The Correlation between Insulin Resistance and Fatty Liver Disease in Qena University Hospital: Cross-Sectional Study

Hasan Sedeek Mahmoud[a], Shimaa Arafat Abd El-Monem[a], Abanoub Safwat Zakhary Agaiby[a*], Mohammed H. Hassan[b]

[a]Tropical Medicine and Gastroenterology Department, Faculty of Medicine, South Valley University, Qena, Egypt.

[b]Medical Biochemistry Department, Faculty of Medicine, South Valley University, Qena, Egypt.

Abstract

Background: Insulin resistance, driven by inactivity and overnutrition, disrupts glucose and lipid metabolism and contributes to metabolic syndrome, obesity, type 2 diabetes mellitus (T2DM), atherosclerosis, and nonalcoholic fatty liver disease (NAFLD). The liver plays a pivotal role, and NAFLD is the most common chronic liver disease, strongly associated with metabolic dysfunction.

Objectives: To determine the features of NAFLD in insulin-resistant, non-diabetic, non-alcoholic individuals and identify associated risk factors.

Patients and methods: This cross-sectional case-control study included 100 non-alcoholic, non-diabetic patients with insulin resistance (55 with NAFLD and 45 with non-NAFLD) and 50 controls, excluding any with liver-affecting conditions. All underwent a battery of lab tests, including CBC, liver and renal function, lipid profile, glucose, HbA1c, insulin, and adiponectin. Liver stiffness and steatosis were assessed using abdominal ultrasound and FibroScan. LSM values staged fibrosis, and CAP ≥ 238 dB/m is used to diagnose steatosis.

Results: Mean age was 49.71±16.71 years; 52.67% were male. NAFLD cases had significantly higher BMI (37.46±2 kg/m²), WC (101.4±6.15 cm), HbA1c (6.04±0.19%), fasting glucose (111.38±7.37 mg/dL), HOMA-IR (9.92±3.86), ALT, AST, bilirubin, and PT/INR ($P < 0.0001$) compared to those without NAFLD and the control groups. Serum adiponectin was lower (26.87±10.35 ng/mL, $P = 0.006$). LSM (9.68±2.66 kPa) and CAP (279.07±18.06 dB/m) were significantly elevated in NAFLD. The ROC curve revealed that HOMA-IR showed 83.6% sensitivity, 77.9% specificity, and 80% accuracy. Adiponectin showed 74.5% sensitivity, 50.5% specificity, and 59.33% accuracy. Elevated HbA1c and dyslipidemia are independent risk factors for NAFLD.

Conclusion: NAFLD in insulin-resistant individuals is linked to significant hepatic and metabolic alterations. CAP, LSM, and HOMA-IR are valuable diagnostic tools for NAFLD.

Keywords: Insulin resistance; NAFLD; Liver steatosis; HOMA-IR; Qena University Hospital

DOI: 10.21608/SVUIJM.2025.398267.2208

*Correspondence: sabanoub685@gmail.com

Received: 1 June,2025.

Revised: 28 June, 2025.

Accepted: 6 July, 2025.

Published: 7 July, 2025

Cite this article as Hasan Sedeek Mahmoud, Shimaa Arafat Abd El-Monem, Abanoub Safwat Zakhary Agaiby, Mohammed H. Hassan.(2025). The Correlation between Insulin Resistance and Fatty Liver Disease in Qena University Hospital: Cross-Sectional Study. *SVU-International Journal of Medical Sciences*. Vol.8, Issue 2, pp: 160-173.

Introduction

Insulin is essential for transitioning the body from nutrient production to storage after food intake by promoting glucose and protein uptake in adipose tissue and skeletal muscle. In response to elevated blood glucose, pancreatic β-cells secrete insulin to facilitate this process. However, sedentary behavior and overnutrition disturb this metabolic balance, contributing to insulin resistance—a condition where tissues become less responsive to insulin. This resistance is a key driver in the pathogenesis of metabolic syndrome, obesity, type 2 diabetes mellitus (T2DM), atherosclerosis, and nonalcoholic fatty liver disease (NAFLD) **(Lee et al., 2022).**

