

Deregulated miR-487b-3p in Patients with Non-Alcoholic Fatty Liver Disease and Its Regulatory Effect on Insulin Resistance

Mingtao Chen¹, Jie Tan¹, Min Huang¹, Ting Zhan¹, Zheng Han¹, Meng Liu¹, Yuanyuan Lu¹, Xia Tian¹

Department of Gastroenterology, Wuhan Third Hospital, Tongren Hospital of Wuhan University, Wuhan, China

Cite this article as: Chen M, Tan J, Huang M, et al. Deregulated miR-487b-3p in patients with non-alcoholic fatty liver disease and its regulatory effect on insulin resistance. *Turk J Gastroenterol.* 2026;37(5):629-636.

ABSTRACT

Background/Aims: This study aims to elucidate both the diagnostic potential of miR-487b-3p for insulin resistance and its regulatory mechanisms in patients with nonalcoholic fatty liver disease (NAFLD).

Materials and Methods: The study group included 60 NAFLD patients and 60 healthy controls. The expression levels of miR-487b-3p and insulin receptor substrate 1 (IRS1) in serum and cells were quantified by RT-qPCR. The diagnostic utility of the microRNA was evaluated through receiver operating characteristic (ROC) analysis. Glucose uptake in HepG2 was measured using fluorescence-based assays. The interaction between miR-487b-3p and IRS1 was confirmed by dual-luciferase reporter assay.

Results: Compared with controls, NAFLD patients exhibited significantly elevated serum miR-487b-3p levels ($P < .05$). The ROC analysis demonstrated high diagnostic accuracy of miR-487b-3p in distinguishing between healthy controls and NAFLD patients. Functional analyses demonstrated that miR-487b-3p overexpression suppressed both glucose uptake and IRS1 expression ($P < .05$), whereas miR-487b-3p inhibition enhanced glucose uptake and IRS1 expression ($P < .05$). Mechanistically, miR-487b-3p negatively regulated IRS1 to modulate glucose uptake.

Conclusion: miR-487b-3p may serve as a potential diagnostic biomarker for NAFLD, and miR-487b-3p negatively regulates IRS1 to modulate glucose uptake, thereby impairing insulin sensitivity in NAFLD patients.

Keywords: Insulin receptor substrate 1, miR-487b-3p, nonalcoholic fatty liver disease

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) constitutes a chronic hepatic disorder characterized by predominant triglyceride deposition within hepatocytes. This condition arises independently of steatogenic medications or hereditary disorders, progressing through a clinical continuum ranging from isolated steatosis to steatohepatitis with potential cirrhosis.¹ Clinical manifestations include but are not limited to fatigue, anxiety, polydipsia, and abdominal distension.² Current estimates indicate a global NAFLD prevalence of 25-30%, affecting over 1.8 billion people worldwide.³ Screening modalities encompass serum ALT, hepatic ultrasonography, liver biopsy, and MRI, however, all have limitations in specificity, sensitivity, and cost-effectiveness.⁴

NAFLD arises from synergistic genetic and environmental influences,⁵ with emerging evidence implicating epigenetic mechanisms in its pathogenesis. Recent studies

highlight ethnicity-specific susceptibility, as demonstrated by Bonacini et al, reporting increased NAFLD risk among Hispanic populations.⁶ MicroRNAs (miRNAs), short non-coding RNAs of 19-28 nucleotides, epigenetically regulate gene expression central to NAFLD development.⁷ Substantial research confirmed that miRNAs modulate peripheral insulin action primarily through targeting key insulin signaling proteins, contributing to insulin resistance. Specific miRNAs exhibit diagnostic and mechanistic significance: circulating miR-122⁸ and miR-192 differentiate NAFLD patients from healthy controls, while miR-193a-5p modulates hepatic oxidative stress responses.⁹ MiR-125b-5p ameliorates hepatic steatosis by downregulating lipid metabolism factors via ESRRA suppression,¹⁰ and miR-690 improves insulin resistance by inhibiting NADK, thereby activating JAK/STAT and insulin signaling pathways to enhance AKT phosphorylation.¹¹ Although primarily recognized as an oncogenic miRNA in gliomas,¹² miR-487b-3p also influences skeletal

*The first two authors contributed equally.

Corresponding author: Xia Tian, e-mail: seganzhanh848@163.com

Received: October 16, 2025 Revision Requested: November 11, 2025 Last Revision Received: December 14, 2025 Accepted: January 6, 2026 Publication Date: March 16, 2026

DOI: 10.5152/tjg.2026.25649



myogenesis and myocyte differentiation. Insulin modulates glucose homeostasis through its effects on skeletal muscle, hepatic tissue, and white adipose depots, where insulin receptor substrate 1 (IRS1) mediates key signaling cascades.¹³

Quantitative PCR analysis revealed significant differential expression of miR-487b-3p between healthy controls and NAFLD patients. In this investigation, the mechanistic role of miR-487b-3p in the pathogenesis of insulin resistance was elucidated. The findings demonstrated that miR-487b-3p directly targets the 3'-untranslated region (3'-UTR) of IRS1 mRNA, consequently modulating cellular glucose uptake capacity. These results provide novel molecular insights into microRNA-mediated regulation of insulin resistance.

