

Decreased Serum Osteocalcin is an Independent Risk Factor for Metabolic Dysfunction-Associated Fatty Liver Disease in Type 2 Diabetes

Yu-Jie Wang¹*, Chun-Hua Jin^{2,*}, Jiang-Feng Ke¹, Jun-Wei Wang¹, Yi-Lin Ma¹, Jun-Xi Lu¹, Mei-Fang Li³, Lian-Xi Li¹

¹Department of Endocrinology and Metabolism, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, Shanghai, People's Republic of China; ²Department of Endocrinology and Metabolism, Shanghai Songjiang District Central Hospital, Songjiang Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Preparatory Stage), Shanghai, People's Republic of China; ³Department of Emergency, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Mei-Fang Li, Department of Emergency, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 600 Yishan Road, Shanghai, 200233, People's Republic of China, Tel +86 18930170426, Email 15821955054@126.com; Lian-Xi Li, Department of Endocrinology and Metabolism, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, 600 Yishan Road, Shanghai, 200233, People's Republic of China, Tel +86 21 64369181x58337, Email lilx@sjtu.edu.cn

Purpose: The association between serum osteocalcin (OCN) levels and metabolic dysfunction-associated fatty liver disease (MAFLD) is still controversial. Moreover, few studies have explored their relationship in type 2 diabetes mellitus (T2DM) patients so far. The present study aimed to investigate the association of serum OCN levels with MAFLD in Chinese T2DM patients.

Methods: This cross-sectional, real-world study included 1889 Chinese T2DM inpatients. MAFLD was diagnosed by abdominal ultrasonography. Participants were divided into four groups according to serum OCN quartiles, among which the clinical characteristics were compared. The association of serum OCN levels with the presence of MAFLD was also analyzed in subjects.

Results: After controlling for sex, age, and diabetes duration, the prevalence of MAFLD significantly decreased across the serum OCN quartiles (55.3%, 52.0%, 48.6%, and 42.1% for the first, second, third, and fourth quartiles, respectively, $P < 0.001$ for trend). A fully adjusted multiple logistic regression analysis showed that serum OCN levels were independently and negatively associated with the presence of MAFLD in T2DM patients (odds ratio, 0.832; 95% confidence interval, 0.719–0.962; $P = 0.013$). Furthermore, there were significant decreases in HOMA-IR ($P = 0.001$ for trend) and C-reactive protein ($P < 0.001$ for trend) levels across the serum OCN quartiles after controlling for sex, age, and diabetes duration.

Conclusion: Serum OCN levels were independently and negatively associated with the presence of MAFLD in Chinese T2DM patients, partially due to the improvement of insulin resistance and inflammation mediated by OCN. Serum OCN may be used as a biomarker to assess the risk of MAFLD in T2DM patients.

Keywords: diabetes mellitus, type 2, metabolic dysfunction-associated fatty liver disease, osteocalcin, insulin resistance, inflammation

Introduction

Osteocalcin (OCN), regarded as a biomarker of bone formation, has been recently found to contribute to the regulation of insulin sensitivity and energy metabolism in rodents.¹ However, two recent research revealed that the deficiency of OCN shows no effect on glucose metabolism in OCN-knockout mouse.^{2,3} Despite certain differences between murine and human OCN, some studies in humans have pointed out the negative association between serum OCN levels and metabolic diseases such as obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome.^{4–8} For example, Liu

et al⁵ demonstrated that lower serum OCN levels were correlated with less favorable metabolic parameters including waist circumference (WC), triglyceride (TG), blood glucose, blood pressure and high-density lipoprotein cholesterol (HDL-C), and a higher risk of metabolic syndrome.

As a metabolic disease, metabolic dysfunction-associated fatty liver disease (MAFLD), formerly named non-alcoholic fatty liver disease (NAFLD), is a new term to describe the liver disease associated with known metabolic dysfunction,⁹ which has been endorsed by multiple institutions and individuals.¹⁰ There have been several studies focusing on the relationship of serum OCN levels with NAFLD in general population, some of which revealed the decreased change of serum OCN levels in NAFLD patients.^{11–17} However, a few studies reported that the close association between serum OCN and NAFLD was no longer significant after adjustments for metabolic factors.^{14–16} Therefore, the relationship between serum OCN levels and MAFLD is inconclusive so far.

Currently, it is well established that T2DM patients tend to have a significantly higher risk of MAFLD than general population.^{18,19} Furthermore, NAFLD and cardiovascular diseases share several metabolic risk factors including T2DM.²⁰ A recent study revealed that NAFLD was associated with an increased risk of cardiovascular diseases, hepatocellular carcinoma, and all-cause mortality in T2DM subjects.²¹ Additionally, few studies have investigated the correlation between serum OCN levels and MAFLD in T2DM patients, and the findings from different studies are inconsistent. For example, Wang et al²² reported that serum OCN levels were negatively associated with advanced NAFLD status in middle-aged and elderly men and postmenopausal women with T2DM. However, another study in postmenopausal women with either T2DM or impaired glucose regulation presented a contrary result, in which a positive correlation between OCN levels and NAFLD fibrosis score was observed.²³

Therefore, the aims of the present study were to explore the clinical characteristics of MAFLD and the association of serum OCN levels with MAFLD in Chinese T2DM patients in a real-world setting.

Methods

Study Design and Population

T2DM patients hospitalized in our department from January 2009 to December 2012 were enrolled consecutively in this study, and information of some patients was obtained from our previous studies.^{24–26} The exclusion criteria were as follows: incomplete clinical data; without data of serum OCN and abdominal ultrasonography; diseases influencing serum OCN levels such as hyperparathyroidism; and liver injury caused by drugs, viral hepatitis, and other reasons except for drinking. Finally, there were 1889 patients including 1089 men and 800 women in the present study. According to serum OCN quartiles, the patients were divided into four groups.