The liver plays a central role in maintaining metabolic homeostasis **(Tanase et al., 2020).** NAFLD has become the most prevalent chronic liver disease globally, often regarded as the hepatic manifestation of metabolic syndrome. It frequently coexists with obesity, dyslipidemia, hypertension, and diabetes. The increasing incidence of obesity and T2DM parallels the global rise in NAFLD prevalence **(Loomba et al., 2021).** Insulin resistance (IR) is a condition where normal insulin levels are insufficient to produce a normal metabolic response or where higher-than-normal insulin concentrations are required for the same effect. IR is closely linked to hepatic fat accumulation and plays a key role in the development of NAFLD. This relationship is supported by clinical, laboratory, and physiological evidence **(Zhao et al., 2023).**

The pathogenesis of NAFLD involves a complex interaction between adipokines and cytokines released from adipose tissue and inflammatory cells. These molecules affect insulin sensitivity in target organs, including the liver. Adipokines such as adiponectin act as insulin sensitizers, improving insulin sensitivity and potentially reducing NAFLD risk **(Qiu et al., 2023).**

This study aimed to assess the features of NAFLD in non-diabetic, non-alcoholic patients with insulin resistance; characterize its features, including grading and metabolic syndrome associations; and identify risk factors contributing to its development (e.g., adiponectin).

Patients and methods

This cross-sectional case-control study was conducted at the Tropical Medicine and Gastroenterology Department and clinic for 6 months, from January 2025 till June 2025. It included 100 non-alcoholic, non-diabetic patients with confirmed insulin resistance and 50 healthy controls of similar age and sex. Fatty liver disease was diagnosed based on FibroScan results (Controlled Attenuation Parameter (CAP) of ≥ 248 dB/m) **(Piccinni et al., 2020).**

Patients with chronic hepatitis C or B, hepatocellular carcinoma (HCC), autoimmune liver diseases, alcohol abuse, use of drugs causing hepatic steatosis, or any condition affecting liver stiffness measurement were excluded.

Sample size justification

We used Epi Info to calculate the sample size based on **Gutierrez-Buey et al., 2017,** which found that HOMA-IR had a 93% specificity for predicting fatty liver disease. The sample size was calculated using a 95% two-sided confidence level, 80% power, and a 5% odds ratio = 1.115, using the following formula:

$$n = \frac{Z^2 \cdot P \cdot (1 - P)}{d^2}$$

Where: P = specificity of HOMA*IR for prediction of fatty liver disease = 93%. Z = 1.96 for a 95% confidence interval. D = 0.05 (desired margin of error).

The final maximum sample size taken from the Epi-Info output was 100 cases (55 with NAFLD and 45 non-NAFLD

cases), with 50 healthy controls added for comparison.

Ethical statement: The study was approved by the local Research Ethics Committee under the ethical code SVU-MED-GIT023-1-23-2-561; informed consent was obtained from the patients before enrollment in the study; all data was kept confidential; and all participants had the right to withdraw from the study without

Methods

A complete clinical assessment was conducted on all patients, including Waist circumference was measured with a flexible tape at the midpoint between the lower ribs and iliac crest, across the navel, with patients standing, anthropometric measurements and BMI was calculated (kg/m^2).

Patients were fasting for 8 hours. 5 ml of venous blood sample was collected under aseptic conditions through antecubital venipuncture. 1.8 ml was collected into 3.2% sodium citrate, and 1 ml was collected into an EDTA tube for CBC, and the rest was collected into plain tubes to obtain serum by centrifugation at 3500 rpm for 10 minutes for clinical chemistry analysis according to the standard operating procedures.

Laboratory investigations included complete blood count (CBC) analyzed by the Sysmex XN-1000 (Sysmex, Japan). Coagulation profile (PT, PC%, INR). Fasting blood glucose was measured using a glucometer, and HbA1c via turbidimetric immunoassay on the Cobas C501 (Roche Diagnostics, Germany). Liver enzymes (AST, ALT, ALP, and GGT) and bilirubin were assessed on the Cobas C701. Albumin, urea, and electrolytes (Na, K, Ca) were measured using the Cobas C311, while creatinine was determined by the Jaffe method (Cobas CREJ2). Lipid profile (total cholesterol, HDL, LDL, VLDL, triglycerides) was analyzed using the Cobas b 101 system.

