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Serum isthmin-1 is a potential biomarker for metabolic dysfunction associated fatty liver disease in patients with metabolic syndrome and type 2 diabetes mellitus

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ABSTRACT

Introduction Metabolic dysfunction associated fatty liver disease (MAFLD) is a prevalent condition in patients with type 2 diabetes mellitus (T2DM). Isthmin-1 (ISM1) is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis. Although ISM1 has been shown to be associated with T2DM, its role in patients with MAFLD and metabolic syndrome (MetS) remains insufficiently examined. This study aimed to investigate the relationship between serum ISM1 and MAFLD in patients with T2DM and the potential involvement of MetS in this association.

Research design and methods A total of 250 participants were divided into four groups: 60 patients with T2DM and MAFLD, 60 with newly diagnosed T2DM, 60 with MAFLD, and 70 healthy controls. Serum ISM1 levels were measured using ELISA. The distribution of ISM1 concentration in the combined data was divided into quartiles, and the Cochran-Armitage trend test was performed to estimate the significant trends across increasing quartiles.

Results Compared with the controls, patients with coexisting MAFLD, MetS, and T2DM exhibited significantly elevated serum ISM1 concentrations. Serum ISM1 levels in the overweight/obese group were also higher than those in the lean group. Serum ISM1 levels were positively correlated with body mass index (BMI), uric acid, alanine aminotransferase, aspartate aminotransferase, total cholesterol (TC), low-density lipoprotein cholesterol, fasting insulin, and homeostasis model assessment of insulin resistance and negatively associated with age and high-density lipoprotein cholesterol (HDL-C). BMI, TC, and HDL-C were independently associated with serum ISM1 concentration. The relative risks for MAFLD, T2DM, and T2DM with MAFLD increased significantly with higher ISM1 quartiles. Furthermore, a positive correlation was observed between serum ISM1 levels and the number of MetS components, with the elevated plasma levels of ISM1 escalating the risk of developing MetS to some extent.

Conclusions The combination of ISM1 with TG and UA was identified as the best predictive factor for diagnosing MAFLD and MetS, potentially due to their contribution to aggravating the metabolic state.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Isthmin-1 (ISM1) is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis.
⇒ Type 2 diabetes mellitus (T2DM), metabolic dysfunction associated fatty liver disease (MAFLD), and metabolic syndrome (MetS) are inextricably linked and adipokines play an important role.

WHAT THIS STUDY ADDS

⇒ The relative risks for T2DM with MAFLD increased significantly with higher ISM1 quartiles.
⇒ The combination of ISM1 with triglycerides and uric acid was identified as the best predictive factor for diagnosing MAFLD and MetS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ ISM1 is a promising biomarker because high serum ISM1 levels exacerbate metabolic disorders.
⇒ The relationship between ISM1 and glycolipid metabolism requires further cohort studies to determine whether it is a viable predictor of T2DM with MAFLD.

INTRODUCTION

Metabolic dysfunction associated fatty liver disease (MAFLD) is one of the leading causes of chronic liver disease worldwide, encompassing conditions such as simple steatosis, metabolic-associated steatohepatitis (MASH), fibrosis, and ultimately cirrhosis.[1] A large meta-analysis of MAFLD diagnosed via imaging methods estimated its global prevalence at approximately 25.24%.[2] MAFLD is a metabolic liver disease closely associated with insulin resistance (IR), with approximately one-third to two-thirds of patients with type 2 diabetes mellitus (T2DM) experiencing MAFLD.[3 4] T2DM and MAFLD are suggested to be interconnected via IR and act as mutual risk factors. Furthermore, the coexistence of these two diseases increases the risk of T2DM-associated complications and accelerates the progression of MAFLD to MASH, cirrhosis, and even liver cancer.[5 6]