All studied subjects were interviewed to obtain their diabetes duration (DD), smoking and drinking habits, history of hypertension (HTN), medications including insulin or insulin analogs (IIAs), lipid-lowering drugs (LLDs), metformin, and insulin sensitizers. The definitions of HTN, smoking, and drinking were consistent with our previous studies.^{27,28}

Medical Examinations and Laboratory Tests

Physical examination included height, weight, hip circumference (HC), WC, and blood pressure. The calculation of waist-to-hip ratio (WHR) and body mass index (BMI) was in line with our previous studies.²⁴ Blood pressure was measured with a standard mercury sphygmomanometer. Obesity was defined as a BMI above 25 kg/m² based on the Asia/Pacific criteria set by the World Health Organization, consistent with our previous study.²⁹

Venous blood samples from the subjects were collected for laboratory testing after an overnight fast and 2 hours after breakfast, respectively. The measured clinical parameters included fasting plasma glucose (FPG), 2-hour postprandial plasma glucose (2h PPG), glycated hemoglobin A1c (HbA1C), fasting insulin (Fins), 2-hour postprandial insulin (2hins), fasting C-peptide (FCP), 2-hour postprandial C-peptide (2h C-P), TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), lipoprotein(a) (Lp(a)), alanine aminotransferase (ALT), γ -glutamyltransferase (GGT), creatinine (Cr), C-reactive protein (CRP). 24-hour urinary albumin excretion (UAE) and estimated glomerular filtration rate (eGFR) were determined based on our previous method.³⁰ The homeostasis model assessment of insulin resistance (HOMA-IR) and HOMA2-IR were calculated using corresponding formula and computer model, respectively.^{24,31}

Additionally, serum OCN levels were determined with electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

Abdominal Ultrasonography

For detection of hepatic steatosis, abdominal ultrasound examination was conducted in each participant, which was in accordance with our previous reports.^{26,32} Based on our recent study,³³ since all participants in the current study had T2DM, subjects with the evidence of liver steatosis on abdominal ultrasonography were diagnosed as MAFLD according to the definition of MAFLD.⁹

Statistical Analysis

Statistical analyses were performed using SPSS version 15.0 for Windows. Continuous variables were tested for normality through the Kolmogorov–Smirnov test. Data were expressed as mean \pm standard deviation for normally distributed variables, and either independent sample *t*-test or one-way analysis of variance (ANOVA) with the least significant difference (LSD) was applied to compare the differences between different groups. Skewed data were described as medians with interquartile ranges, using nonparametric tests to compare the differences among different groups. For categorical variables, percentages and chi-squared tests were used to describe and analyze data, respectively. The association of serum OCN levels with the presence of MAFLD was assessed through binary logistic regression using four models. Statistical significance was considered when a two-sided *P* value was 0.05 or below.

Ethics Statement

This real-world, cross-sectional study was approved by the ethics committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval number: 2018-KY-018(K)). Each participant signed the written informed consent form. In addition, the study was in accordance with the Helsinki Declaration.

Results

Clinical Characteristics of the Study Subjects

The study subjects were divided into quartiles based on serum OCN levels with cutoffs of <9.78, 9.78–12.52, 12.53–16.28 and >16.28 mg/L. According to serum OCN quartiles, the comparisons of the clinical characteristics of the study subjects are shown in Table 1. The results showed that there were more females in the higher serum OCN quartile groups after controlling for age, but no significant difference in age was found among the four groups after adjustment for sex. Additionally, after adjusting for sex and age, the participants in the higher serum OCN quartile were more likely to have a lower prevalence of obesity, along with a lower percentage of the subjects with drinking habits, lower WC, WHR, BMI, and a lower rate of the use of IIAs and metformin (all *P* < 0.05). Interestingly, the prevalence of hypertension in the fourth quartile was significantly lower than in the other three groups (*P* = 0.015). In addition, as the serum OCN quartile increased, the levels of 2h C-P and HDL-C showed an increased trend, and the levels of FPG, 2h PPG, TG, ALT, γ -GT, Cr, eGFR, CRP, and UAE displayed a decreased trend (all *P* < 0.05). No differences were observed in DD, smoking habits, the use of LLDs and insulin sensitizers, SBP, DBP, HbA1c, Fins, 2hins, FCP, TC, LDL-C, Lp(a), and SUA.

Comparisons of MAFLD Prevalence and Serum OCN Levels Stratified by Sex, Age, and DD

The comparisons of MAFLD prevalence and serum OCN levels stratified by sex, age, and DD in T2DM patients are presented in Figure 1. The prevalence of MAFLD in all participants was 49.5%, and with 50.9% in women significantly higher than 48.5% in men after adjusting for DD and age (*P* = 0.003) (Figure 1A). Furthermore, the prevalence of MAFLD markedly decreased with the increase of age (*P* < 0.001 for trend) and prolongation of DD in T2DM patients (all *P* < 0.001 for trend) (Figure 1B and C). Additionally, serum OCN levels in women were significantly higher than in men (*P* = 0.001) (Figure 1D). However, there was no significant difference in serum OCN levels among subjects stratified by age and DD (Figure 1E and F).

