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ORIGINAL RESEARCH

Dynamic Alterations of BMP9 in NAFLD: A Novel Hepatokine Marker of Metabolic Dysfunction and Disease Severity

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Purpose: Bone morphogenetic protein 9 (BMP9), a hepatokine belonging to the transforming growth factor-β (TGF-β) superfamily, plays a critical role in glucose and lipid metabolism. This study aimed to investigate the dynamic changes of BMP9 under metabolic stress in patients with different severities of non-alcoholic fatty liver disease (NAFLD), and to evaluate its associations with metabolic abnormalities and clinical diagnostic value.

Patients and Methods: A total of 84 participants were enrolled and categorized into normal control (Con), mild, moderate, and severe NAFLD groups. Standardized oral fat tolerance tests (OFTT) were conducted to assess dynamic alterations in circulating BMP9 and related metabolic indices. Trend analysis, Spearman correlation, multivariate regression, and receiver operating characteristic (ROC) curve analyses were performed to explore the relationships between BMP9 and metabolic parameters as well as its diagnostic performance.

Results: Both fasting and postprandial BMP9 levels declined with increasing NAFLD severity, with a more pronounced reduction observed in the severe group ($P < 0.05$). In the severe NAFLD group, baseline BMP9 levels were reduced by 47.2%, and the time-integrated area under the concentration–time curve (AUC) was reduced by 52.7% compared with controls. The BMP9 AUC was negatively correlated with BMI, HOMA-IR, TG, and LDL-C, and positively correlated with HDL-C (all $P < 0.05$). Multivariate regression identified HOMA-IR and TG as independent predictors of lower BMP9 levels. Furthermore, ROC analysis demonstrated excellent diagnostic performance of BMP9 AUC in identifying moderate-to-severe NAFLD (ROC-AUC = 0.965).

Conclusion: BMP9 exhibits both basal deficiency and functional impairment in NAFLD and is closely associated with multiple metabolic abnormalities. Its dynamic profile may represent a promising early diagnostic biomarker for NAFLD and could potentially serve as a tool for metabolic disease screening and intervention.

Plain Language Summary: Non-alcoholic fatty liver disease (NAFLD), recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD), is a common condition linked to obesity, diabetes, and heart disease. Finding simple blood markers to detect the disease early is important. In this study, we measured the blood levels of bone morphogenetic protein 9 (BMP9), a hormone produced by the liver, in people with NAFLD. We found that BMP9 levels were significantly lower in patients compared with healthy individuals, and the decrease was related to the severity of liver fat and metabolic problems. After a standardized meal test, BMP9 levels changed in a dynamic way that reflected the body's metabolic response. These findings suggest that BMP9 may serve as a promising biomarker for early detection and monitoring of NAFLD/MASLD.

Keywords: hepatic steatosis, postprandial metabolism, lipid homeostasis, dysglycemia, insulin resistance, bone morphogenetic protein 9

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disorder closely associated with metabolic syndrome. Its global prevalence is estimated at 25–30%, rising to 70–95% among patients with type 2 diabetes mellitus (T2DM), and reaching up to 98% in individuals with morbid obesity.[1] NAFLD has emerged as a leading cause of end-stage liver disease, hepatocellular carcinoma, and cardiovascular events.[2] Although its pathogenesis is multifactorial, insulin resistance and dyslipidemia are recognized as key drivers.[3] In 2023, an international expert consensus recommended a change in nomenclature from NAFLD to metabolic dysfunction-associated steatotic liver disease (MASLD), underscoring the central role of metabolic risk factors in disease pathogenesis.[4,5] In this study, we primarily use the term NAFLD for consistency with existing literature, while acknowledging the updated MASLD terminology.

Recent studies suggest that the liver functions not only as a target organ of metabolic imbalance but also as an endocrine organ. Hepatokines—hormones secreted by the liver—play essential roles in maintaining glucose and lipid homeostasis.[6,7] Among them, bone morphogenetic protein 9 (BMP9), a member of the transforming growth factor-β (TGF-β) superfamily, is predominantly secreted by the liver and has been implicated in the regulation of glucose metabolism, lipogenesis, and vascular endothelial function.[8–10] Animal studies have shown that BMP9 suppresses hepatic lipogenesis and improves insulin sensitivity by downregulating lipogenic transcription factors such as SREBP-1c and enhancing insulin signaling pathways.[11] Consistently, BMP9 knockout models develop fatty liver phenotypes and impaired glucose tolerance, further supporting its hepatoprotective role.[12] Clinically, circulating BMP9 levels are reduced in individuals with obesity and T2DM.[13] However, its expression profile and dynamic changes in human NAFLD have not been fully elucidated.

