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ORIGINAL RESEARCH

Serum Lactate Dehydrogenase Is a Novel Predictor for the Severity in the Patients With MAFLD: A Cross-Sectional Study in Hefei, China

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Background & Aims: The aim of this study was to assess the association between the level of lactate dehydrogenase (LDH) and the severity of metabolic syndrome (MetS) and Metabolic Associated Fatty Liver Disease (MAFLD) and the potential diagnostic value of LDH for identifying at-risk metabolic associated steatohepatitis (MASH).

Methods: This cross-sectional, real-world retrospective study enrolled a total of 1118 obese patients in the department of bariatric surgery at the Second Affiliated Hospital of Anhui Medical University from January 1, 2018, to December 31, 2021. Of these, 855 were enrolled in the study cohort. MAFLD was defined as the presence or absence of fatty liver disease as suggested by histologic biopsy of liver or postoperative pathology slides, or even hematology, and meets one of the following three conditions: overweight or obesity, T2DM, and metabolic dysfunction (MetS). Serum LDH activity levels were measured in 885 patients, and logistic regression was used to analyze the relationship between LDH and metabolic syndrome and the severity of MAFLD.

Results: In the study cohort of 855 obese patients, 604 (70.6%) had MetS. Patients with MetS (214.1[209.0–219.2]) had significantly higher serum LDH levels than those without MetS (188.7[181.6–195.9]). Particularly, serum LDH level was significantly higher in subjects with hypertension, central obesity, diabetes mellitus or hyperglycemia, elevated levels of triglycerides, or reduced levels of high-density lipoprotein than in those without. Moreover, LDH concentrations were grouped according to the total number of MetS components present in each patient, with Serum LDH levels gradually increase with the total number of MetS components. The MASH subjects had significantly higher LDH levels than the other three less severe non-MASH cohorts, including normal liver, simple fatty steatosis, and B.MASH. Logistic regression showed that LDH was significantly and positively correlated with MAFLD, B.MASH, MASH, at-risk MASH, fibrosis grade ≥1, fibrosis grade ≥2 and fibrosis grade ≥3.

Conclusion: Increased LDH levels were significantly and independently associated with the presence and severity of metabolic syndrome and MAFLD, indicating that LDH could be used as a novel biomarker and clinical predictor of severity of metabolic syndrome and MAFLD.

Keywords: lactate dehydrogenase, obesity, metabolic syndrome, inflammation, metabolic associated fatty liver disease, (MAFLD)

Introduction

As one of the metabolic disorders, metabolism-associated fatty liver disease (MAFLD) affects the majority of obese patients and is associated with a range of metabolic syndromes,[1] like type 2 diabetes mellitus (T2DM), obesity, liver damage and other disease stages like chronic liver disease,[2] liver failure, cirrhosis hepatitis and hepatocellular carcinoma.[3] The new definition of

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MAFLD emphasizes the important role of multiple metabolic disorders such as overweight, insulin resistance, dyslipidemia, T2DM and metabolic inflammation in the pathogenesis of fatty liver disease and the importance of intervening in these metabolic cardiovascular high-risk factors to combat liver and its comorbidities. The most accurate and conclusive diagnosis of MAFLD has so far required excluding other potential factors in the development of chronic liver disease, such as syphilis or alcohol consumption.[4] The International Expert Consensus Statement on the New descriptions of MAFLD, proposes comprehensive and simple MAFLD diagnostic criteria, which are based on the presence or absence of fatty liver disease as suggested by histologic biopsy of liver or postoperative pathology slides, or even hematology, and meet one of the following three conditions: overweight or obesity, T2DM, and metabolic dysfunction (MetS).[5,6] However, high cost, invasiveness, inconsistent observer subjectivity, and operator sampling error are considered limitations of liver biopsy.

Metabolism is the main inducing driver of NAFLD, several experts in the field proposed a new nomenclature-MAFLD or metabolism-related fatty liver disease.[7] A cross-sectional study from 14 hospitals included 246 patients with NAFLD, all of whom were confirmed by liver puncture biopsy, and showed that 84% of the patients met the criteria of diagnosis for non-alcoholic steatohepatitis (NASH) and 97% for MAFLD.[8] Relevant studies on the relationship between LDH levels and MAFLD are scarce and controversial. For example, a recent study investigated the effect of a prognostic index for patients with MAFLD and COVID-19 based on lactate dehydrogenase (LDH) and glutamine transaminase, and this prognostic index provides precise risk stratification for poor outcomes in patients with MAFLD. Moreover, Ricardo et al demonstrated that serum LDH and transaminase activity is of diagnostic value in predicting liver steatosis.[9] Due to the diagnosis of MAFLD can be combined with alcohol abuse and other liver diseases, the prevalence of MAFLD should be slightly higher than prevalence of NAFLD in general population.[10]

Currently, several studies have now shown that lactate accumulates in the liver of patients during the development of NAFLD, and LDH is an enzyme that can promote the change of pyruvate to lactate under hypoxia is upregulated under hypoxia, and they have also found that impaired lactate clearance is due to acetylation of an enzyme involved in lactate metabolism thereby exacerbating the progression of NAFLD.[11,12] However, the relationship between the LDH and MAFLD is uncommon and may be equivocal in the general population. A dramatic increase in plasma levels of LDH has been reported in patients with acute liver injury. It can be assumed that LDH may play a differential role in the diagnosis of liver injury. Considering its short half-life, ubiquitous presence in the body, low non-specificity and high sensitivity, LDH can be used as a marker of hepatic ischemic injury to provide diagnostic and prognostic information in the context of liver injury such as NAFLD.[11,13] However, there are few studies on the diagnosis and sensitivity of LDH for MAFLD.

Metabolic syndrome (MetS) is an endocrine disorder that acts as a group of cardiovascular risk factors and includes multiple metabolic abnormalities, including increased insulin resistance leading to diabetes mellitus (DM) or glucose intolerance, hypertension, dyslipidemia and central obesity, which is closely related to obesity, T2DM, hypertension and hyperlipidemia.[14–16] Given the high prevalence of MetS and its associated cardiovascular risk factors, it has become a public health problem on a serious global scale.[17] According to the latest guidelines, China has the largest number of diabetics in the world, with a prevalence of 11.2% for type 2 diabetes.[18] MetS can cause diabetes-related complications, such as retinopathy or diabetic foot, and it occupies one of the main positions among the factors that contribute to death in the elderly. There is growing evidence that inflammation and immune responses play key roles in insulin resistance, lipid metabolism, obesity and the development of MetS, and a number of correlates have been found to be of critical diagnostic and prognostic value or may serve as therapeutic targets.[19]

In this study, which included 885 morbidly obese patients, we investigated the association of LDH with insulin resistance, obesity, metabolism and MetS severity, as well as MAFLD, and we assessed the sensitivity of LDH in differentiating and recognizing the diagnostic aspects of metabolism-associated steatohepatitis (MASH) and at-risk MASH.

Subjects and Methods

Participants

This study complies with the Declaration of Helsinki and was performed according to ethics committee approval. This cross-sectional, real-world retrospective study was approved by the Second Affiliated Hospital of Anhui Medical University Ethics committee (YX2021-099). We informed patients about the study and obtained their verbal and signed written consent. We

enrolled a total of 1118 obese patients in the department of bariatric surgery at the Second Affiliated Hospital of Anhui Medical University from January 1, 2018, to December 31, 2021. Of these, 855 were enrolled in the study cohort. Two hundred and sixty-three were excluded from the study cohort, with exclusion criteria contains liver disease caused by drugs, viral hepatitis and incomplete information. The exclusion process complies with WHO standards, as detailed in Supplementary Figure 1. Concurrent other liver diseases are not considered as exclusion criteria.[6]

MetS Diagnosis Criteria

The diagnosis of MetS is based on 3 or more of the following criteria: abdominal obesity (waist circumference (WC) ≥80 cm in women or ≥90 cm in men); triglycerides (TG) ≥150 mg/dl or ≥1.7 mmol/L; high-density lipoprotein (HDL) cholesterol level <50 mg/dl for women and <40 mg/dl for men (<1.3 mmol/L for women and <1.0 mmol/L for men); Patients were on antihypertensive medication or had a current blood pressure Systolic ≥130 and/or diastolic 85 mmHg; history of T2DM or current fasting glucose ≥5.6 mmol/L with oral medication or insulin injection.[20,21] There are five MetS, so the scores range from 0 to 5, and as higher scores represent more severe MetS.