Table 1 Characteristics of the Subjects According to Osteocalcin Quartiles

Variables	Q1 (n=472)	Q2 (n=469)	Q3 (n=473)	Q4 (n=475)	P value	^a P value
Serum OCN ($\mu\text{mol/L}$)	<9.78	9.78–12.52	12.53–16.28	≥ 16.29	—	—
Male (n, %)	338 (71.6)	281 (59.9)	261 (55.2)	209 (44.0)	<0.001	<0.001
Age (years)	58 \pm 13	59 \pm 12	59 \pm 13	59 \pm 13	0.870	0.245
*DD (months)	108 (36–168)	108 (36–168)	96 (36–108)	96 (36–156)	0.814	0.320
Hypertension (n, %)	263 (55.7)	264 (56.3)	269 (56.9)	229 (48.2)	0.023	0.015
Obesity (n, %)	248 (53.0)	224 (47.8)	223 (47.1)	200 (42.1)	0.010	0.011
Smoking (n, %)	186 (39.4)	168 (35.8)	146 (30.9)	121 (25.5)	<0.001	0.726
Drinking (n, %)	122 (25.8)	105 (22.4)	71 (15.0)	48 (10.1)	<0.001	<0.001
IIAs (n, %)	366 (77.5)	350 (74.6)	319 (67.4)	324 (68.2)	<0.001	<0.001
LLD (n, %)	255 (54.0)	243 (51.8)	242 (51.2)	224 (47.2)	0.198	0.192
Metformin (n, %)	334 (70.8)	310 (66.1)	298 (63.0)	253 (53.3)	<0.001	<0.001
Insulin sensitizers (n, %)	71 (15.0)	74 (15.8)	61 (12.9)	56 (11.8)	0.254	0.094
SBP (mmHg)	133 \pm 16	132 \pm 17	132 \pm 18	132 \pm 17	0.558	0.339
DBP (mmHg)	80 \pm 9	81 \pm 9	80 \pm 10	80 \pm 9	0.534	0.643
WC (cm)	91.9 \pm 11.2	90.4 \pm 9.8	90.8 \pm 10.4	88.9 \pm 9.8	<0.001	0.023
WHR	0.94 \pm 0.07	0.92 \pm 0.06	0.93 \pm 0.06	0.91 \pm 0.06	<0.001	0.001
BMI (kg/m^2)	25.4 \pm 3.5	25.0 \pm 3.4	25.0 \pm 3.4	24.6 \pm 3.2	0.005	0.005
*FPG (mmol/L)	8.56 (6.95–11.06)	7.46 (6.13–9.61)	7.49 (6.11–9.86)	7.22 (5.83–8.96)	<0.001	<0.001
*2h PPG (mmol/L)	14.33 (11.20–18.09)	13.11 (10.23–16.51)	13.02 (10.27–16.07)	12.39 (9.31–15.79)	<0.001	<0.001
HbA1c (%)	9.77 \pm 2.35	9.18 \pm 2.19	8.98 \pm 2.15	8.58 \pm 2.31	<0.001	0.870
*FCP (ng/mL)	1.78 (1.13–2.43)	1.66 (1.11–2.56)	1.87 (1.21–2.53)	1.77 (1.10–2.44)	0.283	0.459
Fins ($\mu\text{U/mL}$)	12.17 (8.10–19.51)	12.11 (7.85–20.22)	11.20 (7.42–18.50)	11.71 (7.11–20.05)	0.233	0.229
2hins ($\mu\text{U/mL}$)	48.41 (32.30–69.93)	51.81 (34.44–77.76)	49.04 (32.57–77.56)	54.46 (35.46–80.42)	0.119	0.113
*2h C-P (ng/mL)	3.29 (2.08–5.48)	3.72 (2.16–5.93)	4.21 (2.41–6.26)	3.96 (2.37–6.06)	0.001	0.005
*TG (mmol/L)	1.57 (1.01–2.56)	1.47 (0.99–2.24)	1.34 (0.94–2.06)	1.34 (0.94–1.98)	<0.001	<0.001
TC (mmol/L)	4.91 \pm 1.49	4.80 \pm 1.21	4.74 \pm 1.02	4.85 \pm 1.08	0.179	0.248
HDL-C (mmol/L)	1.05 \pm 0.30	1.10 \pm 0.29	1.10 \pm 0.30	1.15 \pm 0.35	<0.001	0.031
LDL-C (mmol/L)	2.91 \pm 0.98	2.99 \pm 1.00	2.94 \pm 0.83	2.96 \pm 0.91	0.604	0.535
*Lp (a)	9.70 (5.07–20.45)	10.40 (5.43–19.30)	10.80 (6.00–20.60)	11.80 (6.60–21.75)	0.050	0.179
*ALT (U/L)	20 (15–30)	21 (15–31)	20 (14–30)	18 (13–29)	0.002	0.008
* γ -GT (U/L)	26 (18–39)	25 (18–37)	24 (17–40)	22 (16–33)	<0.001	0.003
*Scr ($\mu\text{mol/L}$)	66 (54–80)	66 (55–77)	67 (54–79)	64 (54–76)	0.701	<0.001
*SUA ($\mu\text{mol/L}$)	325 (259–381)	314 (256–384)	305 (256–360)	294 (247–356)	<0.001	0.086
*UAE (mg/24h)	14.22 (7.42–49.03)	11.49 (6.67–28.87)	11.49 (6.79–28.51)	9.48 (6.18–23.90)	<0.001	<0.001
*eGFR (mL/min/1.73 m^2)	118 (92–142)	112 (94–138)	111 (90–132)	111 (94–133)	0.043	<0.001

Notes: Values are expressed as number (%), mean \pm standard deviation, or median (interquartile range). P values: The P values were not adjusted for age and sex for the trend. ^aP values: The ^aP values were adjusted for age and sex for the trend. *The Mann-Whitney U-test was applied.

Abbreviations: OCN, osteocalcin; DD, diabetes duration; IIA, insulin or insulin analogue; LLD, lipid-lowering drug; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; WHR, waist-to-hip ratio; BMI, body mass index; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; HbA1c, glycated hemoglobin A1c; FCP, fasting C-peptide; PCP, postprandial C-peptide; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); ALT, alanine aminotransferase; γ -GT, γ -glutamyltransferase; Cr, creatinine; SUA, serum uric acid; UAE, urinary albumin excretion; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

Comparisons of MAFLD Prevalence

Figure 2 displays the comparisons of MAFLD prevalence among the serum OCN quartiles and the comparison of serum OCN levels between T2DM patients with and without MAFLD. After adjustment for sex, age and DD, the prevalence of MAFLD obviously reduced across the serum OCN quartiles in T2DM patients (55.3%, 52.0%, 48.6%, and 42.1% for the first, second, third, and fourth quartiles, respectively, $P < 0.001$ for trend) (Figure 2A). Additionally, serum OCN levels in the T2DM patients with MAFLD were significantly lower than in those without MAFLD ($P < 0.001$) (Figure 2B).