Notably, NAFLD involves not only fasting metabolic abnormalities but also impaired postprandial lipid handling, characterized by delayed triglyceride clearance and elevated free fatty acids, both of which contribute to atherogenesis.[14,15] The oral fat tolerance test (OFTT), a standardized method for evaluating postprandial lipid metabolism, reflects the body's adaptive response to lipid intake.[16,17] However, the dynamic profile of BMP9 during OFTT and its potential as a metabolic biomarker remain unexplored.

This study aimed to address three major questions: (1) What are the dynamic changes in BMP9 levels in NAFLD patients under fasting and postprandial conditions? (2) How do BMP9 levels correlate with insulin resistance, dyslipidemia, and other metabolic parameters? (3) What is the diagnostic potential of BMP9 in identifying metabolic abnormalities and NAFLD severity?

Materials and Methods

Study Population

A case-control study was conducted at Linyi Hospital of Traditional Chinese Medicine from June 2024 to June 2025. Participants were recruited from the health examination center, and eligibility was assessed through a standardized screening process. Of 86 initially screened individuals, 63 NAFLD patients were enrolled and stratified by disease severity (mild [n=21], moderate [n=22], severe [n=20]) based on abdominal ultrasound findings, interpreted independently by two senior radiologists blinded to clinical data according to AASLD guidelines.[18] Twenty-one healthy controls without NAFLD, hypertension, T2DM, or other chronic conditions were also recruited (Figure S1).

The study protocol was approved by the Institutional Ethics Committee of Linyi Traditional Chinese Medicine Hospital (Approval No. 20240167) and was registered in the Chinese Clinical Trial Registry (ChiCTR2500105557; https://www.chictr.org.cn/). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

The sample size was estimated based on previously published data on hepatokines in NAFLD, assuming a 25–30% difference in BMP9 levels between groups, 80% power, and $\alpha = 0.05$. Thus, at least 18 subjects per group were required, and approximately 20 were enrolled to account for potential data loss.

Exclusion Criteria

Exclusion criteria for all participants included viral hepatitis, autoimmune liver disease, other chronic liver disorders, cardiovascular disease, malignancy, impaired renal function, a history of abdominal surgery, and alcohol consumption exceeding 20 g/day.

To avoid the risk of fat-loading–induced acute pancreatitis and to minimize measurement errors caused by extreme values, individuals with fasting triglyceride levels > 5 mmol/L were also excluded. All remaining eligible participants subsequently underwent the OFTT.

Data Collection

Anthropometric parameters, including age, sex, and body mass index (BMI), were recorded for all participants. Fasting venous blood samples were obtained to assess glucose, insulin, and lipid profiles. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) according to Matthews et al. HOMA-IR = [Fasting Glucose (mmol/L) × Fasting Insulin (μU/mL)] / 22.5.[19]

Biochemical measurements were performed as follows: fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analyzed using a Hitachi 7600 automatic biochemical analyzer. Fasting insulin (FINS) was measured by electrochemiluminescence immunoassay on a Cobas e601 analyzer. Serum BMP9 concentrations were determined using a commercially available ELISA kit (Jiangsu Meimian Industrial Co., Ltd, China) in accordance with the manufacturer's instructions. Dynamic changes in BMP9 and glucose–lipid metabolism during the OFTT were evaluated by calculating the AUC.

Oral Fat Tolerance Test

A standardized oral fat tolerance test was performed based on the method described in reference,[20] with minor modifications. After a 12 h overnight fast, participants consumed a test meal at 8:00 a.m. containing 100 grams of fat (83% long-chain fatty acids and 17% medium-chain fatty acids). Venous blood samples were collected at 0 (fasting), 2, 4, 6, and 8 h postprandially. All samples were immediately centrifuged at 3000 rpm for 10 minutes at 4°C. The serum was then aliquoted and stored at −80°C for subsequent analysis.

Statistical Analysis

Analyses were performed using SPSS 26.0 and GraphPad Prism 8.0. Continuous variables were expressed as mean ± SD or median (IQR). Between-group comparisons were performed using ANOVA or Kruskal–Wallis tests. Correlations were assessed using Spearman correlation coefficients. Multivariate linear regression was conducted adjusting for confounders. Receiver operating characteristic (ROC) curves evaluated the diagnostic performance of BMP9. $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

A total of 84 participants were enrolled in this study and categorized into four groups: normal control (Con, n = 21), mild NAFLD (n = 21), moderate NAFLD (n = 22), and severe NAFLD (n = 20). There were no significant differences in age or sex distribution among the groups ($P > 0.05$).

With increasing NAFLD severity, both BMI and HOMA-IR showed a significant upward trend (both $P < 0.05$). Furthermore, compared with the control group, the moderate and severe NAFLD groups showed significantly higher levels of BMI, HOMA-IR, FPG and FINS (all $P < 0.05$).