MATERIALS AND METHODS

Patients and Clinical Data

This study enrolled 60 patients with confirmed NAFLD (32 males and 28 females, aged 30-54 years) from Wuhan Third Hospital, Tongren Hospital of Wuhan University Hospital, between October 2024 and April 2025, with the sample size determined through a priori power analysis using G*Power 3.1 (Heinrich-Heine; Dusseldorf, Germany) to achieve 95% statistical power ($\alpha = 0.05$). The NAFLD was diagnosed based on abdominal ultrasound, which was defined as diffuse hyperechoic near-field liver parenchyma (brighter than the kidney and spleen regions), gradual attenuation of far-field echoes, and any of the following findings: unclear visualization of intraparenchymal structures; mild to moderate hepatomegaly with rounded borders; reduced blood flow signals but normal blood flow distribution; or incomplete or unclear visualization of the right hepatic lobe and diaphragm. Laboratory tests and liver biopsy (NAFLD Activity Score (NAS) ≥ 5 ; METAVIR score of fibrosis $\geq F2$) were also performed to confirm NAFLD diagnosis and eligibility for inclusion in the study. Consecutive sampling was employed to select participants. Exclusion criteria included: cirrhosis or portal

hypertension, diabetes mellitus, autoimmune hepatitis, hepatocellular carcinoma, the presence of hepatitis B or C viral markers, alcohol consumption, or ischemic heart disease.

Additionally, 60 age- and sex-matched apparently healthy blood donors (30 males, 30 females) were recruited as controls. Participants in the control group had no serological evidence of viral hepatitis, had laboratory parameters within normal reference ranges, and exhibited no pathological abnormalities on diagnostic abdominal ultrasonography. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Wuhan Third Hospital, Tongren Hospital of Wuhan University (Date: August 24, 2023; No.MS-2023-1014). In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Blood Sampling

Following an overnight fast (≥ 8 hours), venous blood was collected using BD Vacutainer Red Top plain tubes (Dickinson and Company, USA). Samples were clotted at room temperature to separate serum, immediately aliquoted into cryogenic vials (Axygen®, USA), and stored at -70°C in ultra-low temperature freezers (Thermo Scientific, USA) until analysis.

Serum glucose, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) were quantified by the Model 7600 Series AUTOMATIC ANALYZER (Hitachi, Japan). The insulin levels were determined by electrochemiluminescence immunoassay on a Roche e-601 Analyzer (Roche Diagnostics Products Co., Ltd, Germany).

Bioinformatics

Putative target genes of the miR-487b-3p were systematically identified through integrated analysis of the TargetScan (https://www.targetscan.org/vert_71/) and miRDB (<https://www.mirdb.org/miRDB>) datasets. The potential binding sites between miR-487b-3p and IRS1 were further predicted using TargetScan.

RT-qPCR Assay

Total RNA was isolated from serum using the miRNeasy Serum/Plasma Kit (Qiagen, Germany) and from cells using TRIzol reagent (Takara, Japan), strictly adhering to the manufacturers' protocols. The cDNA synthesis was

Main Points

- Serum miR-487b-3p was significantly elevated in nonalcoholic fatty liver disease (NAFLD) patients.
- miR487b-3p is a potential diagnostic marker for NAFLD.
- Overexpression of miR-487b-3p inhibited glucose uptake and insulin receptor substrate 1 (IRS1) expression, while its inhibition promoted these effects.
- miR-487b-3p negatively regulates IRS1 to modulate glucose uptake.

performed using the PrimeScript RT Reagent Kit (Takara Bio, Japan), followed by quantitative PCR analysis with TB Green Premix Ex Taq II (Takara Bio, Japan) on a real-time PCR system. miR-487b-3p was detected using the PrimeScript RT kit. Reverse transcription was performed using its specific stem-loop RT primers, followed by RT-qPCR quantification using SYBR Green II Master Mix. U6 serves as an endogenous standard control.

Receiver-Operating Characteristic Curve Analysis

To evaluate the diagnostic efficacy of miR-487b-3p for NAFLD, ROC curves were constructed based on miR-487b-3p expression levels using SPSS software.

Cell Culture and Transfection

HepG2 cells (Procell, China) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Procell, China) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Beyotime, China), under standardized conditions (37°C, 5% CO₂).

HepG2 cell monolayers were established in 6-well culture plates (4 × 10⁵ cells/well), followed by overnight culture to achieve 70%-80% confluency. Cells were transfected with Lipofectamine 3000 transfection reagent (Fisher Scientific, USA) following the supplier's technical specifications. DNA-lipid complexes were incubated with cells for 6 hours at 37°C/5% CO₂, after which the medium was replaced with fresh DMEM containing 10% FBS. Cells were maintained at 37°C/5% CO₂ for an additional 48 hours post transfection. Transfection efficiency and subsequent analyses were conducted at the 48-hour time point following transfection initiation.