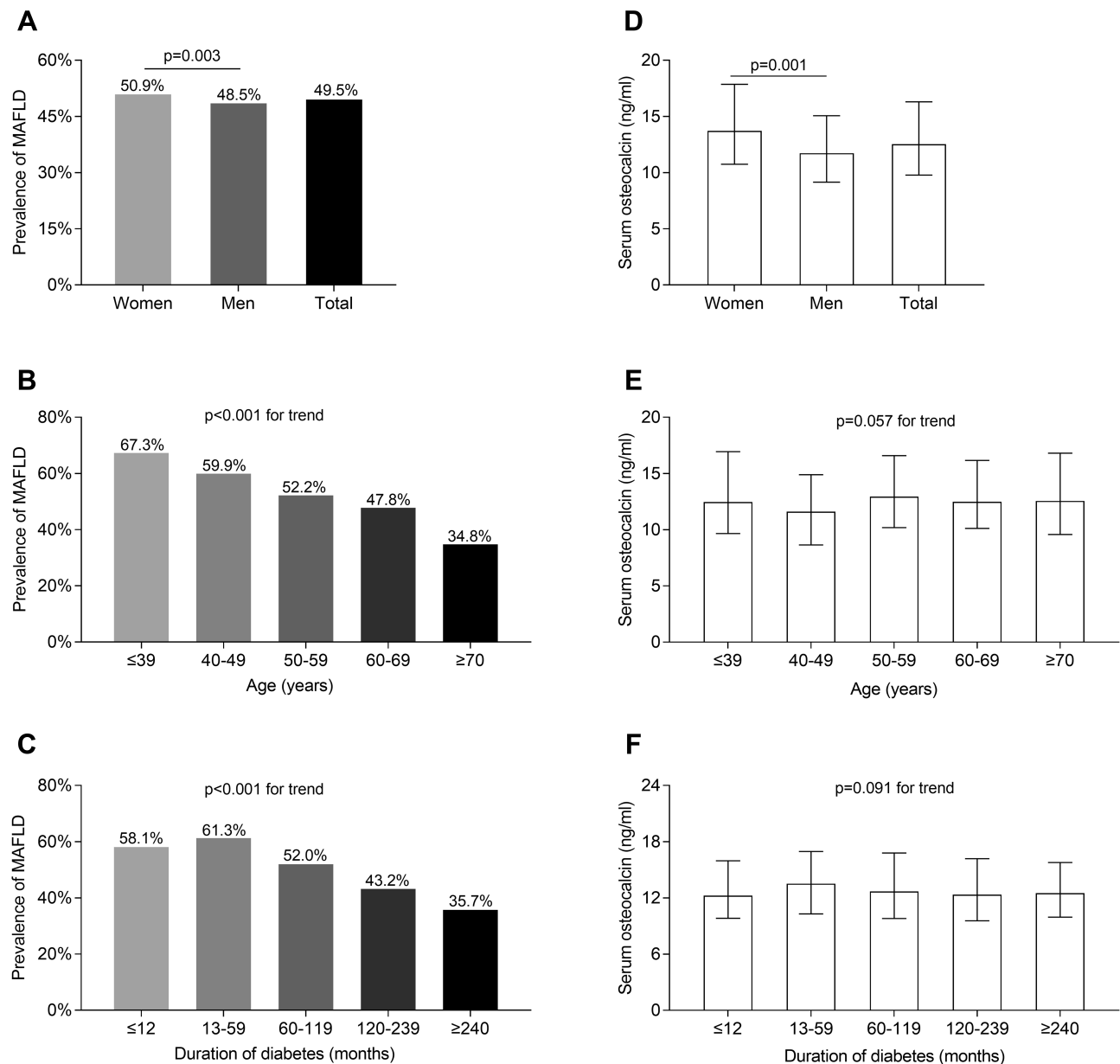


Figure 1 Comparisons of MAFLD prevalence and serum OCN levels stratified by sex, age, and DD. **(A)** Overall prevalence of MAFLD in the subjects and comparison of the MAFLD prevalence between men and women ($P = 0.003$). **(B)** Comparison of the MAFLD prevalence among the subjects stratified by age after controlling for sex and diabetes duration (DD) ($P < 0.001$ for trend). **(C)** Comparison of the MAFLD prevalence among the subjects stratified by DD after controlling for sex and age ($P < 0.001$ for trend). **(D)** Comparison of serum osteocalcin (OCN) levels between men and women ($P = 0.001$). **(E)** Comparison of serum OCN levels among the subjects stratified by age after controlling for sex and DD ($P = 0.057$ for trend). **(F)** Comparison of serum OCN levels among the subjects stratified by DD after controlling for sex and age ($P = 0.091$ for trend).

Comparisons of Liver Enzymes

Figure 3 compares the liver enzymes including ALT and γ -GT among the serum OCN quartiles. As shown in **Figure 3A and C**, both serum ALT and γ -GT levels in the T2DM subjects with MAFLD were significantly higher than in those without MAFLD (all $P < 0.001$) (**Figure 3A and C**). Furthermore, both serum ALT ($P = 0.007$ for trend) and γ -GT levels ($P < 0.001$ for trend) were clearly decreased across the serum OCN quartiles in T2DM patients (**Figure 3B and D**).