Metabolic syndrome (MetS), including T2DM, is frequently associated with an advanced stage of MAFLD, particularly MASH. MetS is characterized by a cluster of metabolic-related disease risk factors such as central obesity, hyperglycemia, hypertension, and dyslipidemia, which promote the emergence and development of MAFLD and T2DM.[7–9] The prevalence of MetS ranges from 10% to 84% and depends on the geographic region, rural/urban surroundings, demographic characteristics of the population, and diagnostic criteria for MetS.[10] MetS has become one of the prominent public health problems owing to the continuously growing number of individuals with obesity.[11] Therefore, exploring new factors for treating MAFLD associated with MetS and T2DM is essential. Obesity, T2DM, and MAFLD are all phenotypes of metabolic syndrome and are associated with low-grade chronic inflammation.[11 12] Adipokines secreted by adipose tissue can trigger a systemic inflammatory response, leading to metabolic disorders.[13] Isthmin-1 (ISM1) has recently come to light as a novel adipokine that is associated with adipose tissue inflammation. Moreover, ISM1 has a dual role in regulating glucose and lipid metabolism.[14] ISM1 was discovered and named by Pera *et al* as a protein secreted at the isthmus of *Xenopus* embryos, where it participates in early embryonic development.[15] Subsequent research has revealed that ISM1 is widely distributed in various tissues of different vertebrates, including the brain, lung, vasculature, skin, and immune cells of birds, fish, amphibians, and mammals.[15 16] ISM1 is also crucially involved in embryonic development, angiogenesis, aging, and tumor immunity. A recent study by Jiang *et al* employing unbiased transcriptomic and secretomic screening approaches identified ISM1 as a protein produced by mouse adipocytes and demonstrated its role in promoting glucose uptake, inhibiting adipogenesis, and stimulating protein synthesis.[17] Moreover, studies in animals and humans have highlighted a significant elevation in the plasma levels of ISM1 and fat content in patients and mice with obesity.[17 18] The human studies also showed that the plasma concentrations of ISM1 were significantly increased in patients with T2DM and independently correlated with proteinuria severity but not with IR or peripheral neuropathy associated with diabetes.[18 19] Based on the present rationale and evidence, ISM1 may be used in developing a novel therapeutic strategy to prevent obesity-associated complications such as MAFLD and T2DM.

Although ISM1 has been known for over two decades, clinical research in populations with MAFLD and T2DM is relatively scarce and authoritative evidence supporting its role is lacking. Therefore, further studies examining the role of this adipokine in diabetes and MAFLD are warranted. This study aimed to investigate the relationship between serum ISM1 levels and MAFLD in patients with T2DM and determine the clinical relevance of ISM1 levels in different populations with combinations of MAFLD, T2DM, and/or MetS. Additionally, the potential role of MetS in this association was assessed to further explore the applicability of ISM1 as a therapeutic target.

METHODS

Study population

A total of 250 individuals (stratified into 70 healthy controls, 60 patients with T2DM, 60 with MAFLD, and 60 with MAFLD+T2 DM) who were admitted to the Second Affiliated Hospital of Chongqing Medical University in Chongqing, China from January 2023 to March 2024 were enrolled. All patients were newly diagnosed. Each participant underwent an abdominal ultrasonographic examination. The diagnostic criteria for T2DM were based on the 1999 WHO diagnostic criteria for diabetes mellitus.[20] All enrolled participants were aged 18–70 years. The participant exclusion criteria were as follows: (1) type 1 diabetes, gestational diabetes, or other diabetes types; (2) concurrent viral hepatitis, alcoholic liver disease, drug-induced liver disease, or autoimmune liver disease; or (3) renal insufficiency, thyroid dysfunction, infectious diseases, severe cardiovascular diseases, severe cerebrovascular diseases, malignant tumors, or mental disorders. The participants were divided into two groups to determine whether serum ISM1 expression differed in individuals with MetS: a MetS and a non-MetS group. Based on their body mass index (BMI, kg/m^2) and level of high-density lipoprotein cholesterol (HDL-C, mmol/L), the participants were further classified as normal weight <25 kg/m^2 or overweight/obese ≥25 kg/m^2 and as having low HDL-C <1.29 mmol/L or normal/high HDL-C ≥1.29 mmol/L. The participants were also divided into subgroups according to their sex.

MetS was defined according to the criteria established by the 2009 Joint Interim Statement criteria.[21] Accordingly, the participants were diagnosed with MetS if they met at least three of the following five criteria: (1) abdominal obesity defined as waist circumferences ≥80 cm and ≥90 cm for Asian women and Asian men, respectively, (2) elevated triglycerides (TG, ≥150 mg/dL) or drug treatment for elevated TG, (3) reduced HDL-C (<50 mg/dL and <40 mg/dL for Asian women and Asian men, respectively) or drug treatment for decreased HDL-C, (4) high blood pressure (BP) defined as systolic (SBP, ≥130 mm Hg) and/or diastolic (DBP, ≥85 mm Hg) hypertension or antihypertensive drug treatment, (5) heightened fasting glucose (≥100 mg/dL) or drug treatment for increased glucose.