Histologic Evaluation of Liver Biopsies

During bariatric surgery, a liver biopsy was performed from the middle of the right lobe using 14-gauge needles. The 4 μm liver biopsy sections were stained with Masson's trichrome or hematoxylin-eosin and obtained by an independent expert pathologist in a blinded manner. Scores for ballooning (0–2), lobular inflammation (0–3), steatosis (0–3) and fibrosis (0–4) were assessed using NAFLD scoring system proposed by Kleiner et al.[22]

MAFLD Diagnosis Criteria

The MAFLD diagnosis criteria is the presence of steatosis and accumulation of more than 5% fat content in the hepatocytes, confirmed by imaging or liver biopsy, and one of the following three diagnostic criteria: overweight or obesity, T2DM, or indicators of metabolic disorder. According to the World Health Organization (WHO) Asian standards, overweight and obesity are classified by body mass index (BMI): overweight is defined as a BMI of 23.0 to 24.9 kg/m^2; obesity I is defined as a BMI of 25 to 29.9 kg/m^2; and obesity II is defined as a BMI of ≥30 kg/m^2. Patients with type 2 diabetes or pre-diabetes, obesity, hypertension, low HDL-C, insulin resistance and plasma C-reactive protein who do not have a BMI of more than 30 kg/m² can also be included in MAFLD. Patients with metabolic syndrome or hyperinsulinism (fasting hyperinsulinemia (≥15 mIU/mL) or glucose challenge hyperinsulinemia (≥80m IU/mL)) can also be included in MAFLD. Borderline MASH (B.MASH) is defined as steatosis of the liver with inflammation or ballooning. Among individuals with metabolic risk factors, a nonalcoholic fatty liver activity score (NAS) ≥4 and a fibrosis grade ≥2 are considered as at-risk MASH.[23,24] Metabolic risk factors include type 2 diabetes or pre-diabetes, obesity, hypertension, low HDL-C, insulin resistance and plasma C-reactive protein levels.

Statistical Analysis

Statistical analyses were performed using SPSS version 26.0 (SPSS, Inc, Chicago, IL). For continuous variables, the characterization of the data depends on whether they follow a normal distribution. Obedience to normal distribution can be analyzed by the Shapiro–Wilk normality test and expressed as mean ± standard deviation; disobedience to normal distribution can be expressed as median and interquartile spacing. Numerical and percentage values can express categorical data. Student's *t*-test was used for comparison of parametric variables. Mann–Whitney *U*-test was available for comparison of non-parametric variables. ROC curve analysis can also identify the optimal cut-off value that can be used to predict the presence of MetS. A probability value of less than 0.05 was defined as statistically significant.

Results

Baseline Characteristics

Of all subjects in this cross-sectional real-world retrospective study, 219 participants were diagnosed in the non-MAFLD group and 636 were in the MAFLD group. A comparison of the five MetS definition components, elevated WC, hypertension, elevated fasting glucose, elevated TG and low LDH-C, as well as several clinical parameters. Overall, Table 1 highlights the

Table 1 Clinical, Biochemical and Histological Characteristics of 855 Obese Patients

Parameters	Overall	Non-MAFLD	MAFLD	p value
	(n=855)	(n=219)	(n=636)	
Age(years)	30.00(24.00–36.00)	31.00(25.00–38.00)	30.00(24.00–36.00)	0.29
Gender, men	327(38.2%)	46(21%)	281(44.2%)	**<0.001**
BMI (kg/m2)	38.31(33.7–43.85)	35.20(31.20–39.71)	39.45(35.00–44.98)	**<0.001**
Waist circumference (cm)	119.5(108.5–131.00)	110.00(101.5–121.50)	121.5(111.58–134.0)	**<0.001**
Hip circumference (cm)	122.00(114.00–132.00)	118.5(111.2–127.0)	123.50(115.00–134.00)	**<0.001**
Waist-to-hip ratio	0.97±0.07	0.94±0.07	0.99±0.07	**<0.001**
Systolic BP (mmHg)	125.00(117.46–136.00)	119.00(117.00–128.00)	128.00(119.00–137.00)	**<0.001**
Diastolic BP (mmHg)	79.00(72.79–96.00)	75.00(72.79–82.00)	81.00(72.84–89.04)	**<0.001**
Glucose (mmol/L)	5.47(4.92–6.48)	5.11(4.76–5.65)	5.61(5.00–7.06)	**<0.001**
HbA1c (%)	5.80(5.40–6.55)	5.50(5.20–5.80)	5.90(5.50–6.80)	**<0.001**
Fasting insulin (mIU/L)	19.55(12.89–27.67)	13.08(9.03–21.20)	21.34(15.08–29.67)	**<0.001**
C-Peptide (ng/mL)	3.50(2.56–4.48)	2.61(2.03–3.53)	3.68(2.93–4.68)	**<0.001**
HOMA-IR	5.13(3.21–7.57)	3.24(2.05–5.28)	5.62(3.94–8.67)	**<0.001**
TC (mmol/L)	4.92(4.36–5.66)	4.82(4.28–5.60)	4.96(4.40–5.70)	**0.05**
TG (mmol/L)	1.61(1.17–2.29)	1.30(0.94–1.68)	1.75(1.30–2.48)	**<0.001**
HDL-C (mmol/L)	0.99(0.87–1.15)	1.05(0.91–1.25)	0.97(0.85–1.12)	**<0.001**
LDL-C (mmol/L)	2.97(2.54–3.51)	2.90(2.45–3.38)	3.01(2.59–3.53)	**0.033**
ALT (U/L)	39.00(23.00–70.00)	22.00(15.00–31.00)	48.00(30.00–83.00)	**<0.001**
AST (U/L)	24.00(18.00–39.00)	18.00(15.00–22.00)	29.00(20.00–49.00)	**<0.001**
AST/ALT ratio	1.52(1.18–1.87)	1.18(1.00–1.47)	1.61(1.31–1.98)	**<0.001**
y-GT (U/L)	35.00(22.00–59.00)	24.00(17.00–39.00)	40.00(26.00–66.00)	**<0.001**
ALP (U/L)	78(64–94)	71.00(60.00–84.00)	80.5(67.00–96.00)	**<0.001**
Cholinesterase (U/L)	9844(8690–11,032)	9040(8288–10,206)	10,100(8938.25–11,219.5)	**<0.001**
TP (g/L)	71.8(68–75.3)	70.90(66.8–74.7)	72.10(68.3–75.5)	**0.006**
ALB (g/L)	42(39.8–44.3)	41.6(39.7–43.7)	42.15(39.9–44.5)	**0.004**
Prealbumin (mg/L)	255.90(221.1–286.1)	251.1(216.3–280.00)	256.8(223.1–288.25)	**0.048**
GLB (g/L)	29.4(26.9–32.11)	28.87(26.67–31.8)	29.57(26.9–32.3)	0.083
A/G	1.48(1.33–1.63)	1.46(1.35–1.60)	1.49(1.32–1.65)	0.301
TB (μmol/L)	11.6(9.0–15.1)	10.8(8.20–13.6)	11.8(9.3–15.48)	**0.005**
DB (μmol/L)	2.8(2–4)	2.5(1.8–3.6)	2.9(2.1–4.1)	**0.003**
IBIL (μmol/L)	8.6(6.6–11.2)	8.1(5.9–10.7)	8.9(6.73–11.4)	**0.029**
TBA (μmol/L)	3.3(2.2–5.4)	2.9(2–4.6)	3.4(2.23–5.6)	0.146

(*Continued*)

Table 1 (Continued).