Regarding lipid profiles, levels of TG, TC and LDL-C increased significantly across the NAFLD severity spectrum (all $P < 0.05$). In contrast, HDL-C tended to decrease, although the between-group difference was not statistically significant.

At baseline, BMP9 levels decreased progressively with NAFLD severity: the median in the control group was 82.68 ng/L (77.37–103.11), while levels in the mild, moderate, and severe NAFLD groups declined to 66.09, 57.63, and 43.68 ng/L, corresponding to reductions of 20.1%, 30.3%, and 47.2% compared to the control group, respectively. Trend analysis showed statistical significance ($P < 0.05$) (Table 1).

Table 1 Clinical and Biochemical Characteristics of Included Subjects

Group	Con (n=21)	Mild NAFLD (n=21)	Moderate NAFLD (n=22)	Severe NAFLD (n=20)	P
N (men/women)	21 (10/11)	21 (10/11)	22 (12/10)	20 (9/11)	0.764
Age (years)	41.10±12.15	36.48±12.59	36.23±11.46	38.7±9.70	0.486
BMI (kg/m^2)	22.60±4.72	22.81±3.54	25.71±4.43*	28.38±7.28*	<0.001
HOMA-IR	1.57±0.61	2.30±1.00*	3.39±1.51*	5.52±3.03*	<0.001
FINS (pmol/L)	49.46±20.02	69.46±31.38	97.21±44.43*	136.86±69.83*	<0.001
FPG (mmol/L)	4.89±0.49	5.06±0.40	5.40±0.45*	5.94±0.88*†	<0.001
TC (mmol/L)	4.60±0.75	4.84±0.74	5.30±0.46*#	5.78±0.87*†	<0.001
TG (mmol/L)	1.77±0.76	2.00±0.53	2.39±0.66*#	2.78±0.59*†	<0.001
HDL-C (mmol/L)	1.34 (1.14–1.47)	1.25 (1.10–1.40)	1.13 (1.01–1.35)	1.02 (0.84–1.24)*	0.015
LDL-C (mmol/L)	2.79±0.57	3.40±0.46*	3.75±0.62*#	4.20±0.54*†	<0.001
BMP9 (ng/L)	82.68 (77.37–103.11)	66.09 (60.61–74.61)*	57.63 (54.85–63.87)*	43.68 (39.5–45.95)*†	0.01

Notes: Data are presented as mean ± SD for normally distributed variables and median (IQR) for skewed variables. *P < 0.05 vs control group; #P < 0.05 vs mild NAFLD group; †P < 0.05 vs moderate NAFLD group.

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; FINS, fasting insulin; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BMP9, bone morphogenetic protein 9.

Characteristics of the Oral Fat Tolerance Test

Following oral fat loading, TG levels in the control group peaked at 4 hours and returned to baseline by 8 hours. LDL-C, TC, and HDL-C levels showed minimal fluctuations. In contrast, patients with NAFLD exhibited marked postprandial lipid dysregulation. Specifically, TG levels peaked later (at 6 hours), peaked at notably higher levels with prolonged clearance time, most pronounced in the moderate and severe groups. In the severe group, mean TG levels at 6 hours reached 7.07 mmol/L and remained elevated at 8 hours. TG AUC increased significantly with disease severity ($P < 0.05$). Similarly, AUCs for LDL-C and TC were significantly elevated in the moderate and severe groups (trend $P < 0.05$). Compared to the control group, LDL-C AUC increased by 25.0% and 43.8% in the moderate and severe NAFLD groups, respectively ($P < 0.05$), while TC AUC increased by 13.2% and 26.2% ($P < 0.05$). Although HDL-C showed only minor dynamic changes, its AUC was significantly lower in the moderate and severe NAFLD groups ($P < 0.05$).

In terms of glucose metabolism, the control group showed mild glycemic excursions (peak rise <6.4%, 4.89 ± 0.49 to 5.20 ± 0.48 mmol/L) with a return to baseline by 8 hours. Insulin secretion followed a normal physiological pattern. NAFLD patients, particularly those with severe disease, exhibited pronounced postprandial hyperglycemia and hyperinsulinemia. In the severe group, the glucose AUC increased by 24.9% compared to controls ($P < 0.05$), and insulin AUC was 3.4-fold higher than in the control group ($P < 0.05$).