Glucose Uptake

HepG2 cells were seeded in 96-well plates at a density of 1 × 10⁴ cells/well. After 24 hours of culture (reaching ~80% confluency), cells were serum-starved for 12 hours in DMEM without serum. They were then treated with 100 nmol/L insulin for 24 hours. Glucose uptake was quantified using an assay kit (Cayman Chemical, Ann Arbor, MI) following the manufacturer's protocol. Fluorescence was measured at 485 nm excitation/535 nm emission using a FlexStation 3 microplate reader (Molecular Devices, CA, USA).

Dual-Luciferase Reporter Assay

Wild-type (WT) and mutant (MUT) sequences of the IRS1 3'UTR containing miR-487b-3p binding sites were cloned downstream of the firefly luciferase open reading frame

to construct dual-luciferase reporter vectors. HepG2 cells were seeded in 24-well plates and co-transfected with the constructed vectors, along with either a miR negative control (miR NC), miR-487b-3p antagomir, or miR-487b-3p agomir, according to the manufacturer's instructions. After 48 hours of transfection, dual-luciferase reporter assays were performed using a commercial kit (TransGen, China).

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 (IBM SPSS Corp.; Armonk, NY, USA). Data analysis was conducted using ROC curve analysis, and the diagnostic threshold was quantified through sensitivity/specificity determination. Data are expressed as mean ± standard deviation (SD). Student's *t*-test or 1-way analysis of variance was used for comparisons between continuous variable groups, and the chi-square test was used for categorical data. Plotted using GraphPad Prism 10.1.2 (GraphPad Software; Boston, Massachusetts, USA).

RESULTS

Baseline Characteristics of the Participants

Table 1 presents the baseline demographic and clinical profiles of the enrolled cohort. Initial analysis revealed that NAFLD showed no significant association with age, marital status, gender, physical activity, smoking habits, or LDL-C levels (all *P* > .05). However, significant correlations were observed with body mass index (BMI), fasting insulin, fasting blood glucose (FBG), homeostasis model assessment of insulin resistance (HOMA-IR), TC, TG, and HDL-C levels (all *P* < .05). These findings indicated that metabolic parameters are critical determinants of NAFLD pathogenesis, warranting prioritized clinical intervention.

Expression of MiR-487b-3p in Serum and Receiver-Operating Characteristic Curve Analysis

Analysis of serum miR-487b-3p expression revealed significantly elevated levels in patient groups compared with the healthy controls (*P* < .001) (Figure 1A). To assess the diagnostic potential of miR-487b-3p for NAFLD, ROC curve analysis was performed. The results revealed that miR-487b-3p exhibited an area under the curve (AUC) of 0.940 (95% CI, 0.899-0.981, *P* < .001), and the cutoff value was 1.365 (sensitivity, 91.67%; specificity, 85.00%) (Figure 1B; Supplementary Table 1). Biomarker's stable discriminative performance was carried out through stratified 5-fold cross-validation (mean AUC = 0.933, SD = 0.048; Supplementary Table 2), demonstrating high

Table 1. Baseline Characteristics of Patients with NAFLD

Clinical Characteristic	Control Group (n = 60)	NAFLD Group (n = 60)	t/ χ^2	P
Age	45.12 ± 5.86	42.70 ± 7.33	-1.865	.065
Gender			0.133	.715
Male	30(50.0%)	32(53.3%)		
Female	30(50.0%)	28(46.7%)		
BMI (kg/m ²)	22.02 ± 2.15	29.29 ± 3.87	12.717	<.001
Smoking			0.048	.827
Yes	14	13		
No	46	47		
Marital status			0.240	.624
Yes	51	49		
No	9	11		
Exercise			0.436	.509
Yes	56	54		
No	4	6		
Fasting insulin (IU/mL)	9.11 ± 2.18	10.99 ± 3.46	3.558	.001
FBG (mmol/L)	5.21 ± 0.67	5.78 ± 1.10	3.442	.001
HOMA-IR	2.13 ± 0.56	2.66 ± 1.15	3.188	.002
TC (mmol/L)	4.15 ± 0.58	4.66 ± 0.73	4.173	<.001
TG (mmol/L)	1.10 ± 0.22	1.65 ± 0.69	5.873	<.001
HDL-C (mmol/L)	1.32 ± 0.22	1.18 ± 0.30	-4.173	<.001
LDL-C (mmol/L)	2.75 ± 0.37	2.73 ± 0.75	-0.129	.897

BMI, body mass index; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; TC, total cholesterol; TG, triglycerides.

diagnostic accuracy in discriminating NAFLD patients from healthy controls.