Parameters	Overall (n=855)	Non-MAFLD (n=219)	MAFLD (n=636)	p value
WBC (×109/L)	7.85(6.52–9.27)	7.5(6.39–8.85)	7.92(6.67–9.35)	**0.01**
CRP (mg/L)	5.70(2.83–11.43)	4.33(1.93–8.80)	5.95(3.01–12.22)	**<0.001**
Metabolic comorbidities (n, %)				
Elevated waist circumference	855(100%)	219(100%)	636(100%)	-
Hypertriglyceridemia	390(45.61%)	53(24.2%)	337(52.9%)	**<0.001**
Low HDL-C	655(76.6%)	162(73.9%)	493(77.5%)	0.285
Hyperglycemia	382(44.6%)	60(27.3%)	322(50.6%)	**<0.001**
Hypertension (n=507)	431(50.4%)	65(29.6%)	366(57.5%)	**<0.001**
Class II obesity	791(92.5%)	186(84.9%)	605(95.1%)	**<0.001**
Diabetes	248(29.0%)	27(12.3%)	221(34.7%)	**<0.001**
MetS	604(70.6%)	108(49.3%)	496(77.9%)	**<0.001**
Liver histology (n, %)				
Normal liver	200(23.3%)	200(91.3%)	0(0%)	-
Steatosis	636(74.4%)	0(0%)	636(100%)	-
Ballooning	336(39.3%)	15(6.8%)	321(50.5%)	**<0.001**
Inflammation	485(56.7%)	55(25.1%)	430(67.6%)	**<0.001**
Fibrosis	406(47.5%)	50(22.8%)	356(60%)	**<0.001**

Note: Data shown as n (%), mean (SD) for normally distributed data, or median (interquartile range) for non-normally distributed data.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDH, lactate dehydrogenase; MetS, metabolic syndrome; TC, total cholesterol; TG, triglyceride; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance; y-GT, Gamma Glutamyl Transpeptidase; TP, total protein; ALB, albumin; GLB, Globulin; A/G, Albumin/Globulin; TB, Total Bilirubin; TB, Total Bilirubin; DB, Direct Bilirubin; IBIL, Indirect Bilirubin; TBA, Total bile acids. *P* <0.05 was considered statistically significant.

baseline characteristics of the subjects in the MAFLD and non-MAFLD groups. The average age of the enrolled subjects in both groups was almost same, 30.00 (24.00–36.00) years of age, of which 38.2% were males, and there was no difference in the age of the subjects in the two cohorts (P = 0.29). However, patients with MAFLD have higher BMI, WC, Hip circumference, Systolic BP(SBP), Diastolic BP(DBP), Glucose, HbA1c, fasting insulin, fasting C-Peptide and HOMA-IR levels than Non-MAFLD cohort (all P < 0.001). This cohort contained a wide range of BMI 38.31 (33.70–43.85kg/m2), of which 791 (92.5%) patients BMI ≥30 kg/m2 and 106 (12.3%) had T2DM. Additionally, serum lipid profiles, such as total cholesterol (TC), TG, HDL-C and LDL-C were significantly elevated in the MAFLD cohort compared to the non-MAFLD cohort (P < 0.001, P = 0.05, P < 0.001 and P = 0.033, respectively). Liver enzymes, including alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), (r-glutamyl transpeptidase) y-GT, and Cholinesterase, were also significantly elevated in the MAFLD cohort compared with the non-MAFLD cohort (all P < 0.001).

According to the Histologic evaluation of liver biopsies, there were 200 (23.3%) for normal liver, 636 (74.4%) for hepatic steatosis, 336 (39.3%) for ballooning, 485 (56.7%) for inflammation and 406 (47.5%) for fibrosis (Table 1). We introduced a Fatty Liver Inhibition of Progression algorithm, in which 211 (24.7%) patients showed normal liver histology, whereas 140 (16.4%), 257 (30.0%) and 247 (28.9%) could be categorized as simple steatosis, B.MASH, and MASH, respectively (Table 2). Based on the fact that all 855 patients met the criteria of overweight or obesity, Of the 855 patients with hepatic steatosis, 636 (74.39%) could be diagnosed with MAFLD (Table 1).

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Table 2 Clinical and Biochemical Characteristics of Normal Liver, Simple Steatosis, Borderline MASH and MASH

Parameters	Normal liver	Simple Steatosis	Borderline MASH	MASH	p
	(n=211)	(n=140)	(n=257)	(n=247)	
Age (years)	31.00(25.00–38.00)	30.00(24.00–37.00)	31.00(26.00–37.00)	29.00(23.00–34.00)	0.088
Gender, men	20(20.4%)	25(35.2%)	107(47.8%)	63(40.1%)	**<0.001**
BMI (kg/m2)	35.2(31.2–39.7)	37.60(33.13–42.87)	39.04(34.81–44.57)	40.6(36.07–45.54)	**<0.001**
Waist circumference (cm)	110.(101.5–121.5)	120(118–131.5)	121(111.65–134.1)	123.5(113.2–135)	**0.007**
Waist-to-hip ratio	0.94±0.07	0.98±0.07	0.99±0.07	0.99±0.07	0.132
Systolic BP (mmHg)	119(117–128)	127.5(120–137)	129(120–138)	126(119–137.9)	**<0.001**
Diastolic BP (mmHg)	75(72.79–82)	81(74–89.03)	82(72.79–89.04)	82(72.79–89.04)	**<0.001**
Glucose (mmol/L)	5.11(4.76–5.65)	5.65(4.89–6.41)	5.61(5.03–7.04)	5.83(5.11–7.4)	**<0.001**
HbA1c (%)	5.5(5.2–5.8)	5.7(5.3–6.3)	5.8(5.5–6.85)	6(5.66–7)	**<0.001**
Fasting insulin (mIU/L)	13.08(9.03–21.2)	18.29(12.45–25.47)	20.92(14.37–29.63)	22.88(17.49–33.93)	**<0.001**
C-Peptide (ng/mL)	2.61(2.03–3.53)	3.3(2.58–4.09)	3.68(2.91–4.68)	3.89(3.25–4.94)	**<0.001**
HOMA-IR	3.24(2.06–5.27)	4.63(2.99–6.73)	5.5(3.9–68.39)	6.41(4.6–10.62)	**<0.001**
TC (mmol/L)	4.82(4.28–5.60)	4.85(4.22–5.33)	4.97(4.5–5.8)	5.02(4.41–5.76)	0.076
TG (mmol/L)	1.30(0.94–1.66)	1.66(1.1–2.41)	1.75(1.31–2.48)	1.81(1.35–2.58)	**<0.001**
HDL-C (mmol/L)	1.05(0.91–1.25)	0.99(0.86–1.18)	0.99(0.85–1.11)	0.95(0.85–1.11)	0.442
LDL-C (mmol/L)	2.9(2.46–3.37)	2.85(2.45–3.36)	3.04(2.6–3.57)	3.07(2.6–3.56)	0.927
ALT (U/L)	22(15–31)	35(23–52.5)	44(28–75)	65.1(40–101)	**<0.001**
AST (U/L)	18(15–22)	24(19–36)	26(20–40)	37(24.5–56)	**<0.001**
AST/ALT ratio	1.17(1–1.47)	1.46(1.17–1.81)	1.63(1.37–2.04)	1.66(1.37–2.01)	**0.018**
γ-GT (U/L)	24(17.5–38.5)	39(25–66.5)	39(25.3–63)	42(27–70)	**<0.001**
ALP (U/L)	71(60–83.5)	75.5(64–98)	81(68–94)	82(67–96)	**0.003**
Cholinesterase (U/L)	9040(8293.5–10,198.5)	9753.5(8039–11,078)	10,156(9039–11,220)	10,112(9066–11,285.5)	**<0.001**
TP (g/L)	70.9(66.85–74.6)	72.1(68.25–76.35)	72.3(68.3–75.5)	71.7(68.3–75.2)	**0.048**
ALB (g/L)	41.6(39.7–43.65)	42.6(40.05–45)	42.1(39.9–44.5)	41.9(39.8–44.35)	**0.045**
Prealbumin (mg/L)	251.1(216.3–279.65)	268.3(224–300)	257.4(227.4–287.1)	252.9(216.55–282.5)	**0.035**
GLB (g/L)	28.87(26.68–31.8)	29.1(26.55–32.15)	29.8(27.2–32.4)	29.36(26.9–32.2)	0.275
Albumin_Globulin	1.46(1.35–1.6)	1.55(1.32–1.70)	1.46(1.32–1.61)	1.49(1.32–1.65)	0.23
TB (μmol/L)	10.8(8.2–13.6)	11.55(8.8–15.4)	12.2(9.3–15.5)	11.6(9.5–15.25)	**0.015**
DB (μmol/L)	2.5(1.8–3.55)	2.7(2–3.8)	3(2.1–4.2)	2.9(2.1–4.1)	**0.01**
IBIL (μmol/L)	8.1(5.9–10.65)	8.65(6.2–11.7)	9(6.8–11.5)	8.7(6.95–11.1)	0.103
TBA (μmol/L)	2.9(2–4.6)	3.05(2.2–4.7)	3.3(2.1–5.7)	3.7(2.6–6.05)	**0.001**
CRP (mg/L)	4.33(1.95–8.8)	4.61(2.36–9.79)	6.04(3.03–12.2)	7.25(3.69–13.12)	**<0.001**

(*Continued*)

Table 2 (Continued).