BMP9 levels in all groups showed a similar dynamic trend: levels declined postprandially, reaching a nadir at 6 hours, followed by gradual recovery. However, both baseline and postprandial BMP9 levels were significantly lower in NAFLD patients compared to controls. In the severe NAFLD group, BMP9 baseline levels were reduced by 47.2%, and the postprandial decline reached 30.7%, significantly greater than the 21.9% observed in the control group. Moreover, BMP9 recovery was markedly impaired. BMP9 AUC in the control group was 580.91 ng/L·h, which decreased by 52.7% in the severe group ($P < 0.05$), showing a significant downward trend with disease progression. These findings suggest that hepatic BMP9 synthesis and secretion are impaired in NAFLD and that dynamic dysregulation of BMP9 is closely linked to metabolic dysfunction (Tables 2, 3 and Figure 1).

Correlation Between BMP9 and Metabolic Parameters

Spearman correlation analysis revealed that BMP9 AUC was significantly negatively associated with multiple metabolic indicators, including HOMA-IR ($r = -0.601$), FINS ($r = -0.533$), FPG ($r = -0.457$), TG ($r = -0.535$), TC ($r = -0.548$), LDL-C ($r = -0.646$) and BMI ($r = -0.255$) (all $P < 0.05$). Conversely, a positive correlation was observed with HDL-C ($r = 0.288$, $P < 0.01$). Baseline BMP9 levels showed similar trends, although correlation coefficients were slightly lower. No significant associations were observed between BMP9 levels and either age or sex, ruling out these potential confounders (Table 4).

Table 2 Dynamic Changes in BMP9 and Metabolic Parameters During OFTT in Groups

TG (mmol/L)	**0 h**	**2 h**	**4 h**	**6 h**	**8 h**
Con	1.77±0.76	2.81±1.23#	3.69±1.33#	2.79±1.03#	2.05±0.43
Mild NAFLD	2.00±0.53	2.90±0.86	4.32±1.20#	5.13±1.58*#	3.69±1.73*#
Moderate NAFLD	2.39±0.66*	3.94±1.12*#	5.45±1.22*#	5.97±1.14*#	4.30±1.62*#
Severe NAFLD	2.78±0.59*	4.34±1.08*	6.24±1.22*#	7.07±1.50*#	5.18±1.15*#
TC (mmol/L)	0 h	2 h	4 h	6 h	8 h
Con	4.60±0.75	4.62±0.59	4.71±0.63	4.70±0.69	4.55±0.56
Mild NAFLD	4.84±0.74	4.90±0.64	5.04±0.65	5.01±0.67	4.94±0.54*
Moderate NAFLD	5.30±0.46*	5.19±0.38*	5.30±0.45*	5.30±0.40*	5.27±0.54*
Severe NAFLD	5.78±0.87*	5.90±0.88*	5.87±0.72*	5.90±0.90*	5.85±0.80*
LDL-C (mmol/L)	0 h	2 h	4 h	6 h	8 h
Con	2.79±0.57	2.95±0.32	2.94±0.36	3.01±0.33	2.84±0.51
Mild NAFLD	3.40±0.46*	3.26±0.46	3.34±0.40*	3.34±0.44	3.33±0.52*
Moderate NAFLD	3.75±0.62*	3.66±0.52*	3.63±0.67*	3.64±0.66*	3.70±0.63*
Severe NAFLD	4.20±0.54*	4.19±0.67*	4.24±0.66*	4.22±0.70*	4.24±0.71*
HDL-C (mmol/L)	0 h	2 h	4 h	6 h	8 h
Con	1.34 (1.14–1.47)	1.35 (1.28–1.49)	1.35 (1.02–1.48)	1.38 (1.13–1.63)	1.36 (1.17–1.55)
Mild NAFLD	1.25 (1.10–1.40)	1.21 (1.11–1.34)	1.29 (1.07–1.54)	1.23 (1.09–1.44)	1.27 (1.04–1.52)
Moderate NAFLD	1.13 (1.01–1.35)	1.11 (0.95–1.19)*	1.16 (1.02–1.29)*	1.17 (1.01–1.29)*	1.12 (1.02–1.23)*
Severe NAFLD	1.02 (0.84–1.24)*	1.03 (0.90–1.17)*	1.07 (0.95–1.17)*	1.12 (0.96–1.18)*	1.09 (0.93–1.13)*
Glucose (mmol/L)	0 h	2 h	4 h	6 h	8 h
Con	4.89±0.49	4.98±0.48	5.00±0.38	5.20±0.48	4.99±0.47
Mild NAFLD	5.06±0.40	4.96±0.40	5.07±0.33	4.98±0.49	5.09±0.53
Moderate NAFLD	5.40±0.45*	5.45±0.65	5.61±0.54*	5.47±0.68	5.42±0.38*
Severe NAFLD	5.94±0.88*	6.68±1.15*#	6.56±1.08*	5.98±1.26*	5.87±1.25*
INS (pmol/L)	0 h	2 h	4 h	6 h	8 h
Con	49.46±20.02	89.30±28.64#	66.95±19.06	70.35±26.35#	65.67±31.83#
Mild NAFLD	69.46±31.38	156.66±65.41*#	110.92±43.43*#	79.45±30.71	80.78±26.77
Moderate NAFLD	97.21±44.43*	251.38±117.29*#	184.15±91.94*#	121.71±44.91*	104.55±44*
Severe NAFLD	136.86±69.83*	475.50±163.29*#	222.87±88.49*#	144.03±48.38*	94.97±28.15*
BMP9 (ng/L)	0 h	2 h	4 h	6 h	8 h
Con	82.68 (77.37–103.11)	75.59 (70.09–92.7)#	70.74 (63.67–85.76)#	64.61 (57.72–78.02)#	83.83 (76.04–95.52)
Mild NAFLD	66.09 (60.61–74.61)	62.89 (58.04–69.80)	58.16 (50.90–63.60)#	52.28 (48.43–58.62)#	60.48 (56.88–72.39)
Moderate NAFLD	57.63 (54.85–63.87)*	51.89 (50.23–58.58)*#	48.51 (44.11–52.56)*#	41.91 (36.96–48.44)*#	55.13 (52.78–58.53)*
Severe NAFLD	43.68 (39.50–45.95)*	37.42 (33.12–44.13)*#	32.45 (28.34–37.60)*#	30.28 (25.16–34.84)*#	42.06 (34.83–47.60)*

