibroblast growth factor-23 levels in insulin resistant states negatively affect the BMD [33]. Interestingly, in the present study, the correlation between right-hip BMD and serum OC levels disappeared when HOMA-IR was inserted during the multiple linear regression analysis. This suggests that the serum OC levels are not a passive marker of bone remodelling, but rather an indirect regulator of bone metabolism via the secretion and sensitization of insulin.

NAFLD was negatively associated with the serum OC levels in this study. OC-deficient mice with metabolic derangements resulting from a Western-style high-fat, high-cholesterol diet have been demonstrated to have a higher frequency of NAFLD, and administration of recombinant OC improved the NAFLD in one previous study [34]. However, population-based studies have failed to demonstrate consistent results regarding the association between serum OC levels and NAFLD [20–23]. Aller *et al.* [20] and Dou *et al.* [22] reported that the serum OC levels were not independently associated with histologic changes in NAFLD and ultrasonography-proven NAFLD, respectively, whereas Yilmaz *et al.* [21] and Sinn *et al.* [23] reported that the serum OC levels were associated with hepatocyte ballooning degeneration and ultrasonography-proven NAFLD in women with normal BMD, respectively. The discrepancies between epidemiological studies and animal and in-vitro studies might be explained by the following. First, in most epidemiologic studies, as well as in the present study, undercarboxylated OC, considered as the metabolic active form, was not measured; instead, the total OC levels were evaluated. Total OC levels might

**Table 5.** Means and adjusted means of the serum osteocalcin levels and right-hip BMD

Parameters	NAFLD group (n = 249)	Non-NAFLD group (n = 610)	P value
Osteocalcin <sup>a</sup> (ng/ml)	16.07 ± 4.42	17.36 ± 4.68	<0.001 <sup>b</sup>
Right-hip BMD <sup>a</sup> (T-score)	0.34 ± 0.92	0.19 ± 0.94	0.038 <sup>b</sup>
Adjusted right-hip BMD <sup>c,d</sup> (T-score)	0.11 ± 0.06	0.29 ± 0.04	0.019 <sup>e</sup>

BMD, bone mineral density; HOMA-IR; homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease.

<sup>a</sup>Values are presented as mean ± SD.

<sup>b</sup>P value by Student's *t*-test.

<sup>c</sup>Values are presented as mean ± SE.

<sup>d</sup>Covariates: osteocalcin, BMI, and HOMA-IR.

<sup>e</sup>P value by analysis of covariance.

insufficiently represent the metabolic role of OC. Second, in many epidemiologic studies, artificial OC-deficient models were unavailable. Inconsistencies between population-based studies might also be due to differences in the ethnicity, sex, age groups and bone remodelling status of the study subjects.

Our results indicated that NAFLD is negatively associated with right-hip BMD. This result was similar to those of Moon *et al.* [12], who investigated postmenopausal women, and of Cui *et al.* [13], who analysed men and women aged more than or equal to 40 years. However, the dependent variables representing bone metabolism were different in these studies. Moon *et al.* [12] used the mean lumbar-vertebral BMD, whereas Cui *et al.* [13] used the femoral-neck, right-hip, lumbar-vertebral BMD and the means of these BMDs. Herein, we selected the *T*-score of the right-hip BMD as it was normally distributed, whereas the *T*-scores of the femoral-neck and lumbar-vertebral BMD were not. Furthermore, the right-hip and femoral-neck BMD are known to better reflect the risk of fracture than is lumbar-vertebral BMD [3].

Our results indicated that NAFLD and BMD correlated independent of the BMI, insulin resistance and serum OC levels. Thus, further studies are required to focus on chronic inflammation, central adiposity, serum vitamin D levels and physical activity to investigate the exact mechanism linking NAFLD to BMD [16,17].

The strengths of our study included the relatively large sample size and clear process in recruiting the NAFLD and normal groups. However, this study had several limitations. First, the retrospective cross-sectional nature of the study failed to explain the causal and temporal relationship between BMD and NAFLD. Second, highly selective subjects were recruited, and further prospective studies with a general population, including women, are needed. Third, ultrasonography was used to diagnose NAFLD. The gold standard for diagnosis of NAFLD is liver biopsy. However, liver biopsy is not frequently used in epidemiological studies and in the clinic because of its invasiveness and complications such as bleeding and pain. On the other hand, ultrasonography is a noninvasive and easily accessible method for the diagnosis of NAFLD. Fourth, chronic inflammation markers, including tumour necrosis factor- $\alpha$  and interleukin-6; central adiposity, including waist circumference and adiponectin levels; serum vitamin D levels; and physical activity, which might all affect the BMD, were not evaluated [16,17]. Lastly, total OC rather than undercarboxylated OC, which is considered the active form, was measured.

In conclusion, this study showed that NAFLD was negatively associated with the right-hip BMD and serum OC levels. General population-based prospective studies to evaluate the causal relationship between bone metabolism and NAFLD are needed in the future. In addition, the mechanisms linking NAFLD to BMD, beyond insulin resistance and OC, should be evaluated.

## Acknowledgements

Author contributions: Hae Jin Yang and Sang Goon Shim designed the research. Hae Jin Yang, Bong Oh Ma, and Ji Yeong Kwak performed the research. Hae Jin Yang and

Bong Oh Ma performed statistical analysis. Hae Jin Yang wrote the manuscript.

## Conflicts of interest

There are no conflicts of interest.

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