Parameters	Normal liver (n=211)	Simple Steatosis (n=140)	Borderline MASH (n=257)	MASH (n=247)	p
Metabolic comorbidities (n, %)					
Elevated waist circumference	211(100%)	140(100%)	257(100%)	247(100%)	-
Hypertriglyceridemia	51(24.1%)	63(45%)	137(53.3%)	139(56.2%)	**<0.001**
Low HDL-C	160(75.8%)	99(70.7%)	189(73.5%)	207(83.8%)	**0.01**
Hyperglycemia	56(26.5%)	53(37.8%)	129(50.1%)	144(58.2%)	**<0.001**
Hypertension (n=225)	63(29.8%)	77(55%)	150(58.3%)	141(57%)	**<0.001**
Class II obesity	179(84.8%)	124(88.5%)	246(95.7)	242(97.9%)	**<0.001**
Diabetes	27(12.7%)	32(22.8%)	86(33.4%)	103(41.7%)	**<0.001**
MetS	105(49.7%)	103(73.5%)	194(75.4%)	202(81.7%)	**<0.001**

Note: Data shown as n (%), mean (SD) for normally distributed data, or median (interquartile range) for non-normally distributed data.
Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDH, lactate dehydrogenase; MetS, metabolic syndrome; TC, total cholesterol; TG, triglyceride. HOMA-IR, Homeostasis Model Assessment-Insulin Resistance; y-GT, Gamma Glutamyl Transpeptidase; TP, total protein; ALB, albumin; GLB, Globulin; A/G, Albumin/Globulin; TB, Total Bilirubin; TB, Total Bilirubin; DB, Direct Bilirubin; IBIL, Indirect Bilirubin; TBA, Total bile acids. *P* <0.05 was considered statistically significant.

Association of Serum LDH Levels With Glucose Dysregulation

When grouping patients according to each of these components, those with hypertension, elevated WC, diabetes or elevated fasting glucose, elevated TG or reduced HDL-C had significantly higher serum LDH levels in patients without five MetS (Table 2). Serum LDH level was significantly higher in patients with T2DM (226.7 [217.7–235.7U/L) (n = 248) than with normal glucose (190.5 [184.4–196.6U/L (n = 324) (P < 0.0001) and prediabetes (207.6 [200.9–214.4U/L (n = 283) (P < 0.0001), normal glucose vs diabetes: P < 0.0001; prediabetes vs diabetes: P = 0.0033) (Figure 1A). The results show a strong association between LDH and dysglycemia.

Association of Serum LDH Levels With Metabolic Parameters

There was a significant positive correlation between levels of LDH levels and BMI (β=0.272, P < 0.001) and several baseline clinical parameters including, WC (β = 0.191, P < 0.001), Hip circumference (β = 0.06, P < 0.001), Systolic BP (β = 0.196, P < 0.001) and Diastolic BP (β = 0.138, P < 0.001) analyzed by simple linear regression after adjustment of age, gender and BMI. Furthermore, some glucose metabolism factors such as fasting glucose (β = 0.20, P < 0.001), fasting insulin (β = 0.171, P < 0.001), fasting C-peptide (β = 0.252, P < 0.001), HbA1c (β = 0.228, P < 0.001) and HOMA-IR (β = 0.196, P < 0.001) have strong positive correlation with LDH after adjustment of age, gender and BMI. And some Serum lipid profiles, such as TC (β = 0.178, P < 0.001), TG (β = 0.124, P < 0.001), HDL-C (β = −0.028, P = 0.401) and LDL-C (β = 0.154, P < 0.001) after adjustment of age, gender and BMI. Liver enzymes, including ALT (β = 0.508, P < 0.001), AST (β = 0.532, P < 0.001), y-GT (β = 0.215, P < 0.001), ALP (β = 0.207, P < 0.001) and Cholinesterase (β = 0.129, P < 0.001) after adjustment of age, gender and BMI. Additionally, participants with a higher serum LDH level had a lower HDL-C level (Table 3). In addition, logistic regression showed an odds ratio of LDH to diabetes is 2.401 (P < 0.001) after adjustment for gender, age, and BMI (Table 4). These results demonstrated an association between LDH and obesity and metabolic parameters.

Association of Serum LDH Levels With Metabolic Syndrome

In the study cohort of 855 obese patients, 604 (70.6%) had MetS. Patients with MetS (214.1[209.0–219.2]) had significantly higher serum LDH levels than those without MetS (188.7[181.6–195.9]) (Figure 1B) and logistic regression

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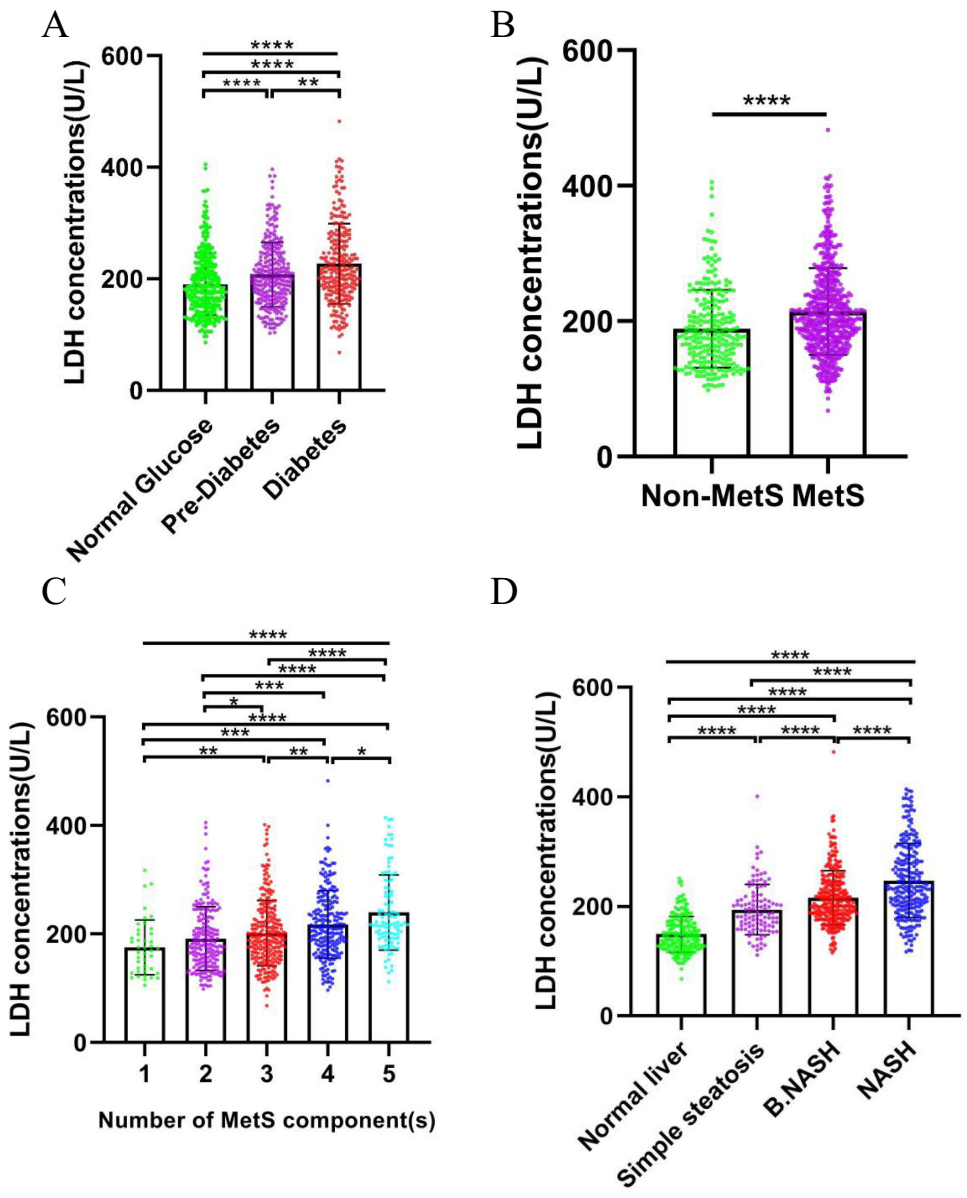