The correlation analysis results demonstrated that the expression levels of miR-487b-3p exhibited significant positive correlations with FBG ($r = 0.6400$, $P < .001$), fasting insulin ($r = 0.5530$, $P < .001$), and HOMA-IR ($r = 0.6478$, $P < .001$) (Figure 1C-E), suggesting that miR-487b-3p may play a regulatory role in glucose metabolism and insulin signaling pathways.

miR-487b-3p Modulates Glucose Uptake in Hepatocytes

Transfection of HepG2 cells with negative controls (NC), anti-NC, miR-487b-3p mimics, or miR-487b-3p inhibitor was performed to assess miR-487b-3p expression

and glucose uptake rates, elucidating their functional relationship. Quantitative RT-PCR analysis demonstrated a statistically significant upregulation of miR-487b-3p expression in the mimics group compared to NC ($P < .05$), while the inhibitor group exhibited marked downregulation relative to anti-NC ($P < .05$) (Figure 2A). Fluorescence-based glucose uptake assays revealed that miR-487b-3p overexpression significantly impaired glucose absorption ($P < .05$), whereas its inhibition markedly enhanced uptake efficiency ($P < .05$) (Figure 2B), collectively establishing miR-487b-3p as a functional modulator of glucose metabolism in cells. These data provide compelling evidence for the regulatory role of miR-487b-3p in cellular glucose homeostasis through bidirectional modulation of uptake mechanisms.

miR-487b-3p Directly Targets the 3'-UTR of Insulin Receptor Substrate 1

Bioinformatic analysis using TargetScan and miRDB identified IRS1 as a potential target of miR-487b-3p. To investigate the regulatory relationship between miR-487b-3p and IRS1, HepG2 cells were transfected with NC, anti-NC, miR-487b-3p mimics, or miR-487b-3p inhibitor, followed by IRS1 mRNA quantification. The RT-PCR analysis revealed that miR-487b-3p overexpression significantly suppressed IRS1 expression compared to NC ($P < .05$), while its inhibition enhanced IRS1 levels versus anti-NC ($P < .05$) (Figure 3A). Subsequent dual-luciferase reporter assays in HepG2 cells, incorporating either wild-type or mutant IRS1 3'-UTR sequences, demonstrated that miR-487b-3p mimics reduced wild-type reporter activity by 42% versus miR-negative control ($P < .01$), whereas the inhibitor increased activity by 35% ($P < .05$). No significant changes occurred in mutant constructs across treatment groups ($P > .05$) (Figure 3B). These findings confirm direct targeting of IRS1 3'-UTR by miR-487b-3p, establishing its post-transcriptional regulatory mechanism through 3'-UTR binding.

DISCUSSION

Emerging as the predominant etiology of chronic liver disease (CLD),¹⁴ NAFLD exhibits a well-documented trajectory toward hepatocellular carcinoma (HCC) and hepatic cirrhosis. Accumulating evidence confirms significantly elevated HCC risk in NAFLD patients.¹⁵ Concurrently, these metabolic disturbances contribute to systemic metabolic dysregulation, including insulin resistance, enhanced gluconeogenesis, and increased blood glucose output. miRNAs are endogenous regulatory RNAs involved in cellular processes¹⁶ that modulate NAFLD