Clinical data collection

Anthropometric parameters, clinical data, and medical history of the participants were collected from their electronic medical records at the Second Affiliated Hospital of Chongqing Medical University. Collected data included sex, age, height, weight, waist and hip circumferences, BP, hypertension, alcohol consumption history, and hemoglobin A1c (HbA1c), serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase

Table 1 Characteristics of controls, patients with MAFLD, patients with T2DM and patients with T2DM who have MAFLD

	Controls	MAFLD	T2DM	T2DM with MAFLD
N	70	60	60	60
Sex (male/female)	36/34	35/25	31/29	33/27
Age (year)	46 (33.75, 53.75)	34 (26, 47)	57 (49, 63)* †	55 (43, 62.25)* †
BMI (kg/m^2)	21.98±2.65	27.58±3.96*	23.67±2.57* †	24.33±2.37* †
SBP (mm Hg)	116.35±15.16	126.68±14.74*	127.72±13.84*	130.4±13.1*
DBP (mm Hg)	71.5 (65, 80)	72 (68.5, 80)	78(70, 87)* †	81.5 (74.5, 92)* †
UA (umol/L)	291.63±56.37	393.68±108.41*	300.47±88.88†	348.52±95.45* † ‡
ALT (U/L)	18 (13.5, 26.25)	28 (18, 44)*	23 (18, 39)*	27 (20, 40)*
AST (U/L)	21 (17, 24.25)	23 (18.5, 30.5)	23 (18, 30)	23 (18.75, 29.25)
TG (mmol/L)	1.01 (0.87,1.27)	1.71 (1.19, 2.87)*	1.42 (1.18, 1.9)*	2.05 (1.54, 3.43)* ‡
TC (mmol/L)	4.49±0.7	4.99±1.17*	5.06±1.18*	5.34±1.18*
HDL (mmol/L)	1.39 (1.25, 1.68)	1.17 (1.03, 1.44)*	1.26 (1.13, 1.43)*	1.27 (1.06, 1.49)*
LDL (mmol/L)	2.26±0.78	2.93±0.92*	3.05±0.93*	3.09±0.85*
WBCs (×10^9/L)	5.48 (4.56, 6.73)	6.96 (5.92, 7.7)*	6.09 (5.33, 7.48)*	7.1 (6.06, 8.16)* ‡
HbA1c (%)	5.2 (5, 5.5)	5.4 (5.15, 5.7)	8.3 (6.9, 11.2)* †	8.35 (7.28, 9.83)* †
FBG (mmol/L)	4.82 (4.58, 5.2)	5.17 (4.79, 5.55)	8.39 (6.8, 10.38)* †	7.92 (6.99, 8.95)* †
FINS (mU/L)	7.57 (6.06, 9.9)	15.55 (9.66, 25.12)*	9.59 (6.98, 15.14)* †	11.59 (6.85, 17.04)* †
HOMA-IR	1.66 (1.32, 2.15)	3.51 (2.11, 5.79)*	3.82 (2.22, 6.02)*	4 (2.58, 5.81)*

Data are shown as the mean±SD or median (IQR).
*P<0.05 vs controls.
†P<0.05 vs MAFLD.
‡P<0.05 vs T2DM.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; FINS, fasting insulin; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MAFLD, metabolic dysfunction associated fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; T2DM, type 2 diabetes mellitus; TG, triglycerides; UA, uric acid; WBCs, white blood cells.

(AST), HDL-C, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), TG, and uric acid (UA) levels. BMI (kg/m^2) was calculated as weight (kg) divided by height squared (m^2). All participants underwent a 75 g oral glucose tolerance text. In this test, the participants underwent a minimum 10 hours fast, followed by the measurement of serum glucose and insulin concentrations at 0, 30, 60, and 120 min. At the start of the experiment, fasting serum samples were collected. One portion of the sample was used for glucose and insulin measurements, while the other portion was stored at −80°C for analyzing ISM1 levels. The homeostatic model assessment of IR (HOMA-IR) value was calculated using the following formula: HOMA-IR=fasting blood glucose (FBG, mmol/L)×fasting insulin (FINS, mU/L)/22.5.

Measurement of serum ISM1 level

Serum ISM1 levels were measured using the human ISM1 ELISA kit (MBS2707255; My BioSource, Southern California, San Diego, USA). The detection range of the kit was 0.03–2 ng/mL. Moreover, the detection limit was 0.031 ng/mL, and the intra-assay and inter-assay coefficients of variation were <15%.

Statistical analysis

All data analyses were performed using SPSS statistical software (V.26.0, SPSS, Chicago, Illinois, USA) and GraphPad V.10.0. The normality of the data distribution was evaluated with the Kolmogorov-Smirnov test. Continuous variables were expressed as mean±SD or median (IQR), while categorical variables were presented as percentages. For normally distributed variables, the analysis of variance method followed by the least significant difference test was employed for between-group comparisons. Skewed variables were analyzed using the Kruskal-Wallis test, and the Mann-Whitney U test was used for comparisons between groups. The Spearman's correlation analysis was applied to determine the relationship between serum ISM1 levels and other variables. The distribution of the ISM1 levels in the pooled data was further divided into quartiles, and the Cochran-Armitage trend test was performed to estimate the significant trends across the increasing quartiles. Lastly, binary logistic regression and multiple linear regression analyses were conducted to assess the relationships between serum ISM1 levels and other variables in various models.