Hepatitis B surface antigen (Catalog No.: MBS022875, MyBioSource, USA) and hepatitis C virus antibodies (Catalog No.: MBS766110, MyBioSource, USA) were evaluated using ELISA kits according to the manufacturer instructions.

Insulin resistance was calculated using the HOMA-IR model: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5. HOMA-IR values between 0.5 and 1.4 are considered normal, ≥1.9 are indicative of early IR, and ≥2.9 indicate IR **(Matthews et al., 1985).**

Adiponectin levels were measured using ELISA kits (Cat. No.: CAN-APN-5000, Diagnostics Biochem Inc., Canada) with inter-assay CV of 6.6% and intra-assay CV of 7.5% based on two-step sandwich enzyme-linked immunoassay.

Imaging included abdominal ultrasonography and FibroScan assessment. Routine pelviabdominal ultrasonography was performed using a Vivid S5 device (GE Healthcare, USA) with a 5 MHz curved array probe. The scan began at the level of the anterior superior iliac spine in a transverse plane and progressed superiorly. Liver images were acquired on both axial and longitudinal planes, primarily from the right abdomen.

FibroScan 502 (Echosens, Paris, France) was used to evaluate liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) after at least 8 hours of fasting. The M probe was used initially, with the XL probe applied for obese patients. LSM values, based on the median of 10 valid readings, were expressed in kilopascals (kPa), with normal values ranging from 2.5 to 7.0 kPa and considered valid if the IQR/median ratio was < 30%. Fibrosis staging was defined as follows: F0 < 2.5 kPa (no fibrosis), F1 = 2.5–7 kPa (minimal), F2 = 7.1–9.4 kPa (moderate), F3

= 9.5–12.4 kPa (severe), and F4 > 12.5 kPa (cirrhosis) **(Serra et al., 2020)**.

CAP values were reported in decibels per meter (dB/m), with a valid measurement requiring 10 successful acquisitions. CAP scores ranged from 100 to 400 dB/m, with steatosis diagnosed at a threshold of ≥ 238 dB/m, (**Table.1**).

Table 1. Steatosis was graded based on the CAP score **(Padda et al., 2021)**

CAP Score	Steatosis grade	Portion of liver affected by fatty change
< 238 dB/m	S0	0 to 10%
238 to 260 dB/m	S1	Less than ⅓ (11% to 33%)
260 to 290 dB/m	S2	Between ⅓ and ⅔ (34% to 66%)
290 to 400 dB/m	S3	More than ⅔ (67%)

Statistical analysis

Data were analyzed using SPSS version 26.0. Qualitative variables were expressed as numbers and percentages, and quantitative data as mean ± standard deviation. The Shapiro-Wilk test was used for evaluation of data normality. The chi-square and Fisher's exact test for categorical variables and the one-way ANOVA test for comparing means across more than two groups. Univariate and multivariate regression analyses were used to determine predictors and possible risk factors for NAFLD. Receiver operator characteristics (ROC) curve was used to determine the power of laboratory estimates to discriminate between NAFLD and non-NAFLD via calculating the area under the curve (AUC) and accuracy, sensitivity, specificity, and positive and negative predictive values. A p-value < 0.05 was considered statistically significant.

Results

The current study included 100 cases with insulin resistance and 50 age- and sex-matched healthy controls. Fifty-five of the 100 cases had a confirmed diagnosis of NAFLD. There was no significant difference in age or sex distribution between cases and controls or among NAFLD, non-NAFLD, and control groups (P > 0.05). However, total cases had significantly higher BMI and waist circumference, with NAFLD cases having the highest mean BMI (37.46 ± 2 kg/m²) and WC (101.4 ± 6.15 cm). (**Table. 2).**