Notes: Data are presented as mean±SD for parametric and median (IQR) for non-parametric data. *P < 0.05 vs Control group, #P < 0.05 vs 0 h in the same group.
Abbreviations: TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; INS, insulin; BMP9, bone morphogenetic protein 9.

Independent Determinants of BMP9 Levels

Multiple linear regression analysis demonstrated that NAFLD status (coded as 0 = control, 1 = NAFLD) was a strong independent predictor of reduced BMP9 levels (fasting: $\beta = -25.59$, $P = 0.001$; AUC: $\beta = -181.02$, $P = 0.001$). Even after adjusting for NAFLD status, higher HOMA-IR (fasting: $\beta = -2.01$, $P = 0.005$; AUC: $\beta = -13.44$, $P = 0.005$) and elevated TG levels (fasting: $\beta = -4.79$, $P = 0.028$; AUC: $\beta = -35.45$, $P = 0.016$) remained significantly and independently associated with lower BMP9 levels. The regression model for BMP9 AUC ($R^2 = 0.617$) explained slightly more variance than that for fasting BMP9 ($R^2 = 0.598$), highlighting the enhanced sensitivity of BMP9 AUC in reflecting metabolic disturbances (Table 5).

Table 3 Area Under the Curve of Metabolic Parameters and BMP9 During OFTT in Groups

Group	Con	Mild NAFLD	Moderate NAFLD	Severe NAFLD
TG AUC	22.40±7.31	30.37±7.63*	37.40±7.61*#	43.25±7.26*†
TC AUC	37.20±4.61	39.69±4.74	42.12±3.07*	46.95±6.35*†
LDL-C AUC	23.44±2.19	26.61±3.18*	29.29±4.70*	33.72±5.07*†
HDL-C AUC	10.59 (9.79–12.35)	9.89 (8.86–11.52)	8.85 (8.14–9.76)*	8.21 (7.75–8.94)*
INS AUC	568.31±155.84	844.27±288.37*	1316.25±492.81*#	1916.64±579.65*†
Glu AUC	40.25±1.78	40.19±1.89	43.87±3.50*#	50.27±7.54*†
BMP9 AUC	580.91 (534.16–726.05)	457.46 (439.50–535.37)	391.08 (370.76–437.68)*#	274.48 (256.49–325.87)*†

Notes: Data are presented as mean ± SD for parametric and median (IQR) for non-parametric data. *P < 0.05 vs control group; #P < 0.05 vs mild NAFLD group; †P < 0.05 vs moderate NAFLD group.

Abbreviations: AUC, area under the curve; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; INS, insulin; BMP9, bone morphogenetic protein 9.

Diagnostic Value of BMP9 Dynamic Indices for Moderate-to-Severe NAFLD

ROC curve analysis demonstrated that BMP9 AUC exhibited excellent diagnostic performance for identifying moderate-to-severe NAFLD (ROC-AUC = 0.965, P < 0.01). The optimal cut-off value was 447.76 ng/L·h, yielding a sensitivity of 92.9% and a specificity of 85.7%. In contrast, fasting BMP9 alone showed no significant diagnostic value (P = 0.232), further supporting the potential of BMP9 dynamic indices as a non-invasive biomarker for NAFLD stratification (Table 6 and Figure 2).