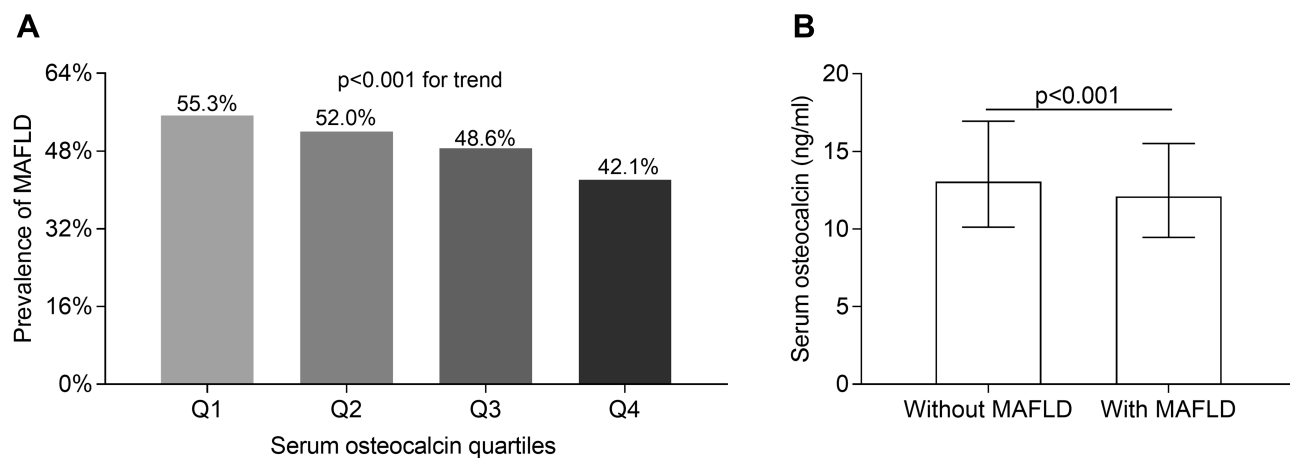


Figure 2 Comparisons of metabolic dysfunction-associated fatty liver disease (MAFLD) prevalence. **(A)** Comparisons of the prevalence of MAFLD across the serum osteocalcin (OCN) quartiles after controlling for sex, age, and diabetes duration ($P < 0.001$ for trend). **(B)** Comparisons of serum OCN levels between the type 2 diabetes mellitus patients with and without MAFLD ($P < 0.001$).