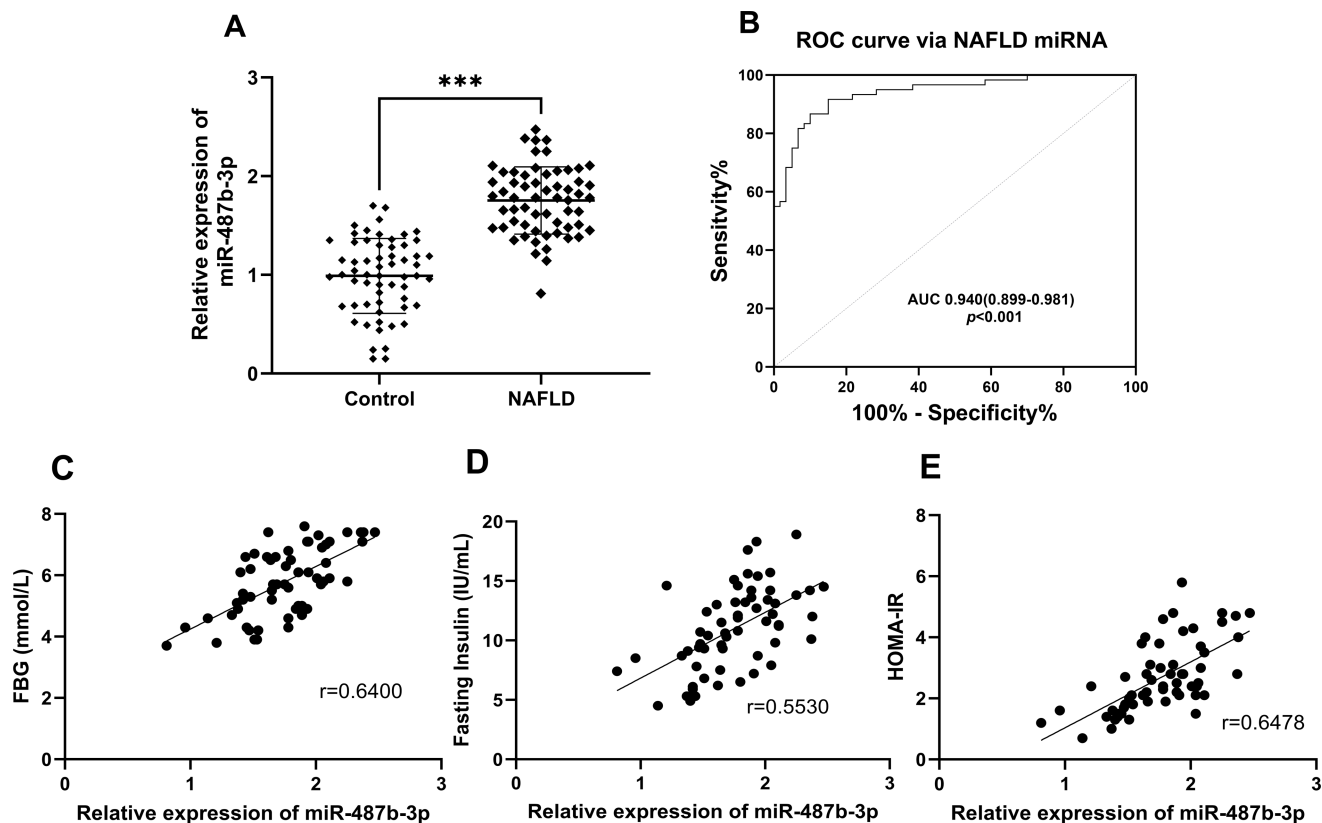


Figure 1. The miR-487b-3p expression in healthy controls and NAFLD groups. A. Relative expression of miR-487b-3p detected by RT-qPCR in healthy controls and NAFLD groups. B. ROC curve via miR-487b-3p levels. C. Correlation between miR-487b-3p and FBG. D. Correlation between miR-487b-3p and fasting insulin. E. Correlation between miR-487b-3p and HOMA-IR.

pathogenesis through target mRNA interactions. For instance, studies have demonstrated that miR-122, a miRNA specifically highly expressed in the liver, whose knockdown effectively reduces excessive lipid production¹⁷ and alleviates inflammation,¹⁸ miR-192 can inhibit triglyceride synthesis in hepatocytes¹⁹ and significantly diminish lipid accumulation,²⁰ while overexpression of miR-34a leads to triglyceride accumulation and impairs mitochondrial membrane potential.²¹ These mechanisms

collectively influence NAFLD progression by directly modulating lipid synthesis and release, as well as inflammatory pathways, and can serve as biomarkers for NAFLD.²²⁻²⁴ While miR-122, miR-192, and miR-34a have been extensively studied in NAFLD, their focus has primarily been on lipid metabolism or inflammatory pathways, with limited direct involvement in the insulin signaling pathway. In this study, ROC analysis further confirmed that miR-487b-3p also has the potential to serve as a biomarker for NAFLD

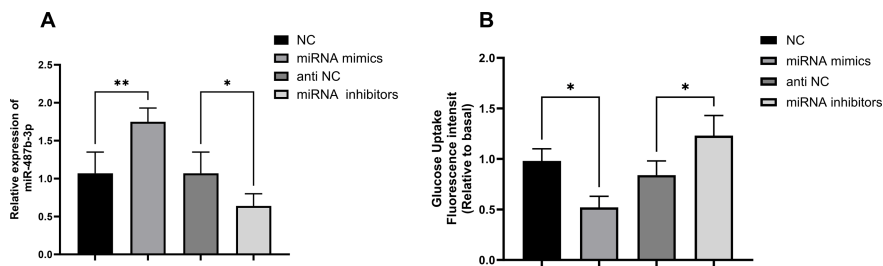


Figure 2. HepG2 cells were transfected with NC, anti-NC, miRNA mimics, and miRNA inhibitors for 48 h. A. Expression levels of miR-487b-3p in HepG2. B. Glucose uptake fluorescence intensity in HepG2.

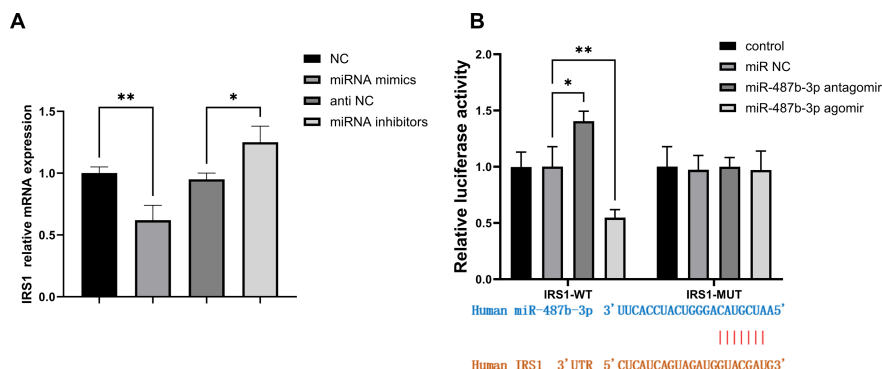


Figure 3. Targeting mechanism of miR-487b-3p on IRS1. A. Expression levels of IRS1 in HepG2. B. Correlation between miR-487b-3p and IRS1 expression in HepG2.

diagnosis. Additionally, it was found that miR-487b-3p was to some extent correlated with fasting insulin, FBG, and HOMA-IR levels, suggesting that miR-487b-3p may influence NAFLD by uniquely targeting the IRS1/PI3K/Akt axis through the insulin signaling pathway, and miR-487b-3p may play a pivotal role in regulating and ameliorating the dysregulated glucose-lipid metabolism and insulin resistance associated with NAFLD.