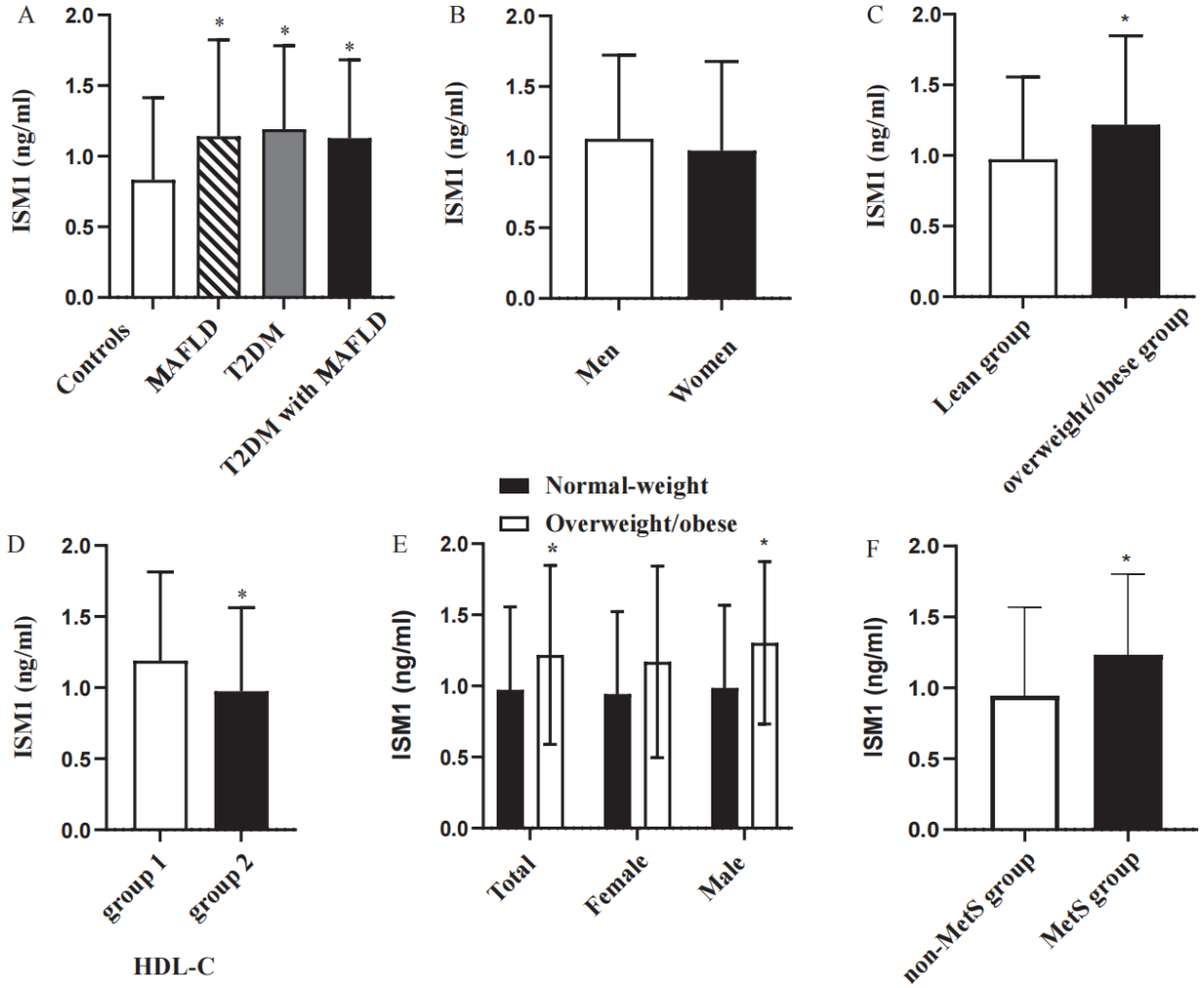

Figure 1 (A) Comparison of serum ISM1 levels among the control, MAFLD, T2DM, and MAFLD with T2DM groups; compared with control, *p<0.05. (B) Comparison of serum ISM1 levels between males and females in the four groups; compared with females, *p<0.05. (C) Comparison of serum ISM1 levels between the lean and overweight/obese groups, compared with lean group, *p<0.05. (D) Comparison of serum ISM1 levels between the two groups between low HDL-C levels (group 1) and high HDL-C levels (group 2), compared with low HDL-C group, *p<0.05. (E) Comparison between normal weight and overweight/obese groups (based on sex), compared with lean group, *p<0.05. (F) Comparison of serum ISM1 levels between the MetS group and non-MetS group, compared with NO-MetS group, *p<0.05. HDL-C, high-density lipoprotein cholesterol; ISM1, isthmin-1; MAFLD, metabolic dysfunction associated fatty liver disease; MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus.