Figure 1 Clinical, biochemical and histological characteristics of 855 obese patients. (**A**) LDH levels from subjects with normal glucose (n = 324), subjects with prediabetes (n = 283), and patients with type-2 diabetes (n = 248) (Kruskal–Wallis test and Dunn's test between each group). (**B**) Comparison of LDH levels between subject with (n = 604 and without (n =251) MetS. (**C**) LDH stratified by the number of the components of MetS. n = 42, 209, 268, 231, and 105 for 1, 2, 3, 4, and 5 components of MetS, respectively. Data are presented as median (interquartile range). (**D**) Histo_Diagnosis (n=200,109,299,247 for Histo_Diagnosis grades of 0, 1, 2, 3 respectively). *P < 0.05, **P < 0.01, and ***P < 0.001,****P < 0.0001.

showed an odds ratio of LDH to MetS is 2.806 (P < 0.001) after adjusting for gender, age, and BMI (Table 4). In order to explore the association between LDH and MetS in greater depth, the LDH concentrations were grouped according to the total number of MetS components present in each patient, with Serum LDH levels gradually increasing with the total number of MetS components, thus proving the internal consistency of the above outcomes (Figure 1C). The LDH concentration rises progressively as the number of MetS components increases. For those with 1 to 5 components of MetS, the LDH levels were 175.3 (159.5–191.0) U/L (n = 42),191.5 (183.5, 199.5) U/L (n = 209), 201.4 (194.1, 208.6) U/L (n = 268), 217.3 (209.3,225.4) (n = 231), and 239.6 (226.2, 253.0) U/L (n = 105), respectively (P < 0.001, Kruskal–Wallis test). In addition, there was a clear association between LDH and the total number of MetS components, suggesting that higher LDH levels indicate more severe MetS (Figure 1C). For Histo diagnosis grades, the NASH

Table 3 Correlation Between Circulating Levels of LDH With Various Anthropometric and Biochemical Parameters (n = 658)

	LDH			LDH (adjusted for gender, age, and BMI)		
	β	η2	P value	β	η2	P value
Age(years)	−0.21	0.044	<0.001			
Gender, men	−0.191	0.036	<0.001			
BMI (kg/m2)	0.272	0.074	<0.001			
Waist circumference (cm)	0.311	0.097	<0.001	0.191	0.132	**<0.001**
Hip circumference (cm)	0.255	0.065	<0.001	0.06	0.121	**0.025**
Waist-to-hip ratio	0.193	0.037	<0.001	0.208	0.131	**<0.001**
Systolic BP (mmHg)	0.23	0.053	<0.001	0.196	0.155	**<0.001**
Diastolic BP (mmHg)	0.166	0.027	<0.001	0.138	0.138	**<0.001**
Glucose (mmol/L)	0.164	0.027	<0.001	0.2	0.157	**<0.001**
HbA1c (%)	0.222	0.049	<0.001	0.228	0.168	**<0.001**
Fasting insulin (mIU/L)	0.251	0.063	<0.001	0.171	0.147	**<0.001**
C-Peptide (ng/mL)	0.341	0.117	<0.001	0.252	0.172	**<0.001**
HOMA-IR	0.255	0.065	<0.001	0.196	0.156	**<0.001**
TC (mmol/L)	0.166	0.028	<0.001	0.178	0.151	**<0.001**
TG (mmol/L)	0.128	0.016	<0.001	0.124	0.134	**<0.001**
HDL-C (mmol/L)	−0.089	0.008	0.009	−0.028	0.12	0.401
LDL-C (mmol/L)	0.156	0.024	<0.001	0.154	0.143	**<0.001**
ALT (U/L)	0.537	0.289	<0.001	0.508	0.342	**<0.001**
AST (U/L)	0.574	0.329	<0.001	0.532	0.377	**<0.001**
AST/ALT ratio	0.243	0.059	<0.001	0.174	0.145	**<0.001**
y-GT (U/L)	0.255	0.065	<0.001	0.215	0.163	**<0.001**
ALP (U/L)	0.271	0.073	<0.001	0.207	0.16	**<0.001**
Cholinesterase (U/L)	0.172	0.03	<0.001	0.129	0.136	**<0.001**
TP (g/L)	0.19	0.036	<0.001	0.141	0.139	**<0.001**
ALB (g/L)	0.172	0.03	<0.001	0.17	0.145	**<0.001**
Prealbumin (mg/L)	0.068	0.005	0.046	0.081	0.126	**0.017**
GLB (g/L)	0.133	0.018	<0.001	0.072	0.125	**0.03**
Albumin_Globulin	−0.004	0	0.907	0.034	0.121	0.319
TB (μmol/L)	0.149	0.022	<0.001	0.105	0.13	**0.002**
DB (μmol/L)	0.171	0.029	<0.001	0.121	0.133	**<0.001**

(*Continued*)

Table 3 (Continued).

	LDH			LDH (adjusted for gender, age, and BMI)		
	β	η2	P value	β	η2	P value
IBIL (μmol/L)	0.112	0.012	0.001	0.074	0.125	**0.026**
TBA (μmol/L)	0.093	0.009	0.006	0.098	0.129	**0.003**

Note: *P* <0.05 was considered statistically significant.

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDH, lactate dehydrogenase; TC, total cholesterol; TG, triglyceride.

Table 4 Logistic Regression Analysis Showing the Association of LDH Level With Metabolic Comorbidities and Severity of MAFLD

		OR (95% CI)	P value
Diabetes	LDH	2.401(2.332–2.473)	**<0.001**
	Sex(male)	0.464(0.454–0.475)	**<0.001**
	Age	1.074(7.073–1.075)	**<0.001**
	BMI	1.013(1.012–1.014)	**<0.001**
MetS	LDH	2.806(2.734–2.879)	**<0.001**
	Sex(male)	0.584(0.570–0.598)	**<0.001**
	Age	1.062(1.061–1.064)	**<0.001**
	BMI	0.998(0.997–0.999)	**0.002**
MAFLD	LDH	10.228(9.939–10.526)	**<0.001**
	Sex(male)	0.433(0.419–0.447)	**<0.001**
	Age	1.015(1.013–1.017)	**<0.001**
	BMI	1.038(1.036–1.040)	**<0.001**
B-MASH	LDH	6.475(6.311–6.644)	**<0.001**
	Sex(male)	0.853(0.834–0.873)	**<0.001**
	Age	1.011(1.01–1.013)	**<0.001**
	BMI	1.037(1.036–1.039)	**<0.001**
MASH	LDH	4.929(4.772–5.092)	**<0.001**
	Sex(male)	1.290(1.262–1.318)	**<0.001**
	Age	0.993(0.992–0.994)	**<0.001**
	BMI	1.103(1.012–1.014)	**<0.001**
At-risk MASH	LDH	22.124(19.792–24.731)	**<0.001**
	Sex(male)	0.553(0.537–0.569)	**<0.001**
	Age	0.981(0.979–0.982)	**<0.001**
	BMI	1.013(1.012–1.015)	**<0.001**

(*Continued*)

Table 4 (Continued).