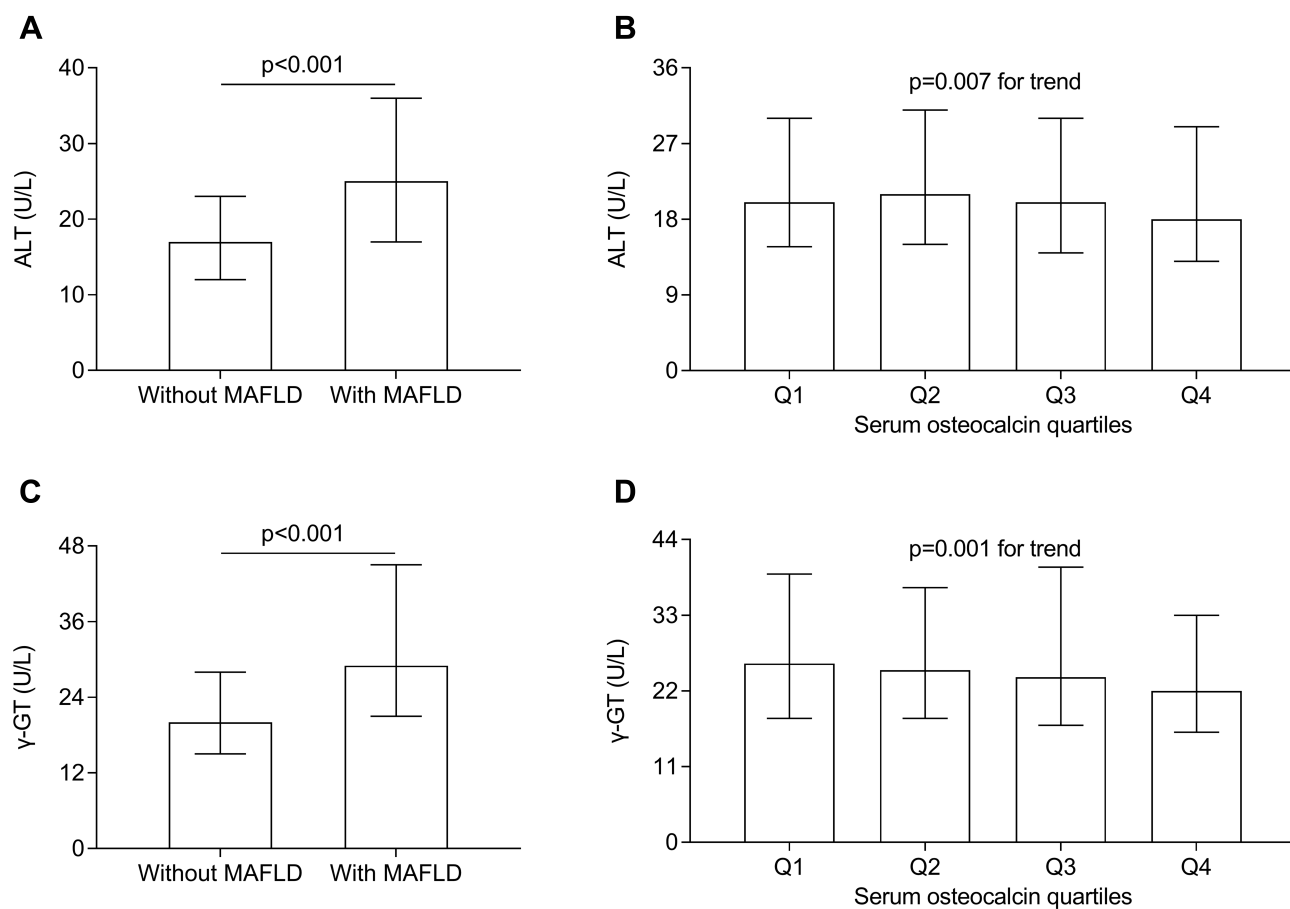


Figure 3 Comparisons of liver enzymes. **(A)** Comparisons of alanine aminotransferase (ALT) between type 2 diabetes mellitus (T2DM) patients with and without metabolic dysfunction-associated fatty liver disease (MAFLD) after controlling for age, sex, and diabetes duration (DD) ($P < 0.001$). **(B)** Comparisons of ALT across the serum osteocalcin quartiles after controlling for age, sex, and DD ($P = 0.007$ for trend). **(C)** Comparisons of γ -glutamyltransferase (γ -GT) between T2DM patients with and without MAFLD after controlling for age, sex, and DD ($P < 0.001$). **(D)** Comparisons of γ -GT across the serum OCN quartiles after controlling for age, sex, and DD ($P = 0.001$ for trend).

Comparisons of Insulin Resistance and CRP

The comparisons of insulin resistance (IR) parameters including HOMA-IR and HOMA2-IR and serum CRP levels among the serum OCN quartiles are presented in Figure 4. After controlling for age, sex, and DD, the subjects with MAFLD had obviously increased values of HOMA-IR and HOMA2-IR and serum CRP levels compared with those without MAFLD (all $P < 0.001$)

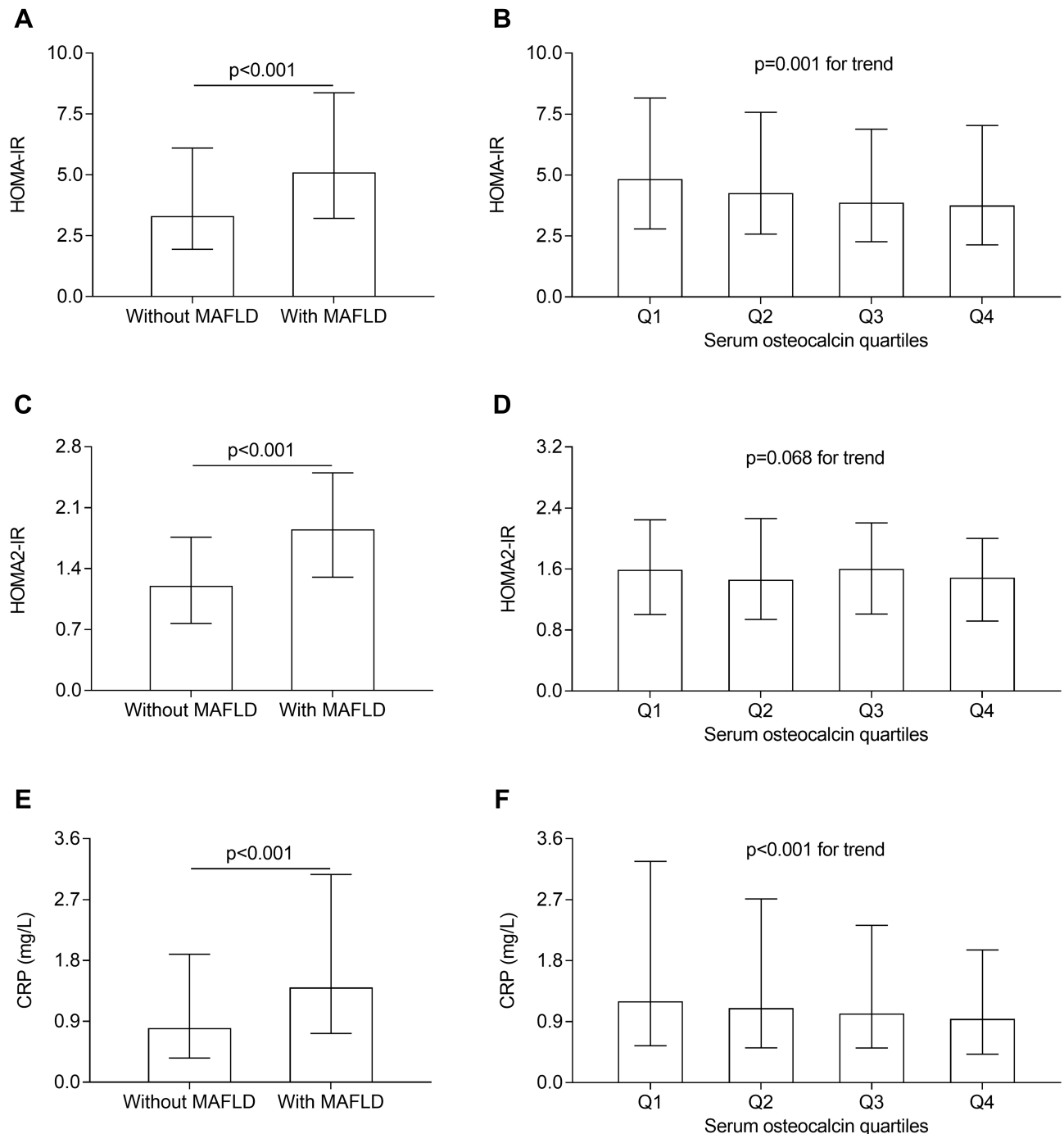


Figure 4 Comparisons of insulin resistance parameters and C-reactive protein (CRP). **(A)** Comparison of homeostasis model assessment of insulin resistance (HOMA-IR) between the type 2 diabetes mellitus (T2DM) patients with and without metabolic dysfunction-associated fatty liver disease (MAFLD) after controlling for age, sex, and diabetes duration (DD) ($P < 0.001$). **(B)** Comparisons of HOMA-IR across the serum osteocalcin (OCN) quartiles after controlling for age, sex, and DD ($P = 0.001$ for trend). **(C)** Comparison of HOMA2-IR between the T2DM patients with and without MAFLD after controlling for age, sex, and DD ($P < 0.001$). **(D)** Comparisons of HOMA2-IR across the serum OCN quartiles after controlling for age, sex, and DD ($P = 0.068$ for trend). **(E)** Comparison of serum CRP levels between the T2DM patients with and without MAFLD after controlling for age, sex, and DD ($P < 0.001$). **(F)** Comparisons of serum CRP levels across the serum OCN quartiles after controlling for age, sex, and DD ($P < 0.001$ for trend).