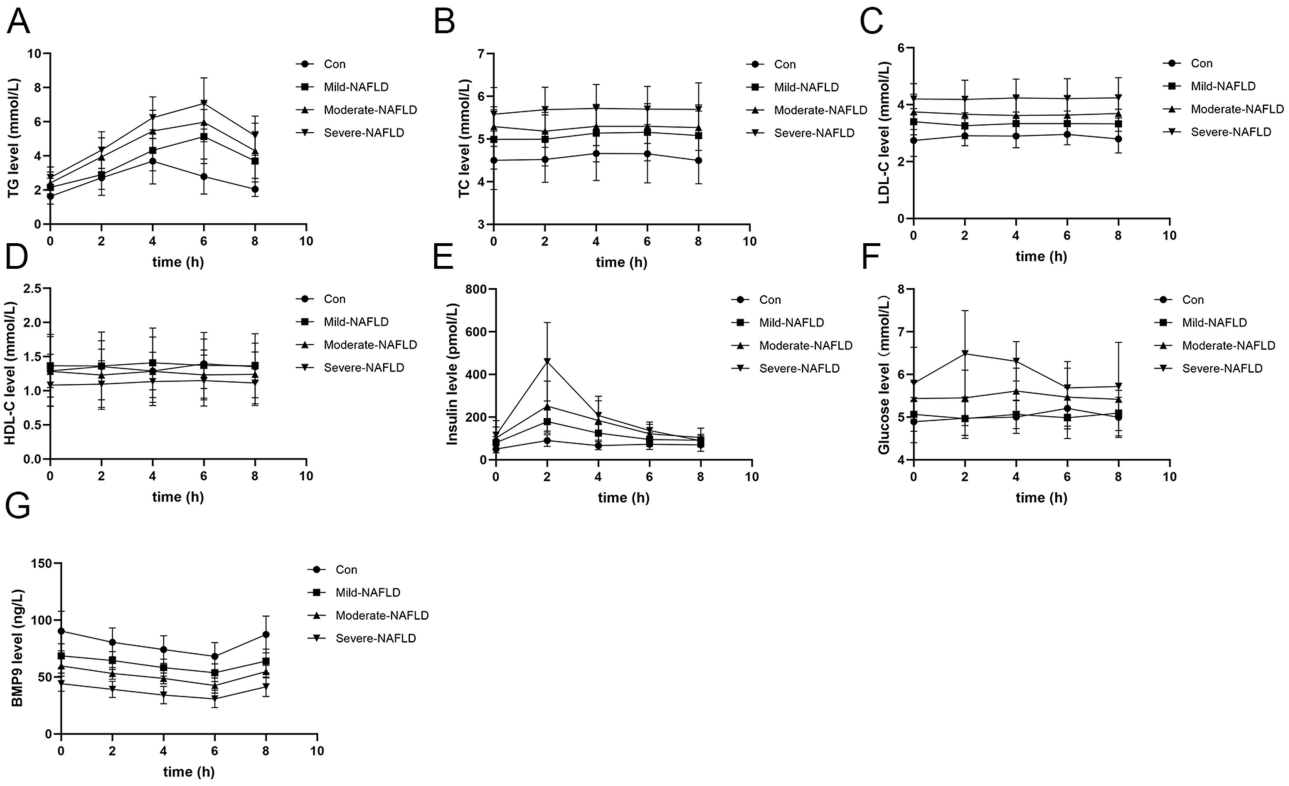

Figure 1 Dynamic changes in metabolic parameters after a high-fat meal in control and NAFLD groups. (**A–D**) Lipid profile alterations, including (**A**) triglycerides (TG), (**B**) total cholesterol (TC), (**C**) low-density lipoprotein cholesterol (LDL-C), and (**D**) high-density lipoprotein cholesterol (HDL-C), (**E**) Insulin (INS) levels, (**F**) Glucose level and (**G**) Bone morphogenetic protein 9 (BMP9) concentrations.

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Table 4 Spearman's Rank Correlation Analysis of BMP9 Levels with Metabolic Parameters

	BMP9		BMP9 AUC	
	r	P	r	P
Group	−0.883	0.001	−0.907	0.001
Gender	−0.063	0.572	−0.053	0.634
Age	0.033	0.767	0.043	0.699
BMI (kg/m^2)	−0.246	0.024	−0.255	0.019
HOMA-IR	−0.603	0.001	−0.601	0.001
FINS (pmol/L)	−0.537	0.001	−0.533	0.001
FPG (mmol/L)	−0.460	0.001	−0.457	0.001
TC (mmol/L)	−0.498	0.001	−0.548	0.001
TG (mmol/L)	−0.508	0.001	−0.535	0.001
LDL-C (mmol/L)	−0.579	0.001	−0.646	0.001
HDL-C (mmol/L)	0.259	0.017	0.288	0.008

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; FINS, fasting insulin; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMP9, bone morphogenetic protein 9; AUC, area under the curve; r, correlation coefficient.