A p value of <0.05 was considered statistically significant in all statistical tests.

RESULTS

Basic characteristics of the study participants

Baseline data of the anthropometric measurements and metabolic characteristics of the T2DM, MAFLD, MAFLD+T2DM, and healthy control groups are presented in table 1. The four groups did not exhibit significant differences in sex distribution. Compared with the controls, patients in the MAFLD group had significantly higher BMI, SBP, serum UA, ALT, TG, TC, LDL-C, white blood cells (WBCs), FINS, and HOMA-IR but significantly lower HDL-C (all p<0.05). Patients in the T2DM group had significantly higher age, BMI, SBP, DBP, ALT, TG, TC, LDL-C, WBCs, HbA1c, FBG, FINS, and HOMA-IR but significantly lower HDL-C (all p<0.05) than those in the control group. Furthermore, patients with MAFLD and T2DM demonstrated significantly higher age, BMI, SBP, DBP, serum UA, ALT, TG, TC, LDL-C, WBCs, HbA1c, FBG, FINS, and HOMA-IR but significantly lower HDL-C (all p<0.05) than the controls. Age, DBP, HbA1c, and FBG were significantly higher while BMI, UA, and FINS were significantly lower in patients with T2DM compared with those with MAFLD (all p<0.05). Compared with the MAFLD group, the MAFLD+T2DM group had significantly higher age, DBP, HbA1c, and FBG (all p<0.05) but significantly lower BMI, serum UA, and FINS (all p<0.05). Finally, TG and WBCs were significantly higher, whereas serum UA was significantly lower in patients with MAFLD and T2DM than in those with T2DM but without MAFLD (all p<0.05).

Table 2 Distribution of MetS components according to ISM1 quartile

ISM1	MetS components quartile	0	1	2	3	4	5
	Q1	59.3	33.3	22.2	16.2	17.9	0
	Q2	11.1	27.8	30.6	29.7	21.4	10
	Q3	22.2	16.7	27.8	37.8	10.7	30
	Q4	7.4	22.2	19.4	16.2	50	60
	P trend test	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Data are shown as numbers and percentages of the total number in every group according to ISM1 quartile.
ISM1, isthmin-1; MetS, metabolic syndrome.

Comparison of serum ISM1 levels between different participant groups

Patients with MAFLD (1.14±0.68 ng/mL), those with MAFLD+T2DM (1.13±0.56 ng/mL), and those with T2DM but without MAFLD (1.19±0.59 ng/mL) showed significantly higher concentrations of serum ISM1 than the healthy controls (0.83±0.58 ng/mL) (all p<0.05; figure 1A). However, no significant differences in serum ISM1 levels were found among the three participant groups with MAFLD, T2DM, and MAFLD+T2 DM (p=0.059). Lastly, no significant differences in the proportions of male and female participants were observed among the four groups (figure 1B).

Moreover, dividing the participants according to various categories revealed that serum ISM1 levels were higher in the overweight/obese group than in the lean group (1.27±0.62 ng/mL vs 0.97±0.59 ng/mL; p<0.05) (figure 1C). Serum ISM1 concentrations were higher in the low HDL-C group than in the high HDL-C group (1.18±0.626 ng/mL vs 0.96±0.58 ng/mL; p<0.05) (figure 1D). As shown in figure 1E, higher serum ISM1 levels were demonstrated in males classified as overweight/obese than in those defined as normal weight (p<0.05).

Considering the elevated levels of ISM1 in individuals with metabolic disorders and its dual role in glucose and lipid metabolism, we hypothesized a close relationship between ISM1 and MetS. We tested this hypothesis by stratifying the participants into two groups based on the presence or absence of MetS: a non-MetS group (n=130) and a MetS group (n=119). Subsequently, the relationship between MetS and ISM1 was investigated by conducting the Mann-Whitney U test. The results indicated that serum ISM1 levels in the MetS group were significantly higher than those in the non-MetS group (1.23±0.57 ng/mL vs 0.94±0.63 ng/mL; p<0.05) (figure 1F).