Table 2 The Association of Serum Osteocalcin Levels with MAFLD

	B Statistic	OR	95% CI	P value
Model 1	-0.208	0.812	0.729–0.904	<0.001
Model 2	-0.193	0.825	0.739–0.920	0.001
Model 3	-0.219	0.804	0.713–0.906	<0.001
Model 4	-0.184	0.832	0.719–0.962	0.013

Notes: Model 1: adjusted for age, sex, diabetes duration, smoking, drinking, history of hypertension and obesity. Model 2: further adjusted for the use of lipid-lowering drugs, metformin, insulin or insulin analogues and insulin sensitizers. Model 3: further adjusted for systolic blood pressure, diastolic blood pressure, waist circumference, waist-to-hip ratio and body mass index. Model 4: further adjusted for total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, lipoprotein(a), creatinine, estimated glomerular filtration rate, serum uric acid, urinary albumin excretion, C-reactive protein, glycated hemoglobin A1c, fasting plasma glucose, 2-hour postprandial plasma glucose, fasting insulin, 2-hour postprandial insulin, homeostasis model assessment of insulin resistance (HOMA-IR), fasting C-peptide, 2-hour postprandial C-peptide and HOMA2-IR.

Abbreviations: OCN, osteocalcin; MAFLD, metabolic dysfunction-associated fatty liver disease; OR, odds ratio; CI, confidence interval.

(Figure 4A, C, and E). Higher serum OCN quartiles were associated with lower IR evaluated by HOMA-IR ($P = 0.001$ for trend) but not HOMA2-IR ($P = 0.068$ for trend) (Figure 4B and D). In addition, serum CRP levels were also significantly reduced across the serum OCN quartiles in T2DM patients ($P < 0.001$ for trend) (Figure 4F).

Association of Serum OCN Levels with MAFLD

Table 2 shows the association between serum OCN levels and the presence of MAFLD in T2DM patients. After adjusting for age, sex, DD, smoking, drinking, HTN and obesity (Model 1), serum OCN levels were independently and negatively correlated with the presence of MAFLD ($P < 0.001$ for trend). Subsequently, after further adjustment for the use of LLDs, metformin, IIAs and insulin sensitizers (Model 2), and for SBP, DBP, WC, WHR and BMI (Model 3), and for TC, TG, LDL-C, HDL-C, Lp (a), Cr, eGFR, SUA, UAE, CRP, HbA1C, FPG, 2h PPG, Fins, 2-hins, HOMA-IR, FCP, 2h PCP and HOMA2-IR (Model 4), the correlation between serum OCN levels and the presence of MAFLD were still stable in T2DM subjects ($P = 0.001$, $P < 0.001$, $P = 0.013$ for trends in Models 2, 3, and 4, respectively).

Discussion

The new nomenclature “MAFLD” was proposed recently to define the liver disease associated with metabolic dysfunction.⁹ Compared with the traditional term “NAFLD”, the definition of MAFLD places more emphasis on the pathophysiologic mechanisms of fatty liver, an underlying state of systemic metabolic dysfunction. Additionally, excessive alcohol consumption and other concomitant liver diseases are no longer the exclusion criteria for MAFLD.⁹ Investigations based on data from the United States demonstrated that MAFLD definition identified a group of patients with a higher risk of advance liver fibrosis, all-cause, cardiovascular-disease-related and other-cause mortality than NAFLD definition.^{34,35}

Although the populations of MAFLD and NAFLD do not overlap completely, the prevalence of MAFLD and NAFLD was relatively consistent.^{35,36} For example, a study based on the Hong Kong census database showed that MAFLD was diagnosed in 263/1016 (25.9%) subjects, while NAFLD in 261/1016 (25.7%) subjects.³⁶ Likewise, the present study showed that the MAFLD prevalence in T2DM subjects was 49.5%, which was close to the prevalence (52.6%) of NAFLD reported in our previous study.²⁵ Like other studies including our recent studies,^{24–26,33,37,38} the current study also showed negative correlations of the MAFLD prevalence with age and DD in T2DM patients. One possible explanation for this phenomenon was that early and young T2DM patients had a greater degree of hyperinsulinemia, which led to the uptake of free fatty acids in hepatocytes.^{39,40}

The prevalence of MAFLD in T2DM patients was significantly higher than in general population.^{18,19} Moreover, the co-existence of MAFLD and T2DM further increases the risk of poor prognosis, engendering severe healthy burden in those patients. For example, a retrospective cohort study found that NAFLD was associated with an increased risk of

cardiovascular diseases, hepatocellular carcinoma, and all-cause mortality in T2DM subjects.²¹ Therefore, it is essential to predict and evaluate the risk of MAFLD to prevent and reduce the harm caused by fatty liver in T2DM patients.

OCN is the most abundant non-collagenous protein produced specifically by osteoblast and plays an important role in bone formation and turnover. Interestingly, several studies, both in vivo and in vitro, have revealed the role of OCN in ameliorating insulin resistance (IR) and regulating energy metabolism.^{1,41,42} Moreover, correlations between OCN and metabolic diseases such as obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome were continuously discovered in recent years.⁴⁻⁸ For example, Saleem et al⁸ demonstrated that serum OCN levels were negatively associated with measures of IR and the presence of metabolic syndrome, but positively associated with serum adiponectin levels.