Table 2. Demographic data in the studied groups

Variables	Total Cases (N = 100)	NAFLD cases (N = 55)	Non NAFLD cases (N = 45)	Control (N = 50)	P-Value
Age (Years)	50.9 ± 15.57	50.82 ± 15.7	51 ± 15.41	47.34 ± 18.54	P_F=0.4738
		P1= 0.9984, P2=0.5403, P3=0.5399			P_{TC}=0. 2506$^{w.t}$
Sex					
• Female	50 (50%)	29 (52.73%)	21 (46.67%)	21 (42%)	P_{X1}=0.5434^{X}
• Male	50 (50%)	26 (47.27%)	24 (53.33%)	29 (58%)	P_{X2}=0.3583 ^{X}
BMI (Kg/m^2)	35.84 ± 2.82	37.46 ± 2	33.87 ± 2.39	20.97 ± 1.48	P_F<0.0001*
		P1= <0.0001*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^{U}
WC (Cm)	98.5 ± 7.28	101.4 ± 6.15	94.96 ± 6.98	86.02 ± 15.72	P_F<0.0001*
		P1= 0.0085*, P2<0.0001*, P3=0.0002*			P_{TC}<0.0001^{U}

*: significant; F: one-ANOVA test and post-Hoc test (Tukey) for pair wise comparison; X: Chi square test. w.t: Welch's t test. U: Mann-Whitney U test; P_{TC}: total cases vs. controls; P_{X1}: cases subgroups vs. controls. PX2: total cases vs. controls; P1 = NFLD vs. non-NAFLD cases; P2 = NAFLD vs control; P3 = non-NAFLD cases vs. control; BMI: Body mass index, WC: Waist circumference.

The study found no significant differences in hemoglobin, platelet count, urea, creatinine, and direct bilirubin levels across all groups. The mean WBC count was higher in cases than controls, but subgroup differences were not significant. The mean ALT, AST, and total bilirubin levels were elevated in NAFLD and non-NAFLD cases, with NAFLD levels significantly higher than non-NAFLD. Serum albumin was lower in cases. PT and INR were significantly prolonged in both case subgroups, with no difference between NAFLD and non-NAFLD. (**Table. 3).**

Table 3. Lab investigations in the studied groups

Variables	Total Cases (N = 100)	NAFLD cases (N = 55)	Non NAFLD cases (N = 45)	Control (N = 50)	P. Value
Hb (g/dL)	14.65 ± 5.12	15.09 ± 6.82	14.11 ± 0.98	14.16 ± 0.71	P_F=0.4096
		P1 = 0.4801, P2 = 0.4948, P3= 0.9983			P_{TC}= 0.7178^U
PLT (× 10^3 cells /µl)	273.73 ± 25.15	277.6 ± 23.33	268.99 ± 26.46	268.1 ± 25.94	P_F=0.1107
		P1= 0.2147, P2 = 0.1389, P3 = 0.9841			P_{TC}=0.1237^U
WBC (× 10^3 cells /µl)	7.28 ± 1.79	7.32 ± 2.05	7.23 ± 1.4	6.66 ± 0.44	P_F=0.0561
		P1= 0.9494, P2=0.0628, P3=0.1537			P_{TC}=0.0046*^{U}
Liver function test					
ALT (U/L)	92.45 ± 20.5	97.73 ± 18.77	86 ± 20.68	34.46 ± 3.15	P_F<0.0001*
		P1= 0.0014*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
AST (U/L)	49.05 ± 11.01	52.04 ± 9.87	45.4 ± 11.23	25.06 ± 2.4	P_F<0.0001*
		P1= 0.0007*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
Alb (g/dL)	3.42 ± 0.28	3.4 ± 0.27	3.43 ± 0.29	3.7 ± 0.25	P_F<0.0001*
		P1= 0.8397, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
Total Bilirubin (mg/dL)	0.75 ± 0.09	0.77 ± 0.09	0.73 ± 0.09	0.68 ± 0.05	P_F<0.0001*
		P1= 0.1087, P2<0.0001*, P3=0.0011*			P_{TC}<0.0001^U
Direct Bilirubin (mg/dL)	0.14 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.12 ± 0.04	P_F=0.0349*
		P1= 0.7422, P2=0.1456, P3=0.0344*			P_{TC}=0.2047^U
PT (Sec)	14.48 ± 0.58	14.49 ± 0.57	14.48 ± 0.58	11.44 ± 0.33	P_F<0.0001*
		P1= 0.9973, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
INR	1.14 ± 0.05	1.14 ± 0.05	1.14 ± 0.05	0.9 ± 0.03	P_F<0.0001*
		P1= 0.8476, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
Renal function test					
Serum Urea (mg/dL)	18.57 ± 2.89	18.9 ± 2.76	18.17 ± 3	17.82 ± 2.74	P_F=0.145
		P1= 0.4123, P2=0.1336, P3=0.8254			P_{TC}=0.1279^U
Serum creatinine (g/dL)	0.97 ± 0.14	0.98 ± 0.13	0.95 ± 0.14	0.93 ± 0.14	P_F=0.1111
		P1= 0.3722, P2=0.1006, P3=0.7939			P_{TC}=0.1161^U
Serology test					
HBs Ag		0 (%)	0 (%)	0 (%)	-
HCV Ab		0 (%)	0 (%)	0 (%)	-