		OR (95% CI)	P value
F1	LDH	1.907(1.862–1.954)	**<0.001**
	Sex(male)	0.592(0.580–0.604)	**<0.001**
	Age	1.008(1007–1.009)	**<0.001**
	BMI	1.022(0.020–1.023)	**<0.001**
F2	LDH	2.639(2.552–2.730)	**<0.001**
	Sex(male)	0.500(0.489–0.512)	**<0.001**
	Age	1.007(1.005–1.008)	**<0.001**
	BMI	1.018(1.017–1.019)	**<0.001**
F3	LDH	2.933(2.763–3.113)	**<0.001**
	Sex(male)	0.373(0.360–0.386)	**<0.001**
	Age	1.005(1.003–1.007)	**<0.001**
	BMI	1.027(1.026–1.029)	**<0.001**

Abbreviations: BMI, body mass index; F, fibrosis; MAFLD, metabolic associated fatty liver disease; MASH, metabolic associated steatohepatitis; MetS, metabolic syndrome; *P* <0.05 was considered statistically significant.

subjects had significantly higher LDH levels than the other three less severe non-NASH cohorts, including normal liver, simple fatty steatosis, and B.NASH (all P < 0.0001, Kruskal–Wallis test, Dunn's test) (Figure 1D).

Association of Serum LDH Levels With Severity of MAFLD

We examined the relationship between LDH and liver function abnormalities and the severity of MAFLD. LDH levels were significantly and positively correlated with ALT (β = 0.508, P < 0.001), AST (β = 0.532, P < 0.001), y-GT (β = 0.215, P < 0.001), ALP (β = 0.207, P < 0.001), and cholinesterase (β = 0.129, P < 0.001), as indicated by linear regression scores (Table 3). Compared to non-MAFLD patients, LDH was significantly higher in MAFLD group. (P < 0.0001, Mann–Whitney *U*-test) (Figure 2A).

In addition, the MASH subjects had significantly higher LDH levels than the other three less severe non-MASH cohorts, including normal liver, simple fatty steatosis, and B.MASH (all P < 0.0001, Kruskal–Wallis test, Dunn's test) (Figure 2B). After adjusting for gender, age, and BMI, analysis of the association between LDH and various histopathological features of MAFLD revealed that LDH was positively associated with the severity of steatosis (R^2 = 0.3497, P < 0.0001), inflammation (R^2 = 0.1852, P < 0.0001), ballooning (R^2 = 0.1762, P < 0.0001), and fibrosis (R^2 = 0.1045, P < 0.0001) (Supplementary Figure 2). For steatosis, patients with steatosis scores of 2 or 3 had significantly higher LDH than patients with steatosis scores of 0 or 1, with LDH levels from 154.7 (149.6, 159.7) U/L (steatosis score 0), 201.5 (196.7, 206.2) U/L (steatosis score 1), 242.9 (233.4, 252.5) U/L (steatosis score 2) to 264.0 (252.3, 275.7) U/L (steatosis score 3) (all P < 0.0001, Kruskal–Wallis test; Dunn's test) (Figure 2C). Similarly, for inflammation, LDH levels in patients with scores of 0, 1, 2, and 3 increased in a step-wise way from 178.1 (172.7, 183.6) U/L, 220.9 (215.2, 226.6) U/L, 254.8 (238.5, 271.1) U/L to 272.9 (239.9, 3.5.9) U/L (P < 0.0001, Kruskal–Wallis test), respectively (Figure 2D). For ballooning, LDH levels increased progressively with increasing score of ballooning, from 187.0 (182.4, 191.5) U/L, 230.0 (222.8, 237.1) U/L to 274.4 (252.4, 296.4) U/L with scores of 0, 1, and 2, respectively (P < 0.0001, Kruskal–Wallis test) (Figure 2E). Additionally, LDH levels were also significantly increased with higher stages of fibrosis, from 190.9 (185.7, 196.0) U/L (no fibrosis), 208.2 (200.9, 215.5) U/L (fibrosis grade 1), 239.8 (226.0, 253.7) U/L (fibrosis grade 2), 248.8 (231.5, 266.1) U/L (fibrosis grade 3) and 266.3 (124.3, 408.2) U/L (fibrosis grade 4) (P < 0.0001, Kruskal Wallis

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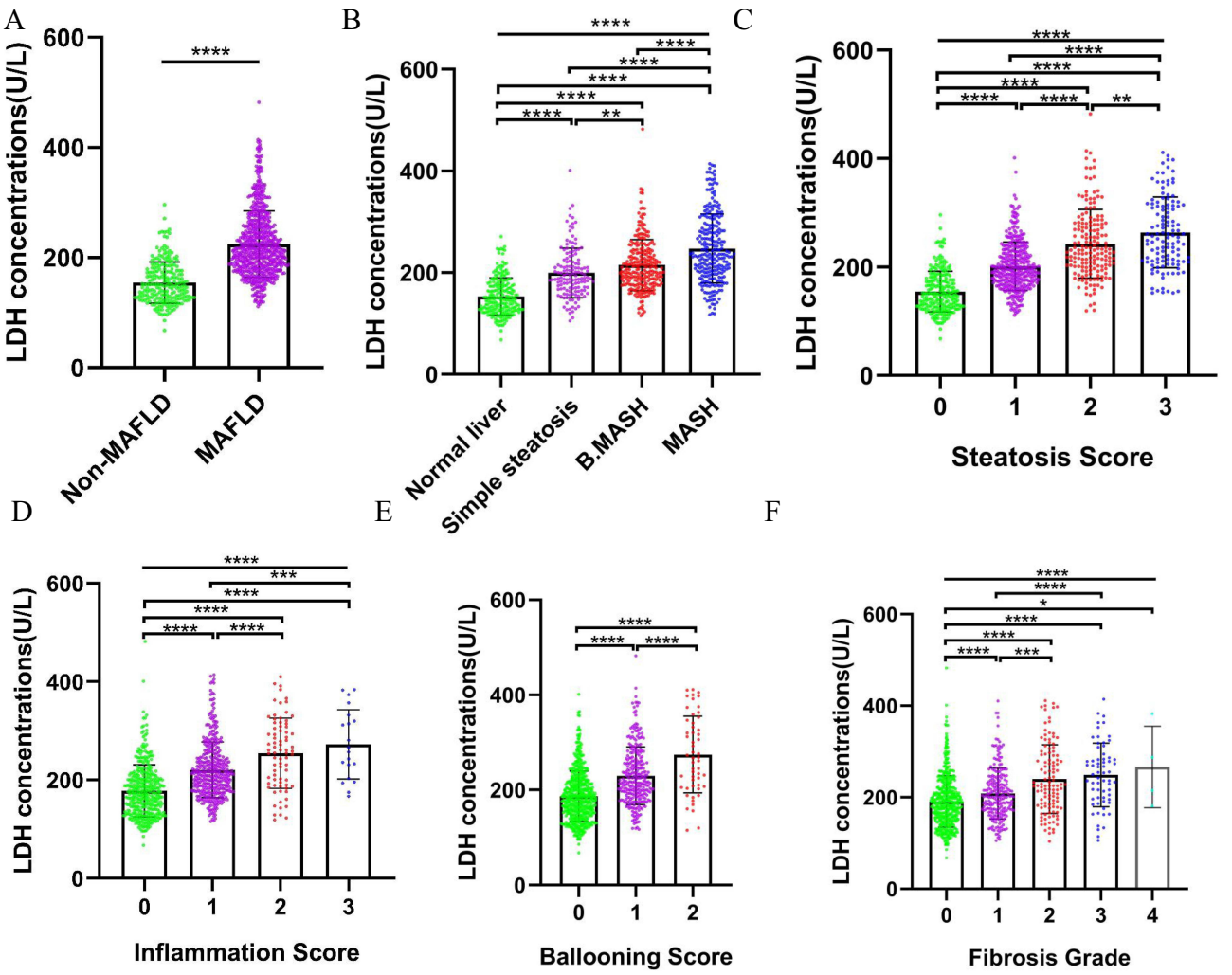

Figure 2 Clinical and biochemical characteristics of normal liver, simple steatosis, borderline MASH and MASH. (**A**) Comparison of LDH levels between non-MALD (n = 219) and MAFLD (n = 636) individuals with obesity. (**B**) LDH levels stratified by normal liver 153.4 (148.4, 158.3) U/L (n = 211), simple steatosis 199.6 (191.5, 207.8) U/L (n = 140), (**B**)MASH 215.1 (208.9,221.2) U/L (n = 257), and MASH 247.5 (239.0,255.9), U/L (n = 247). (Kruskal–Wallis test; Dunn's test). LDH stratified by (**C**)steatosis score (n =219, 343, 172, 121 for steatosis scores 0, 1, 2, 3 respectively). (**D**) ballooning score (n = 519, 282, 54 for ballooning scores of 0, 1, 2 respectively). (**E**) Inflammation score (n = 370, 388, 77, 20 for inflammation scores of 0, 1, 2, 3 respectively), (**F**) fibrosis grade (n = 449, 223, 115, 68 for fibrosis grades of 0, 1, 2, 3 respectively). *P < 0.05, **P < 0.01, and ***P < 0.001,****P < 0.0001.