The pathogenesis of NAFLD primarily involves chronic nutrient oversupply and insulin resistance, which collectively promote intrahepatic fatty acid retention.²⁵ Insulin resistance (IR) represents the core pathophysiological mechanism of NAFLD, and the pathophysiological foundation of IR stems from impaired hormonal signaling transduction within responsive cellular populations.²⁶ Several studies indicated that attenuated stimulation of PI3K/Akt activity by insulin contributes to fasting hyperglycemia,²⁷ characterized by reduced insulin signaling for hepatic glucose production suppression, affecting both the insulin receptor and downstream mediators,²⁸ alongside diminished cellular glucose uptake efficiency, ultimately leading to hyperglycemia. An imbalance between energy intake and expenditure precipitates the emergence of IR;²⁹ thus, insulin resistance precedes type 2 diabetes development, which is manifested by fasting hyperglycemia.³⁰ Furthermore, hepatic de novo lipogenesis negatively correlates with systemic insulin sensitivity while and is associating with 24-hour plasma glucose and insulin concentrations.³¹ In this study, we demonstrated that miR-487b-3p modulates cellular glucose uptake, suggesting its potential role in influencing NAFLD through insulin signaling pathways associated with glucose metabolism.

Insulin receptor substrates (IRS) serve as pivotal molecular switches mediating hepatic insulin signaling and participate in orchestrating signal cascades.³² Among them, the IRS/PI3K/Akt signaling axis plays a critical role in glucose regulation. Upon insulin binding, IRS proteins are activated, subsequently recruiting and activating phosphatidylinositol 3-kinase (PI3K), which stimulates the downstream effector protein kinase B (Akt).³³ The activated Akt directly influences the translocation of the glucose transporter, facilitating its movement from the cytoplasm to the plasma membrane to enhance glucose uptake, thereby modulating cellular glycogen storage, suppressing gluconeogenesis, and promoting lipid conversion.³⁴ As a member of the IRS family, IRS1 mediates most metabolic actions of INSR activation. Its deficiency reduces glucose uptake in myocytes and impairs muscle glycogen synthesis, causing systemic glucose retention and consequently hepatic insulin resistance. Several studies showed that activation of the IRS1/PI3K/Akt pathway can alleviate insulin resistance in hepatocytes,³⁵ and hepatic insulin responsiveness is closely associated with hepatic lipid accumulation and steatosis.³⁶ Disruption of the IRS-1/PI3K/Akt pathway contributed to dysregulated lipid and glucose metabolism.³⁷

In this study, we observed that miR-487b-3p can directly target the 3' UTR of IRS1 mRNA, and that this interaction may potentially influence the IRS-1/PI3K/AKT signaling pathway, thereby possibly leading to impaired glucose uptake in hepatocytes. These findings suggested that circulating miR-487b-3p may amplify insulin resistance through a "liver-circulation" feedback mechanism, thereby perpetuating a metabolic vicious cycle. Consequently, targeting miR-487b-3p can ameliorate NAFLD pathology through multiple mechanisms,

including the alleviation of IRS1 inhibition to activate the PI3K/Akt pathway, thereby enhancing cellular glucose uptake, improving fasting blood glucose levels, and restoring insulin sensitivity. It may mitigate or prevent lipid accumulation and inflammation arising from insulin signaling dysfunction. Whether miR-487b-3p exerts its role in NAFLD through the IRS1/PI3K/AKT signaling pathway remains a key focus of the future research.

In the current study, patients with type 2 diabetes mellitus (T2DM) were excluded, although IR is most closely associated with this subgroup. T2DM patients typically exhibit more severe IR and metabolic disturbances, accompanied by β -cell dysfunction and insufficient insulin secretion, leading to elevated fasting blood glucose levels. Given that these factors may confound the investigation of insulin signaling pathway mechanisms (e.g., glucose regulation), patients with T2DM were excluded during enrollment. However, they will be incorporated into future mechanistic validation experiments to systematically evaluate the synergistic effects of relevant miRNAs on the pathological progression of NAFLD in the context of IR combined with T2DM. Of course, validation in a larger independent cohort remains a necessary next step to provide supporting evidence.

In conclusion, the study demonstrated that miR-487b-3p negatively regulates IRS1 expression, thereby impairing glucose uptake in liver cells and contributing to insulin resistance pathways. Moreover, miR-487b-3p may serve as a diagnostic biomarker and a potential therapeutic target for NAFLD.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Artificial Intelligence Usage Statement: The authors declared that no Artificial Intelligence Tool was used in the preparation of the manuscript.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Wuhan Third Hospital, Tongren Hospital of Wuhan University (Approval no: MS-2023-1014; Date: August 24, 2023).