Table 5 Multiple Linear Regression Analysis of BMP9

Variable	BMP9		BMP9 AUC	
	β (95% CL)	P	β (95% CL)	P
HOMA-IR	−2.01 (−3.4, −0.61)	0.005	−13.44 (−22.81, −4.07)	0.005
TG	−4.79 (−9.04, −0.54)	0.028	−35.45 (−64.00, −6.90)	0.016
Gender	−0.02 (−0.28, 0.23)	0.856	0.71 (−39.06, 40.49)	0.972
Age	−1.20 (−7.12, 4.73)	0.689	−0.57 (−2.30, 1.15)	0.510
Group	−25.59 (−33.12, −18.06)	0.001	−181.02 (−231.57, −130.48)	0.001

Notes: Group, binary variable (0 = normal control, 1 = NAFLD).
Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; BMP9, bone morphogenetic protein 9.

Table 6 Binary Logistic Regression Analysis for Predicting Moderate-to-Severe NAFLD

Characteristic	OR	95% CI	P
BMP9	1.127	0.921, 1.346	0.232
BMP9 AUC	0.936	0.898, 0.974	0.001

Notes: Outcome variable: Group (0 = controls/mild NAFLD; 1 = moderate-to-severe NAFLD).
Abbreviations: OR, odds ratio; CI, confidence interval; AUC, area under the curve; BMP9, bone morphogenetic protein 9.

Discussion

This study is the first to systematically evaluate the dynamic pattern of the liver-derived cytokine BMP9 in patients with NAFLD of varying severity using a standardized OFTT. We observed a stepwise decline in both fasting and postprandial BMP9 levels, which were closely associated with multiple metabolic abnormalities. These findings suggest that patients with NAFLD have a reduced endocrine reserve capacity in response to metabolic stress, providing new insights into the pathophysiology of the disease.

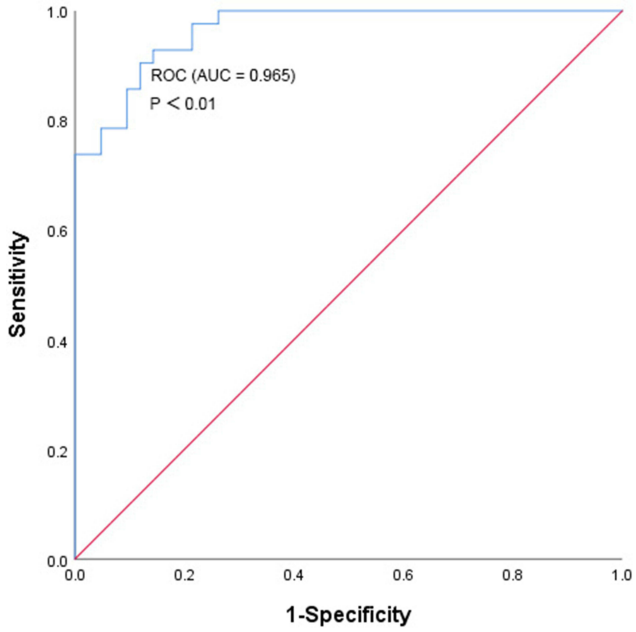

Figure 2 Receiver operating characteristic (ROC) curve analysis of BMP9 AUC.

The liver plays a central role in glucose and lipid homeostasis, partly through the secretion of hepatokines that regulate insulin action, lipid turnover, inflammatory signaling, and systemic energy balance.[8,9] As a member of the TGF-β superfamily, BMP9 is a liver-derived endocrine factor involved in diverse biological processes including cell proliferation, apoptosis, angiogenesis, and metabolic regulation.[21] Previous research has mainly focused on animal models or static fasting BMP9 measurements. For instance, Yang et al reported that BMP9-deficient mice are more susceptible to diet-induced hepatic steatosis, mediated by PPARα activation and lipogenesis.[12] Human studies have also shown decreased fasting BMP9 levels in NAFLD patients,[22] but the hormone's response during dynamic metabolic challenges has not been investigated.