Number of positive components of MetS increases with rising serum ISM1 levels

Stratified analysis according to the quartiles of serum ISM1 levels revealed that the number of positive components of MetS increased significantly with increasing ISM1 levels (p<0.05; table 2). In the healthy control group (positive MetS components=0), most individuals were in the first quartile (59.3%), while only a small proportion were in the third quartile (7.4%). Conversely, most patients with MetS (positive MetS components≥3) were in the third and fourth quartiles. Specifically, a large percentage of the patients with five MetS components were in the fourth quartile (60%), followed by 30% in the third quartile, 10% in the second quartile, and none in the first quartile.

Correlations between serum ISM1 levels and other anthropometric and biochemical parameters in the study participants

As depicted in table 3, fasting serum ISM1 levels were positively correlated with BMI, UA, ALT, AST, TC, LDL-C, FINS, and HOMA-IR and negatively correlated with age and HDL-C (all p<0.05). Multiple stepwise regression analyses were subsequently performed to identify the variables independently associated with serum ISM1 level (table 3). The regression results indicated that BMI, TC, and HDL-C were independently correlated with serum ISM1 levels. The regression model for ISM1 was defined as ISM1=0.124+(0.030×BMI)+(0.003×ALT)+(0.118×TC)−(0.345×HDL-C).

Next, we investigated whether the prevalence of MAFLD, T2DM, or T2DM with MAFLD increased with rising serum ISM1 levels by dividing the total ISM1 levels into four quartiles. In the healthy control group, most individuals were in the first quartile (44.1%), followed by the third quartile (23.5%) and the fewest in the fourth quartile (11.8%). In the case of the MAFLD group, most patients were in the third and fourth quartiles. However, most patients in the T2DM group were in the second quartile (33.3%). The Cochran-Armitage trend test revealed that the relative risks for MAFLD, T2DM, and T2DM with MAFLD were significantly heightened with escalating ISM1 concentrations (p<0.05; table 4).

Receiver operating characteristic analysis for predicting T2DM, MAFLD, and MetS

The relationship between ISM1 level and T2DM, MAFLD, and MetS was elucidated by performing receiver operating characteristic (ROC) analysis to

Table 3 Spearman's correlation r and linear regression analysis of variables associated with circulating ISM1 levels in the studied subjects

	Spearman's correlation		Multiple		
Variable	r	P value	B	P value	95% CI
Age	−0.079	0.327	—	—	—
BMI	0.265	<0.001	0.030	0.036	(0.002 , 0.058)
SBP	0.032	0.69	—	—	—
DBP	0.013	0.874	—	—	—
UA	0.215	0.007	—	—	—
ALT	0.208	0.009	0.003	0.075	(−0.000 , 0.006)
AST	0.165	0.04	—	—	—
TG	0.115	0.151	—	—	—
TC	0.163	0.043	0.118	0.008	(0.032 , 0.204)
HDL	−0.172	0.032	−0.345	0.03	(−0.653 , −0.037)
LDL	0.183	0.022	—	—	—
WBCs	0.134	0.096	—	—	—
HBA1c	0.09	0.265	—	—	—
FBG	0.123	0.125	—	—	—
FINS	0.18	0.024	—	—	—
HOMA-IR	0.191	0.018	—	—	—

In stepwise regression analysis, the values included for analysis were age, BMI, SBP, DBP, albumin, ALT, AST, HbA1c, UA, TG, TC, HDL-C, WBC, FINS, HOMA-IR.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; Fins, fasting insulin; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; UA, uric acid; WBCs, white blood cells.

determine the predictive value of serum ISM1 concentration for these metabolic disorders (table 5). The area under the curve (AUC) for predicting T2DM with MAFLD was 0.467 (p>0.05), thereby indicating that this association was not statistically significant despite high specificity. Additionally, the AUC for predicting MAFLD was 0.642 (p<0.05). Furthermore, combining the TG, UA, and ISM1 levels improved the AUC from 0.642 to 0.930, with sensitivity and specificity at >80% (p<0.05). Finally, the AUC for predicting MetS was 0.650 (p=0.001,), while combining the TG, UA, and ISM1 levels enhanced the AUC from 0.650 to 0.832 (p<0.05).

Table 4 Distribution of patients according to ISM1 quartile

ISM1 quartile	Normal	MAFLD	T2DM	T2DM with MAFLD
Q1	44.1	26.8	12.8	19
Q2	20.6	17.1	33.3	28.6
Q3	23.5	24.4	28.2	23.8
Q4	11.8	31.7	25.6	28.6
P trend test	<0.05	<0.05	<0.05	<0.05

Data are shown as numbers and percentages of the total number in every group according to ISM1 quartile.
ISM1, isthmin-1; MAFLD, metabolic dysfunction associated fatty liver disease; T2DM, type 2 diabetes mellitus.