Informed Consent: Verbal and written informed consent was obtained from the participants who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.T.C., J.T.; Design – M.T.C., J.T., M.H., T.Z.; Supervision – M.H., T.Z.; Resources – Z.H., X.T.;

Materials – M.T.C., J.T., Z. H.; Data Collection and/or Processing – M.T.C., J.T., Y.Y.L., X.T.; Analysis and/or Interpretation – M.T.C., J.T., M.L.; Literature Search – X.T.; Writing – M.T.C., J.T.; Critical Review – Y.Y.L., X.T.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The author declared that this study received no financial support.

REFERENCES

1. Streba LAM, Vere CC, Rogoveanu I, et al. Nonalcoholic fatty liver disease, metabolic risk factors, and hepatocellular carcinoma: an open question. *World J Gastroenterol.* 2015;21(14):4103-4110. [\[CrossRef\]](#)
2. Khoonsari M, Mohammad Hosseini Azar M, Ghavam R, et al. Clinical manifestations and diagnosis of nonalcoholic fatty liver disease. *Iran J Pathol.* 2017;12(2):99-105. [\[CrossRef\]](#)
3. Alalwani J, Eljazzar S, Basil M, et al. The impact of health status, diet and lifestyle on non-alcoholic fatty liver disease: narrative review. *Clin Obes.* 2022;12(4):e12525. [\[CrossRef\]](#)
4. Anderson EL, Howe LD, Jones HE, et al. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PLOS One.* 2015;10(10):e0140908. [\[CrossRef\]](#)
5. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA.* 2015;313(22):2263-2273. [\[CrossRef\]](#)
6. Bonacini M, Kassamali F, Kari S, et al. Racial differences in prevalence and severity of non-alcoholic fatty liver disease. *World J Hepatol.* 2021;13(7):763-773. [\[CrossRef\]](#)
7. Nobili V, Alisi A, Valenti L, et al. NAFLD in children: new genes, new diagnostic modalities and new drugs. *Nat Rev Gastroenterol Hepatol.* 2019;16(9):517-530. [\[CrossRef\]](#)
8. Ghazy AM, Soliman AHE, Mohammed AEA, et al. Micro-RNAs-122 and Pro-neurotensin biomarker for non-invasive diagnosis of Non-alcoholic fatty liver disease (A Case control study). *Clin Ter.* 2025;176(3):344-349. [\[CrossRef\]](#)
9. Johnson K, Leary PJ, Govaere O, et al. Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: diagnostic and mechanistic relevance. *JHEP Rep.* 2022;4(2):100409. [\[CrossRef\]](#)
10. Gao F, Ma Y, Yu C, et al. miR-125b-5p regulates FFA-induced hepatic steatosis in L02 cells by targeting estrogen-related receptor alpha. *Gene.* 2025;959:149419. [\[CrossRef\]](#)
11. Ying W, Gao H, Dos Reis FCG, et al. MiR-690, an exosomal-derived miRNA from M2-polarized macrophages, improves insulin sensitivity in obese mice. *Cell Metab.* 2021;33(4):781-790.e5. [\[CrossRef\]](#)
12. Ames HM, Yuan M, Vizcaíno MA, et al. MicroRNA profiling of low-grade glial and glioneuronal tumors shows an independent role for cluster 14q32.31 member miR-487b. *Mod Pathol.* 2017;30(2):204-216. [\[CrossRef\]](#)
13. Long YC, Cheng Z, Copps KD, et al. Insulin receptor substrates Irs1 and Irs2 coordinate skeletal muscle growth and metabolism via the Akt and AMPK pathways. *Mol Cell Biol.* 2011;31(3):430-441. [\[CrossRef\]](#)
14. Jang W, Song JS. Non-invasive imaging methods to evaluate non-alcoholic fatty liver disease with fat quantification: a review. *Diagnostics (Basel, Switzerland).* 2023;13(11):1852. [\[CrossRef\]](#)