test) (Figure 2F). Logistic regression showed that LDH was significantly and positively correlated with MAFLD (OR 10.228 P < 0.0001), B.MASH and MASH (OR 6.475, P < 0.0001), MASH (OR 4.929, P < 0.0001), at-risk MASH (OR 22.124, P < 0.0001), fibrosis grade ≥1 (OR 1.907, P < 0.0001), fibrosis grade ≥2 (OR 2.639, P < 0.0001) and fibrosis grade ≥3 (OR 2.933 P < 0.0001) (Table 4). Notably, The average age of this cohort was 30 years old and LDH levels in patients with Age of ≤29, 30–39 and 40–49 decreased in a step-wise way from 219.2 (212.8, 225.6) U/L, 220.7 (194.0, 207.4) U/L, to 183.0 (172.4, 193.5) U/L (P < 0.0001, Kruskal–Wallis test), respectively, which means younger people were more likely to develop MAFLD compared with elder people within 50 years old (Figure 3A).[25] Possible reasons for the higher prevalence of MAFLD in younger patients are: younger patients are more likely to have sedentary lifestyles, lack of exercise, and high-sugar diets that predispose them to obesity, leading to a higher prevalence of MAFLD.[26] These associations remained significant after adjusting for gender, age, and BMI. At the same LDH levels, male participants were significantly more likely to have various disease outcomes than female participants (Table 4 and Figure 3B). In addition, men have higher serum LDH levels than women, suggesting that MAFLD is influenced by a number of factors in addition to gender and LDH (Figure 3C). Moreover, the prevalence of MAFLD is higher in men than in women, which corresponds to the higher LDH levels in men. In conclusion, the findings suggest that LDH levels correlate significantly

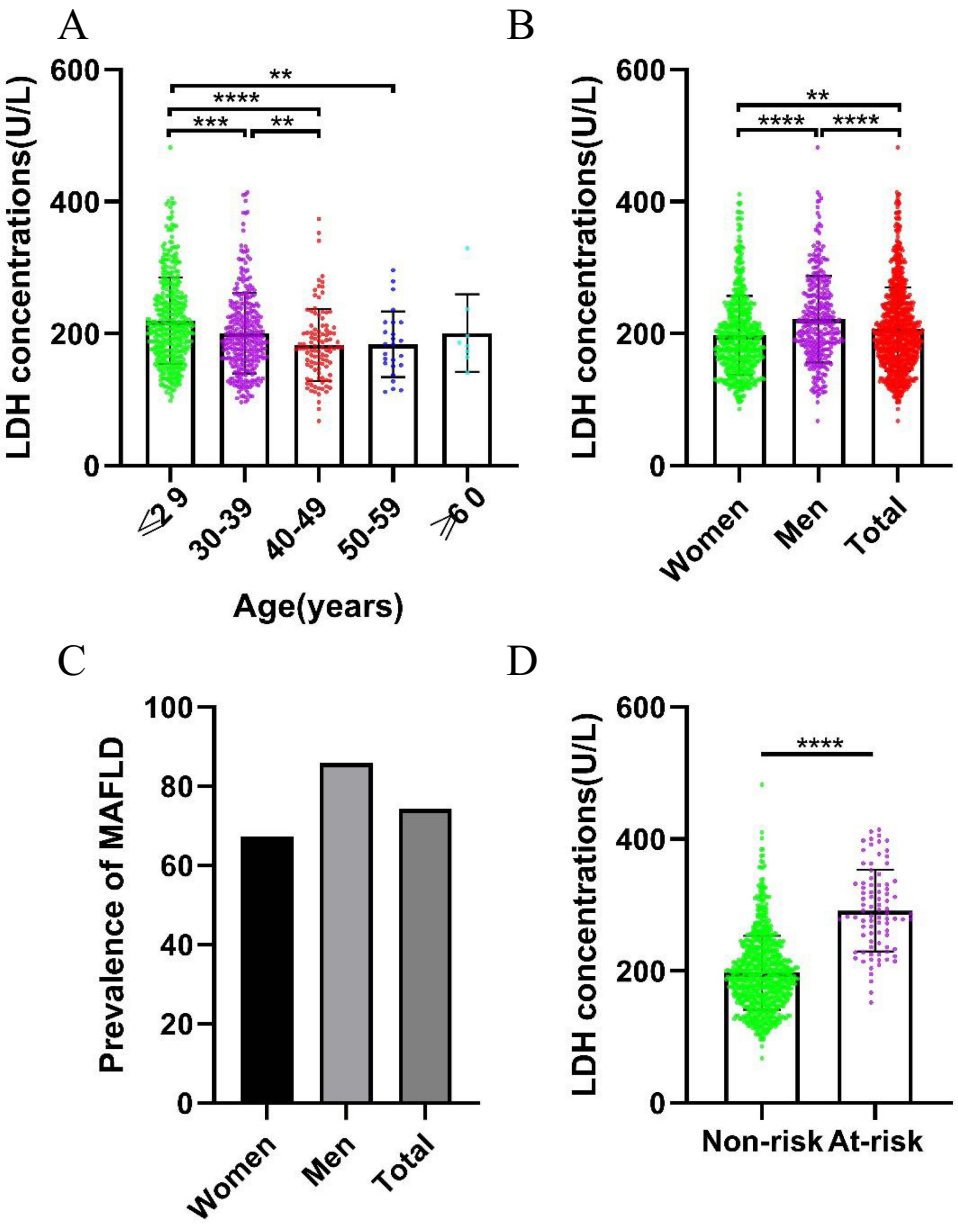

Figure 3 Comparisons of MAFLD prevalence and serum LDH levels stratified by gender and sex. (**A**)Comparisons of serum iron levels among patients stratified by age (P < 0.001 for trend). (**B**)Overall serum LDH levels and comparisons of serum LDH levels stratified by genders (P < 0.0001). (**C**)Overall prevalence of MAFLD and comparisons of the prevalence of MAFLD stratified by gender. (**D**) Comparison of LDH levels between non-risk (n = 769) and At-risk (n = 86) individuals (P < 0.0001). **P < 0.01, and ***P < 0.001,****P < 0.0001.

with all histologic features of MAFLD and can be used as a noninvasive biomarker to distinguish MASH from benign steatosis.

Accuracy of Noninvasive Biomarker for Predicting Clinical at-Risk MASH Patients

Next, we investigated the predictive value of LDH in identifying high-risk MASH patients defined as NAS≥4 and fibrosis grade ≥2 in patients with metabolic-related high risk factors. At-risk MASH patients have a higher risk of disease progression and are candidates for clinical trials and emerging drug therapies. The FDA has identified this subgroup as the primary inclusion criteria for the non-cirrhosis NASH trial. The LDH level in patients with at-risk MASH (291.4 (278.2, 304.6) U/L), which was significantly higher than in patients without at-risk MASH 197.2 (193.2, 201.2) U/L (P < 0.0001, Mann–Whitney *U*-test) (Figure 3D). ROC curve analysis of LDH concentrations predictive of at-risk MASH showed an AUC value of 0.874 (95% CI

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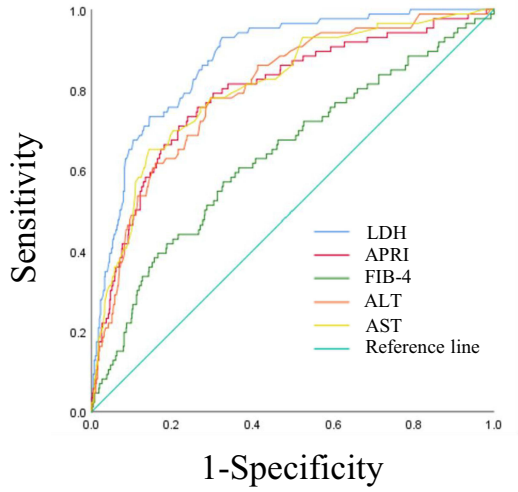

	AUC ROC	95%CI
LDH	0.874	0.839-0.909
APRI	0.794	0.740-0.847
FIB-4	0.638	0.572-0.703
ALT	0.799	0.751-0.847
AST	0.806	0.757-0.856

Figure 4 LDH level and other clinical risk factors for the identification of at-risk MASH patients in study cohort (n = 658). AUC of LDH, APRI, FIB-4, ALT and AST for identification at-risk MASH patient is 0.874, 0.797, 0.638, 0.799, 0.806 respectively. Delong's test P <0.05.