*: significant; one-ANOVA test and post-Hoc test (Tukey) for pair wise comparison, U: Mann-Whitney U test; P_{TC}: total cases vs. controls; P_{X1}: cases subgroups vs. controls; PX2: total cases vs. controls; P1 = NFLD vs. non-NAFLD cases; P2 = NAFLD vs. control; P3 = non-NAFLD cases vs. control; Hb: hemoglobin; PLT: platelet count; WBC: white blood cell count; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PT: prothrombin time; INR: international normalized ratio.

NAFLD cases showed significantly higher fasting glucose (111.38 mg/dL), HOMA-IR (9.92), HbA1c (6.04%), cholesterol (255.41 mg/dL), triglycerides (168.24 mg/dL), LDL (131.6 mg/dL), and VLDL (38.22 mg/dL), with lower adiponectin (26.87 ng/mL) and HDL (35.81 mg/dL) compared to controls ($P < 0.05$). No significant differences were found in Na, K, or Ca levels, (**Table. 4).**

Table 4.Glycaemic and lipid profiles and serum electrolyte levels in the studied groups

Variables	Total Cases (N = 100)	NAFLD cases (N = 55)	Non NAFLD cases (N = 45)	Control (N = 50)	P. Value
Fasting blood glucose (mg/dL)	109.24 ± 8.84	111.38 ± 7.37	106.62 ± 9.74	87.88 ± 3.75	P_F<0.0001*
		P1= 0.0045*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
HOMA IR	8.07 ± 4.17	9.92 ± 3.86	5.8 ± 3.34	0.99 ± 0.3	P_F=0.0001*
		P1< 0.0001*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
HbA1C	5.93 ± 0.23	6.04 ± 0.19	5.79 ± 0.2	5.53 ± 0.58	P_F<0.0001*
		P1= 0.0031*, P2<0.0001*, P3=0.0034*			P_{TC}=0.0002^U
Adiponectin (ng/ml)	29.97 ± 11.35	26.87 ± 10.35	33.76 ± 11.37	36.13 ± 10.9	P_F=0.0001*
		P1= 0.006*, P2=0.0001*, P3=0.5454			P_{TC}=0.0089^U
Cholesterol (mg/dL)	251.19 ± 37.45	255.41 ± 30.86	246.03 ± 43.63	114.47 ± 6	P_F<0.0001*
		P1= 0.2884, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
triglycerides (mg/dL)	165.64 ± 30.65	168.24 ± 13.5	162.46 ± 42.96	73.62 ± 7.48	P_F<0.0001*
		P1= 0.5, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
HDL (mg/dL)	37.03 ± 5.08	35.81 ± 3.11	38.51 ± 6.44	54.04 ± 1.26	P_F<0.0001*
		P1= 0.0038*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
LDL (mg/dL)	128.28 ± 19.23	131.6 ± 17.93	124.21 ± 19.97	91.18 ± 7.12	P_F<0.0001*
		P1= 0.0614, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
VLDL (mg/dL)	37.26 ± 9.48	38.22 ± 8.28	36.08 ± 10.66	22.64 ± 4.47	P_F<0.0001*
		P1= 0.3996, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
serum electrolytes					
Na (mEq/dL)	140.36 ± 2.79	140.29 ± 2.73	140.44 ± 2.87	140.2 ± 2.79	P_F=0.9136
		P1= 0.9604, P2=0.9851, P3=0.9066			P_{TC}=0.7896^U
K (mEq/dL)	4.2 ± 0.44	4.2 ± 0.46	4.2 ± 0.41	4.37 ± 0.4	P_F=0.0815
		P1= 0.99, P2=0.1143, P3=0.1427			P_{TC}=0.0265*^{U}
Ca (mg/dL)	9.55 ± 0.59	9.52 ± 0.6	9.58 ± 0.57	9.44 ± 0.62	P_F=0.5153
		P1= 0.8714, P2=0.7694, P3=0.4875			P_{TC}=0.3163^U