15. He X, Ma J, Yan X, et al. *CDT1 is a potential therapeutic target for the progression of NAFLD to HCC and the exacerbation of cancer.* *Curr Genomics.* 2025;26(3):225-243. [\[CrossRef\]](#)
16. Ma X, Denyer T, Javelle M, et al. *Genome-wide analysis of plant miRNA action clarifies levels of regulatory dynamics across developmental contexts.* *Genome Res.* 2021;31(5):811-822. [\[CrossRef\]](#)
17. Long JK, Dai W, Zheng YW, et al. *miR-122 promotes hepatic lipogenesis via inhibiting the LKB1/AMPK pathway by targeting Sirt1 in non-alcoholic fatty liver disease.* *Mol Med (Camb Mass).* 2019;25(1):26. [\[CrossRef\]](#)
18. Hu Y, Du G, Li G, et al. *The miR-122 inhibition alleviates lipid accumulation and inflammation in NAFLD cell model.* *Arch Physiol Biochem.* 2021;127(5):385-389. [\[CrossRef\]](#)
19. Ma L, Song H, Zhang CY, et al. *MiR-192-5p ameliorates hepatic lipid metabolism in non-alcoholic fatty liver disease by targeting Yy1.* *Biomolecules.* 2023;14(1):34. [\[CrossRef\]](#)
20. Liu XL, Cao HX, Wang BC, et al. *miR-192-5p regulates lipid synthesis in non-alcoholic fatty liver disease through SCD-1.* *World J Gastroenterol.* 2017;23(46):8140-8151. [\[CrossRef\]](#)
21. Wang L, Sun M, Cao Y, et al. *miR-34a regulates lipid metabolism by targeting SIRT1 in non-alcoholic fatty liver disease with iron overload.* *Arch Biochem Biophys.* 2020;695:108642. [\[CrossRef\]](#)
22. Jampoka K, Muangpaisarn P, Khongnomnan K, et al. *Serum miR-29a and miR-122 as potential biomarkers for non-alcoholic fatty liver disease (NAFLD).* *MicroRNA.* 2018;7(3):215-222. [\[CrossRef\]](#)
23. Liu XL, Pan Q, Zhang RN, et al. *Disease-specific miR-34a as diagnostic marker of non-alcoholic steatohepatitis in a Chinese population.* *World J Gastroenterol.* 2016;22(44):9844-9852. [\[CrossRef\]](#)
24. Atic AI, Thiele M, Munk A, et al. *Circulating miRNAs associated with nonalcoholic fatty liver disease.* *Am J Physiol Cell Physiol.* 2023;324(2):C588-C602. [\[CrossRef\]](#)
25. Chen Y, Hu X, Dou J, et al. *DJ-1 protein ameliorates nonalcoholic fatty liver disease via ER-stress pathway and reduces inflammation.* *Biochem Biophys Res Commun.* 2025;776:152175. [\[CrossRef\]](#)
26. Rafiee M, Sadeghi F, Mirzapour A, et al. *Association of MicroRNA-103 expression with type 2 diabetes mellitus.* *Caspian J Intern Med.* 2025;16(1):106-113. [\[CrossRef\]](#)
27. Dong X, Park S, Lin X, et al. *Irs1 and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth.* *J Clin Invest.* 2006;116(1):101-114. [\[CrossRef\]](#)
28. Petersen MC, Shulman GI. *Mechanisms of insulin action and insulin resistance.* *Physiol Rev.* 2018;98(4):2133-2223. [\[CrossRef\]](#)
29. Sakurai Y, Kubota N, Yamauchi T, et al. *Role of insulin resistance in MAFLD.* *Int J Mol Sci.* 2021;22(8):4156. [\[CrossRef\]](#)
30. Peng T, Miao R, Xiong H, et al. *Prediction of insulin resistance in nondiabetic population using LightGBM and cohort validation of its clinical value: cross-sectional and retrospective cohort study.* *JMIR Med Inform.* 2025;13:e72238. [\[CrossRef\]](#)
31. Smith GI, Shankaran M, Yoshino M, et al. *Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease.* *J Clin Invest.* 2020;130(3):1453-1460. [\[CrossRef\]](#)
32. Aravinthan A, Challis B, Shannon N, et al. *Selective insulin resistance in hepatocyte senescence.* *Exp Cell Res.* 2015;331(1):38-45. [\[CrossRef\]](#)
33. Perry RJ, Samuel VT, Petersen KF, et al. *The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes.* *Nature.* 2014;510(7503):84-91. [\[CrossRef\]](#)
34. Hatting M, Tavares CDJ, Sharabi K, et al. *Insulin regulation of gluconeogenesis.* *Ann N Y Acad Sci.* 2018;1411(1):21-35. [\[CrossRef\]](#)
35. Li QH, Lu MX, Feng YH, et al. *Rutin inhibits hepatic gluconeogenesis and increases glycogen synthesis through the IRS/PI3K/akt signaling pathway in insulin resistant hepatocytes.* *J Asian Nat Prod Res.* 2025:1-15. [\[CrossRef\]](#)
36. Watt MJ, Miotto PM, De Nardo W, et al. *The liver as an endocrine organ-linking NAFLD and insulin resistance.* *Endocr Rev.* 2019;40(5):1367-1393. [\[CrossRef\]](#)
37. Wang B, Wu S, Huang Z, et al. *Enteromorpha polysaccharides alleviate high-fat diet-induced glucolipid metabolism disorders via the K18-mediated IRS-1/PI3K/AKT/GSK3 β pathway.* *J Food Sci.* 2025;90(11):e70713. [\[CrossRef\]](#)

Supplementary Table 1. ROC Curve Analysis of miR-487b-3p

Metric	Value
Sensitivity (%)	91.67
Specificity (%)	85.00
Cutoff value	1.365
AUC	0.940
95%CI	0.899-0.981
P value	<.001

Supplementary Table 2. Mean AUC Values of ROC Curves for the Serum Biomarker miR-487b-3p

	AUC1	AUC2	AUC3	AUC4	AUC5
AUC values	0.9375	0.9444	0.8542	0.9861	0.9444
Mean	0.9333				
Standard deviation	0.048				