0.839–0.906) (Figure 3B), which was higher than other established panels including APRI (AUC value of 0.794, 95% CI 0.740–0.847), FIB-4 (AUC of 0.638, 95% CI 0.572–0.703), ALT (AUC of 0.799, 95% CI 0.751–0.847) and FIB-4 (AUC of 0.806, 95% CI 0.757–0.856) respectively (Figure 4). The LDH cutoff value derived from the point on the ROC curve with the largest Youden J index was 174.875 U/L, and the sensitivity of using this LDH threshold value to identify at-risk MASH was 81.1%, with a specificity of 73.1%. In conclusion, these findings suggest that LDH is a reliable and accurate predictor of histologically confirmed high-risk MASH.

Discussion

In the present study, several histological studies of liver tissue have observed an increase and positive association of LDH with NASH and advanced fibrosis.[11] LDH levels are associated with the presence and progression of NAFLD. The relationship between serum LDH levels and the severity of NAFLD in morbidly obese patients has not been studied. For example, a previous study demonstrated that serum LDH activity is of diagnostic value in predicting liver fibrosis in hepatocellular carcinoma.[27] Likewise, besides few relevant investigations, a cohort of covid-19 patients also showed a correlation between LDH and liver fibrosis.[9]

In this study, we assessed whether levels of the noninvasive serum marker LDH accurately predicted the risk of MASH in 855 patients diagnosed by liver biopsy. Of the 4 indicators tested, LDH, AST and APRI had the highest accuracy for the prediction of risk MASH, with FIB-4 having the lowest AUROC, thus we can draw a conclusion that LDH's performance is non-inferior to validated noninvasive fibrosis indices such as FIB4, ALT, AST and APRI in cohort. This demonstrates that LDH can be an effective and optimal biomarker for at-risk MASH patients. Patients with LDH levels above cut-off values (174.875 U/L) were more likely to be at- risk MASH. Additionally, LDH levels were found to be strongly correlated with a range of parameters associated with insulin resistance and abnormal glucose regulation, independent of age, gender and BMI, suggesting that LDH may be involved in the regulation of glucose metabolism and/or insulin sensitivity. In addition, a progressive increase in LDH with increasing MetS composition was observed, suggesting that LDH dysregulation may contribute to the development of metabolic complications associated with obesity. LDH is a metabolic enzyme involved in anaerobic glycolysis in the human body. When hepatocytes are damaged, LDH is released in the cytoplasm, and the activity of LDH in peripheral blood is significantly increased, which is a sensitive indicator reflecting the degree of hepatocyte damage. In the present study, we provide clinical evidence that LDH is closely associated with metabolic disturbances and MAFLD. As the composition of MS is closely related to the development of liver fibrosis. We hypothesize that the LDH may be related to the severity of the metabolic syndrome.

Abnormalities in glucose and lipid metabolism are important features of MetS. In this cross-sectional observational study, we found a significant association between serum LDH levels and the occurrence and severity of MetS and its various components. In diabetic patients, circulating LDH levels were significantly elevated and correlated significantly with fasting glucose and HbA1c. This suggests that LDH may serve as a new biomarker and clinical predictor of MetS.

Studies have shown that a persistent state of chronic systemic inflammation is an important pathophysiological feature of MetS. LDH is an important serum biomarker that has been shown to be associated with acute and neoplastic conditions.[28] We found higher levels of LDH in centrally obese patients. Previous studies have shown that MAFLD is diagnosed and evaluated by a set of positive diagnostic criteria to establish a definitive diagnosis of the individual disease,[6] and MetS and MASH share similar pathophysiologic processes,[29] so it is reasonable to assume that LDH levels, a new biomarker of MASH, may also be a biomarker of MetS. In the present study, serum LDH levels were elevated with increasing metabolic abnormalities. Logistic regression analysis showed that high LDH levels were an independent risk factor for the development of MetS. Thus, our findings provide evidence that serum LDH may be a potential biomarker for MetS. To the best of our knowledge, this study provides the first evidence of a reported correlation between serum LDH levels and the presence and severity of MetS in an obese Chinese population.

Macrophages derived from monocytes in adipose tissue secrete a variety of pro-inflammatory factors, such as tumor necrosis factor-α, interleukin-6, and interleukin-1. Thus, obesity itself is an inflammatory state, which may be reflected or influenced by LDH. In addition, diabetes, hypertension and hyperlipidemia are chronic inflammatory processes involving multiple organs.[30,31] LDH itself may be involved in the pathophysiological processes of various components of the MetS by influencing the inflammatory and immune responses, thus participating in the development and progression of the MetS and reflecting its severity.

It would be interesting to understand whether LDH activity correlates with soluble CD163 (sCD163), a biomarker of liver fibrosis in a variety of etiologies, as well as macrophage activity, and to reflect the spectrum of the disease in the wider community. In our current study cohort, serial histologic characterization of MAFLD revealed significantly higher serum LDH in normal livers, simple steatosis, and junctional MASH in the MASH group compared with the non-MASH group. In addition, serum LDH was strongly correlated with the severity of each histopathologic feature of MAFLD (steatosis, inflammation, and fibrosis scores), independent of gender, age, and body mass index. In conclusion, these findings support the use of serum LDH as an alternative marker to monitor the severity and progression of MAFLD in obese patients. In contrast, LDH, as a single biomarker, had an AUC of 0.874 (95% CI 0.839–0.906) in identifying patients with high-risk MASH (defined as NAS $\geq$ 4 and fibrosis grade $\geq$ 2 in patients with metabolic risk factors). The results show a correlation between LDH and many variables, which is valuable, but correlation does not imply causation. We will combine LDH with other markers for greater accuracy in the further study. Independent validation studies with large sample sizes in different cohorts are needed to further assess the accuracy of LDH in the noninvasive diagnosis of high-risk MASH and advanced fibrosis.

Study Limitation

The limitation of this study is that the participants recruited were predominantly obese and had a high prevalence of MetS due to the associated metabolic syndrome. Therefore, we need further confirmation in the normal population. Furthermore, as the data were from a cross-sectional population, the study did not explain the causal relationship between LDH and MetS and MAFLD. And we acknowledge confounding factors of other relevant molecules; therefore, further large population-based prospective studies are necessary to confirm the independent predictive and protective effects of the LDH on the development of MetS and MAFLD.

In conclusion, our current study provides clinical evidence that serum levels of LDH were increased in subjects with metabolic syndrome and MAFLD. Elevated serum LDH levels may also be a marker of ongoing inflammation in MetS patients. Further large-scale prospective studies are needed to elucidate and confirm the mechanistic and prognostic role of serum LDH in MetS.

Conclusion

This study is the first clinical study to confirm that serum LDH levels are significantly elevated in MetS patients and that serum LDH is an independent predictor of the presence of MetS in our study population. Based on our findings, serum LDH promises as a potential biomarker for MetS, but further studies are needed to establish its clinical utility.

Abbreviations

MAFLD, metabolism-associated fatty liver disease; NAFLD, nonalcoholic fatty liver disease; LDH, lactate dehydrogenase; T2DM, type 2 diabetes mellitus; MetS, metabolic dysfunction; TG, triglycerides; WC, waist circumference; HDL, high-density lipoprotein; B.MASH, Borderline MASH; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TC, total cholesterol; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; y-GT, r-glutamyl transpeptidase); FIB-4, Fibrosis-4; APRI, aspartate transaminase–platelet ratio index; AUROC, area under the curve of receiver operating characteristic curves.

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Disclosure

The authors declare no conflicts of interest in this article.

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