*: significant; one-ANOVA test and post-Hoc test (Tukey) for pair wise comparison; U: Mann-Whitney U test; P_{TC}: total cases vs. controls; P_{X1}: cases subgroups vs. controls; PX2: total cases vs. controls; P1 = NFLD vs. non-NAFLD cases; P2 = NAFLD vs. control; P3 = non-NAFLD cases vs. control; HOMA IR: homeostatic model assessment of insulin resistance; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; Na: sodium; K: potassium; Ca: calcium.

Liver stiffness was higher in NAFLD cases (8.34 ± 2.57 kPa) than controls (5.62 ± 0.21 kPa). Fibrosis staging showed a shift toward advanced fibrosis in NAFLD, with 40% having F3 and 18.18% having F4. The mean CAP values were elevated in NAFLD, reflecting higher hepatic fat. Steatosis grading showed significant fat accumulation and fibrosis in NAFLD, with 63.64% of NAFLD cases had moderate (S2) and 27.27% had severe (S3) steatosis, (**Table.5).**

Table 5. FibroScan findings in the studied groups

Variables	Total Cases (N = 100)	NAFLD cases (N = 55)	Non NAFLD cases (N = 45)	Control (N = 50)	P. Value
LSM Value (kPa)	8.34 ± 2.57	9.68 ± 2.66	6.71 ± 1.11	5.62 ± 0.21	P_F<0.0001*
		P1= <0.0001*, P2<0.0001*, P3=0.00613*			P_{TC}<0.0001^U
Fibrosis stage					
• F0	7 (7%)	0 (0%)	7 (15.56%)	50 (100%)	$P_{X1,2}$<0.0001*^{f}
• F1-F2	59 (59%)	23 (41.82%)	36 (80%)	0 (0%)	$P_{X1,2}$<0.0001*^{f}
• F3	23 (23%)	22 (40%)	1 (2.22%)	0 (0%)	$P_{X1,2}$<0.0001*^{f}
• F4	11 (11%)	10 (18.18%)	1 (2.22%)	0 (0%)	P_{X1}=0.0005*^{f} P_f=0.0163*^{f}
CAP value (dB/m)	265.92 ± 20.19	279.07 ± 18.06	249.84 ± 6.14	234.12 ± 2.1	P_F <0.0001*
		P1= <0.0001*, P2<0.0001*, P3<0.0001*			P_{TC}<0.0001^U
Degree of steatosis					
• S0	0 (0%)	0 (0%)	0 (0%)	50 (100%)	$P_{X1,2}$<0.0001*^{f}
• S1	49 (49%)	5 (9.09%)	44 (97.78%)	0 (0%)	$P_{X1,2}$<0.0001*^{f}
• S2	36 (36%)	35 (63.64%)	1 (2.22%)	0 (0%)	$P_{X1,2}$<0.0001*^{f}
• S3	15 (15%)	15 (27.27%)	0 (0%)	0 (0%)	P_{X1}<0.0001*^{f} P_{X2}=0.0026*^{f}

*: significant; one-ANOVA test and post-Hoc test (Tukey) for pair wise comparison; f: fisher exact test; U: Mann-Whitney U test; P_{TC}: total cases vs. controls; P_{X1}: cases subgroups vs. controls; PX2: total cases vs. controls; P1 = NFLD vs. non-NAFLD cases; P2 = NAFLD vs. control; P3 = non-NAFLD cases vs. control; LSM value: liver stiffness measurement; CAP value: controlled attenuation parameter.