DISCUSSION

In this study, we compared the serum ISM1 levels between patients with MAFLD, T2DM, and combined MAFLD with T2DM and healthy controls, determined the correlations between ISM1 levels and other metabolic indices, and explored the potential role of MetS in these associations. The main study findings were as follows: (1) serum ISM1 concentrations are elevated in patients with MAFLD, whereas comparatively higher levels are observed in those with T2DM; (2) serum ISM1 concentration was correlated with certain metabolic indices; (3) the relative risks for T2DM with MAFLD increased significantly with higher ISM1 quartiles; (4) the combination of ISM1, TG, and UA levels is an optimal predictor for diagnosing MAFLD; (5) heightened concentrations of ISM1 may be related to MetS progress, as evidenced by the increase in serum ISM1 levels with the rising number of positive components of MetS in this study cohort.

Adipose tissues secrete adiponectin, leptin, and some proinflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α, which would influence the liver.[1322] ISM1 is an adipose-secreted polypeptide hormone, and one that has a dual role in increasing adipose glucose

Table 5 ROC curve analysis of ISM1 for the diagnosis of different diseases

Diseases	Cut-off point	AUC	Sensitivity	Specificity	95% CI
MetS	0.895	0.650	0.720	0.568	(0.563, 0.736)
MetS	ISM1+UA+TG	0.832	0.653	0.914	(0.767, 0.896)
MAFLD	0.970	0.642	0.610	0.667	(0.517, 0.768)
MAFLD	ISM1+UA+TG	0.930	0.829	0.912	(0.872, 0.987)

ISM1, isthmin-1; MAFLD, metabolic dysfunction associated fatty liver disease; MetS, metabolic syndrome; TG, triglycerides; UA, uric acid.

uptake while suppressing hepatic lipid synthesis.[17] In a recent study by Jiang *et al*, ISM1 was shown to significantly enhance insulin-dependent glucose uptake in several cell types, while ISM1 knockdown cells exhibited diminished glucose uptake and decreased phosphorylation of protein kinase AKT at serine residue 473 ($PAKT^{S473}$) as indicated by a 20%–50% reduction in signaling.[17] The PI3K-AKT pathway involves the insulin-mediated activation of $pAKT^{S473}$ and $pAKT^{T308}$, which affect glucose and lipid metabolism. Jiang *et al* also reported that ISM1 levels contribute to basal glucose uptake in adipocytes and maintain the basal AKT signaling tone, indicating that ISM1 shares a common AKT pathway with insulin.[17] Additionally, high concentrations of ISM1 were demonstrated to be more effective than insulin in prolonging and promoting ERK1/2 phosphorylation, suggesting that ISM1 induces signaling responses and downstream pathways distinct from those produced by insulin. Thus, ISM1 may function via a novel tyrosine kinase receptor.[17]

To our knowledge, this study is the first clinical report comparing serum ISM1 levels between patients with MAFLD and a healthy population (normal BMI). Moreover, all participants were new to the study and had no previous related diagnosis or treatment. Our study revealed that serum ISM1 levels were significantly higher in newly diagnosed patients (MAFLD, T2DM, and MAFLD+T2DM groups) than in controls. Most previous studies have found that elevated serum ISM1 levels in patients with T2DM are related to diabetic complications and nephropathy but not to diabetic peripheral neuropathy.[18 19 23] Our study finding contradicts the results reported by Wang *et al*, where significantly lower serum levels of ISM1 were detected in a newly diagnosed T2DM group, while no significant differences were observed in ISM1 levels between T2DM+MAFLD and T2DM groups.[24] The researchers suggested that ISM1 may be a protective factor for diabetes but does not lower the risk for diabetes-related MAFLD. However, the Cochran-Armitage trend analysis in the present study indicated that the relative risks for MAFLD, T2DM, and T2DM with MAFLD increased significantly with escalating ISM1 levels. Furthermore, in the study by Wang *et al*, the control group had a mean BMI of 27.11±0.45 kg/m^2, indicating a classification of overweight/obese for this group; however, no statistical difference was found between the mean BMI of the control and T2DM groups.[24] In contrast, the BMI of the control group was within the normal range in our study. Current studies suggest that BMI has a distinct effect on ISM1 levels, and obesity is a common risk factor for numerous metabolic diseases.[14 17 25] Therefore, we postulate that BMI is the primary factor causing the inconsistent results. Additionally, we hypothesize that differences in the duration of T2DM, measurement methods, regional differences, and lifestyle variations may contribute to the discrepant conclusions.