More importantly, we observed a significantly negative association between serum OCN levels and MAFLD in T2DM in this study. Currently, there have been several studies exploring the relationship of OCN with NAFLD in general population,¹¹⁻¹⁷ most of which supported the view that serum OCN levels were inversely associated with the presence of NAFLD. Luo et al¹² presented the negative correlation of serum OCN levels with NAFLD in postmenopausal Chinese women with normal blood glucose levels. Another study in adult males also showed that compared with the subjects in the fourth serum OCN quartile, the risk of NAFLD increased by 2.25-, 2.28-, and 1.83-fold in the subjects in the first, second and third quartile, respectively, which indicated serum OCN levels were inversely related to the presence of NAFLD.¹³ However, some studies in general population demonstrated that the significant association between serum OCN levels and NAFLD disappeared after adjustment for metabolic parameters such as BMI and HOMA-IR.^{15,16} We speculated that the discrepancies may be caused by the different clinical characteristics of the study subjects, such as race and concomitant diseases.

However, similar studies focusing on T2DM population have been scarce and controversial so far. A study in a cohort of 4937 subjects by Wang et al²² reported that N-mid fragment of OCN was negatively associated with advanced NAFLD status including nonalcoholic steatohepatitis and significant fibrosis in middle-aged and elderly men and postmenopausal women with T2DM. Nonetheless, another study in postmenopausal women with T2DM and impaired glucose regulation presented a positive correlation of serum OCN levels and NAFLD fibrosis score, a non-invasive test evaluating the severity of hepatic fibrosis caused by NAFLD.²³ Consistent with these studies in which negative association was observed between serum OCN and MAFLD,^{11-13,17} after adjusting for multiple confounding factors, especially parameters of metabolic dysfunction such as HOMA-IR and obesity, serum OCN levels were still independently and negatively correlated with the presence of MAFLD in T2DM patients in the present study. Consequently, our study provided new and confirming evidence for the inverse association of serum OCN levels with MAFLD in a relatively large number of T2DM subjects.

Serum ALT and γ -GT have been used as sensitive biomarkers for the evaluation of hepatocellular injury, which are commonly elevated in MAFLD patients.^{43,44} In this study, both serum ALT and γ -GT levels showed a downward trend with the increase of serum OCN quartiles. The negative correlations of serum OCN levels with ALT and γ -GT have also been reported by several previous studies.^{13,45-47} Also, a previous study showed that low levels of serum OCN were associated with liver fibrosis in morbidly obese patients,⁴⁸ indicating that serum OCN levels might reflect not only the presence but also the severity of MAFLD. In fact, experiments in animal models have provided confirming evidence that OCN treatment alleviates hepatic steatosis by reducing hepatic expression of sterol regulatory element-binding proteins (SREBP-1) and its downstream proteins in wild-type mice fed with Western diet, which play an important role in hepatic lipogenesis and TG accumulation.¹¹ Moreover, Gupte et al demonstrated that OCN reduced expression of proinflammatory and profibrotic genes such as *Cd68*, *Mcp1*, *Spp1* and *Colla2* in liver, and protected against high-fat, high-cholesterol diet-induced NASH in rodents.⁴⁹

The significant adverse association between serum OCN and MAFLD in T2DM may contribute to IR and systemic chronic low-grade inflammation, which are two crucial mechanisms involved in the pathogenesis of MAFLD.⁵⁰ IR leads to disorders of glucose and lipid metabolism and the ectopic accumulation of free fatty acid in hepatocytes, which play an important role in the development and progression of MAFLD.⁵¹ Another important characteristic of MAFLD was systemic low-grade chronic inflammation, including increased levels of pro-inflammatory cytokines and activation of

immune cells, which contributed to the aggravation of IR and hepatocellular injury and fibrosis.^{50,52} Consistently, several studies have reported negative correlations of serum OCN levels with IR and systemic inflammation.^{4,8,53} For example, Guo et al⁴ demonstrated that lower serum OCN levels were associated with higher levels of IR and worse β -cell function in patients with dysglycemia. Furthermore, a cross-sectional study of 246 T2DM patients showed that serum OCN levels were significantly and negatively associated with inflammation indicators such as CRP, ferritin, basophil count and monocyte count.⁵³ Similarly, the current study also showed increased IR and CPR levels in the T2DM subjects with MAFLD and an inverse correlation between serum OCN levels and HOMA-IR and CRP. Given that animal studies have demonstrated OCN can improve insulin sensitivity in metabolic tissues and reduce inflammation in mouse adipose and liver tissues,^{49,54,55} OCN may have a direct protective effect against MAFLD through ameliorating IR and chronic inflammation. Therefore, the close association of serum OCN levels with MAFLD in T2DM patients may be partially due to the alleviation of IR and inflammation mediated by OCN.

There were limitations to this study. First, this cross-sectional study could not verify the causal relationship between serum OCN and MAFLD. Second, the subjects in the study were hospitalized patients with T2DM in a single-center, which might not fully reflect characteristics of other populations such as community patients with T2DM. Third, variables not included in the analysis might affect the results. However, we controlled for as many clinical factors as possible to minimize the confounding bias. Finally, data on the severity of liver fibrosis in our study such as liver transient elastography or liver biopsy were lacking in the present study. Therefore, large-scale prospective studies are needed to further determine whether serum OCN levels can be used as an independent predictor of MAFLD in diabetic population.

Conclusion

In summary, serum OCN levels were independently and negatively correlated with the presence of MAFLD in Chinese patients with T2DM, which may partially contribute to the alleviation of IR and inflammation mediated by OCN. Serum OCN levels may be used as a potential indicator to assess the risk of MAFLD in T2DM patients, but further prospective studies are needed to obtain more convincing evidence.

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Disclosure

The authors report no conflicts of interest in this work.

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