In this study, BMP9 levels in healthy individuals decreased to a nadir at 6 hours after fat ingestion and gradually returned toward baseline, suggesting potential tissue uptake and involvement in lipid handling. In contrast, NAFLD patients—especially those with moderate to severe disease—exhibited lower baseline levels, more pronounced postprandial declines, and impaired recovery. This "endocrine reserve dysfunction" reflects a reduced hepatic adaptive capacity to lipid overload and is consistent with the concept of metabolic inflexibility, whereby individuals with NAFLD fail to respond appropriately to metabolic challenges—potentially preceding overt abnormalities in traditional biochemical markers.[23] These findings are consistent with those of Li et al, who investigated the dynamic behavior of another hepatokine, fibroblast growth factor 21 (FGF21), during an OFTT. They demonstrated that postprandial FGF21 AUCs were significantly correlated with lipid profiles, inflammatory markers, and MAFLD severity, and emphasized the clinical utility of dynamic biomarkers over static measurements.[17]

Importantly, we applied OFTT to generate BMP9 time–concentration profiles and proposed the area under the curve as a novel dynamic biomarker of hepatic endocrine function. Compared to fasting levels, BMP9 AUC more accurately differentiated NAFLD severity. ROC analysis showed excellent diagnostic performance for moderate-to-severe NAFLD, surpassing conventional liver enzymes and non-invasive indices such as FibroScan and NAFLD-LFS. Furthermore, BMP9 AUC could be incorporated into OFTT-based metabolic function panels, offering a non-invasive and physiologically relevant tool for NAFLD risk stratification in both clinical and community settings.

These findings support the concept of "functional biomarkers" assessed under metabolic stress. Unlike static measurements, dynamic markers capture both the baseline endocrine status and its capacity to respond to nutrient challenge, thus providing earlier and more physiologically relevant information. This strategy has also been validated for FGF19, FGF21 and other hepatokines,[17,24,25] and our study extends this framework to BMP9.

Correlation analysis showed that BMP9 AUC was significantly negatively associated with BMI, HOMA-IR, TG, and LDL-C, and positively correlated with HDL-C, supporting its involvement across multiple components of the metabolic syndrome. Multivariate regression confirmed insulin resistance and hypertriglyceridemia as independent predictors of reduced BMP9 levels, even after adjustment for NAFLD status. This suggests that BMP9 reduction may not simply reflect NAFLD progression but may contribute to NAFLD pathogenesis, potentially through Smad1/5–PPARα signaling axis or FGF21-mediated regulation of hepatic lipogenesis and VLDL secretion.[12,26,27] Moreover, BMP9 has been implicated in hepatic progenitor cell maturation and liver regeneration via the HGF/c-Met pathway, which may indirectly support metabolic homeostasis.[28] In addition, the impaired BMP9 dynamic profile shows promise for early risk stratification and targeted screening of individuals at high metabolic risk. In vitro evidence also supports its therapeutic potential: exogenous BMP9 has been shown to improve insulin sensitivity and reduce lipid accumulation in HepG2 cells,[11] raising the possibility of BMP9-based intervention strategies for NAFLD.

Nevertheless, this study has several limitations. First, its cross-sectional design precludes causal inference. Second, it was conducted at a single center with a relatively small sample size, which may limit generalizability, reduce statistical power, and increase the risk of overestimating effect sizes. Third, mechanistic pathways underlying BMP9 synthesis, secretion, and signaling were not directly investigated. These findings should therefore be regarded as preliminary and require validation in larger, multicenter and longitudinal cohorts, ideally complemented by mechanistic studies in animal models, liver biopsies, and single-cell transcriptomic approaches.

Despite these limitations, our results highlight the potential of dynamic BMP9 profiling during OFTT as a novel biomarker for NAFLD risk stratification and a possible therapeutic target. Future work should further clarify its role in metabolic regulation and assess the clinical utility of BMP9-based interventions in the prevention and treatment of NAFLD, including their potential application in lifestyle modification and rehabilitation programs aimed at improving metabolic health.

Conclusion

This study is the first to dynamically assess BMP9 expression through a lipid tolerance challenge in patients with NAFLD. Our findings reveal a dual defect pattern—reduced baseline levels and impaired metabolic responsiveness—closely linked to markers of metabolic dysfunction. BMP9 AUC demonstrated high diagnostic accuracy and mechanistic relevance, supporting its potential role as both a biomarker and therapeutic target in NAFLD. By showing that impaired BMP9 dynamics represent a novel feature of NAFLD, this study extends current knowledge of hepatokine biology in metabolic liver disease. These results not only highlight BMP9 as a candidate marker for early diagnosis and disease monitoring but also suggest a possible role in guiding personalized management strategies. Further validation in large-scale and longitudinal studies is warranted.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Declaration

The study protocol was approved by the Institutional Ethics Committee of Linyi Hospital of Traditional Chinese Medicine (Approval No. 20240167). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

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Author Contributions

Fan Cui: Conceptualization, Investigation, Methodology, Visualization, Writing-original draft, Writing – review & editing. Fangmin Chen: Conceptualization, Investigation, Data curation, Methodology, Visualization, Writing – original draft. Fanchao

Dong: Conceptualization, Investigation, Data curation, Methodology, Writing – original draft. Jianjun Dong: Conceptualization, Supervision, Visualization, Writing – review & editing. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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