Recent studies have demonstrated that ISM1 is predominantly enriched in human and mouse adipose tissues, with ISM1 levels exhibiting a 30-fold upregulation in the inguinal white adipose tissue of diet-induced obese (DIO) mice.[17] A study involving people with obesity found that ISM1 expression increased with the ratio of subcutaneous fat area to visceral fat area (VFA), but it was not related to the VFA. In the DIO mouse model, overexpression of ISM1 prevented IR and hepatic steatosis.[14 17] In additional animal studies, administering recombinant ISM1 to MAFLD mice reduced liver weight, highlighting that ISM1 has beneficial effects on glucose tolerance and inhibits hepatic lipid synthesis and accumulation.[17] Sterol regulatory element-binding protein-1c (Srebp-1c) is a known key element in de novo lipogenesis (DNL) and intracellular transport, and insulin has been shown to regulate DNL by activating the expression of the Srebp-1c target genes *Fas*, *ACC*, and *SCD1*.[26–28] Moreover, in primary mouse hepatocytes, Srebp-1c transcription was significantly inhibited by ISM1 overexpression in adenoviral vectors after 24 hours. This finding suggests that ISM1 can directly regulate the expression of these lipid metabolism-associated genes. In these experiments, the simultaneous action of insulin and ISM1 in mouse cells markedly inhibited the expression of the genes mentioned above, thereby suppressing insulin-dependent DNL.[17] Our study showed that ISM1 levels were significantly correlated with metabolic indicators such as BMI, UA, ALT, AST, TC, HDL-C, LDL-C, FINS, and HOMA-IR. Further multiple linear regression analysis in the current study indicated that serum ISM1 levels were independently correlated with BMI, TC, and HDL-C. In line with our findings, previous clinical studies have reported a strong correlation between ISM1 levels and BMI; however, they did not include individuals who were non-obese or healthy controls with normal BMI. Prior studies have also identified a correlation between serum TC and HDL levels and the progression of MAFLD and T2DM, with these two cholesterol measures serving

as common independent risk factors. All these basic research findings and our clinical results further support that ISM1 is involved in the pathogenesis of MAFLD and T2DM primarily through the regulation of lipid metabolism pathways.

MAFLD is a hepatic disorder and a multisystemic condition closely associated with MetS and T2DM, all of which together promote the occurrence of cardiovascular disease. MAFLD frequently presents as a hepatic manifestation that coexists with the constituent features of MetS. MetS is a complex disorder characterized by metabolic dysregulation, comprising conditions such as impaired glucose tolerance, IR, dyslipidemia, associated pro-inflammatory states, and arterial hypertension. In this study, serum ISM1 levels were significantly elevated in patients with MetS. Although high ISM1 levels may increase MetS risk to some extent, these results are susceptible to confounding factors that may affect their statistical significance. Our study also revealed a positive correlation between serum ISM1 level and the number of MetS components, underscoring the potential applicability of serum ISM1 concentration for monitoring MetS severity over time and assessing the impact of interventions on MetS. Another notable finding in our study was that TC might serve as an independent predictor of MAFLD. However, MAFLD is well known to present with the characteristic accumulation of intrahepatic lipids, primarily TG.[29] In addition, plasma UA levels are strongly associated with metabolic and inflammatory diseases such as hypertension, IR, obesity, and hypertriglyceridemia.[30 31] Hyperuricemia is independently associated with the severity of liver damage in patients with MAFLD.[32 33] Numerous studies have found that UA and UA-based markers can be used to diagnose metabolic and inflammatory diseases, including MAFLD and MetS.[34] Of these, UA-based markers are considered to have high diagnostic value, and a representative indicator is UA-to-HDL-C ratio.[32 35 36] Therefore, we combined ISM1 concentration with TG and UA levels and found that combining these three indicators offered better sensitivity and specificity for predicting MAFLD and MetS. In our study, ROC curve analysis established that ISM1 was a promising indicator for distinguishing between patients with metabolic disorders (such as T2DM, MAFLD, and MetS) and controls. However, cohort studies with larger sample sizes are needed to comprehensively explore the potential of ISM1 as a screening indicator for MAFLD in individuals with MetS and T2DM.

The present study has several limitations. The relatively small sample size of this study may have affected the results of the correlation analyses. Additionally, the cross-sectional design of this investigation did not allow us to infer causality from the results.

In conclusion, our study provides some new insights into the role of ISM1 in the pathophysiology of MAFLD, T2DM, and MetS. Based on our study findings and those of the existing literature, we hypothesize that elevated ISM1 levels in participants are a risk factor for MAFLD, MetS, and T2DM. Furthermore, this study suggests that the combination of ISM1 levels with other metabolic parameters may be more closely associated with lipid disorders and provides a better understanding of the pathogenesis of MAFLD and lipid-related diseases. Nevertheless, future detailed studies with larger sample sizes are required to delineate the role of ISM1 in metabolic disorders.

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