HOMA IR had an AUC of 0.904, with high sensitivity (83.60%) and specificity (77.90%), but moderate PPV (68.66%) and NPV (89.16%), leading to an accuracy of 80% and a Kappa agreement of 0.5883 (P < 0.0001). Adiponectin exhibited an AUC of 0.698, with sensitivity at 74.5%, specificity at 50.5%, PPV at 46.59%, and NPV at 77.42%, resulting in an accuracy of 59.33% and a negative Kappa agreement of 0.2226 (P < 0.0001). LSM Value had a high AUC of 0.93, with 81.80% sensitivity, 88.40% specificity, 80.36% PPV, 89.36% NPV, and 86% accuracy, along with a Kappa agreement of 0.6997 (P < 0.0001). CAP value showed the highest performance, with an AUC of 0.997, sensitivity of 90.90%, specificity of 98.90%, PPV of 98.04%, NPV of 94.95%, accuracy of 96%, and a Kappa agreement of 0.9125 (P < 0.0001) (**Table.6, Fig.1**).

Table 6. ROC curve for HOMA-IR, adiponectin, LSM, and CAP values for discrimination of NAFLD from (non-NFLD and controls)

Variables	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa agreement	P-Value
HOMA IR	4.95	0.904	83.60%	77.90%	68.66%	89.16%	80%	0.5883	< 0.0001*
Adiponectin	32.235 ng/ml	0.698	74.5%	50.5%	46.59%	77.42%	59.33%	0.2226	< 0.0001*
LSM value	6.95 kPa	0.930	81.80%	88.40%	80.36%	89.36%	86%	0.6997	< 0.0001*
CAP value	260.5 dB/m	0.997	90.90%	98.90%	98.04%	94.95%	96%	0.9125	< 0.0001*

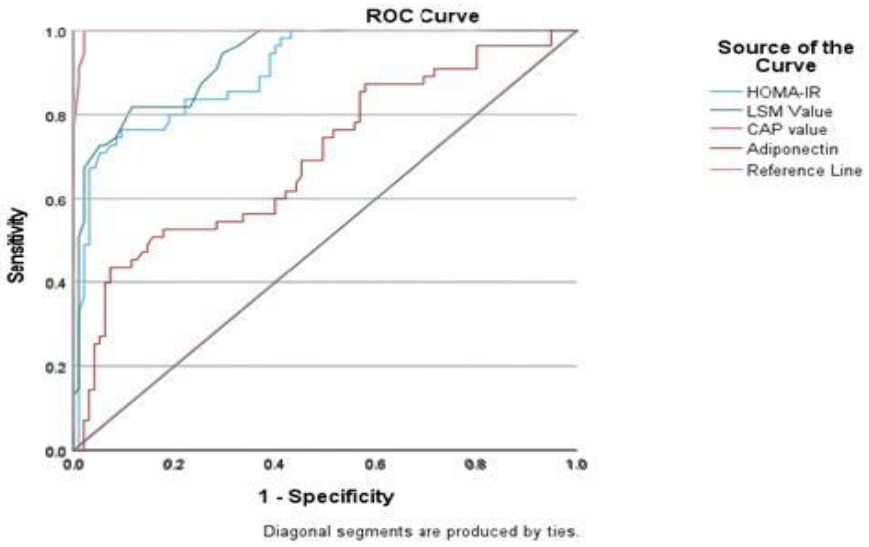

Fig.1. ROC curve for HOMA-IR, adiponectin, LSM, and CAP values for discrimination of NAFLD from (non-NFLD and controls).

Positive associations with NAFLD were observed with higher levels of PLTs, ALT, AST, total bilirubin, PT, INR, serum creatinine, HOMA-IR, fasting blood glucose, HbA1c, cholesterol, triglycerides, LDL, VLDL, BMI, waist circumference (WC), liver stiffness measurement (LSM), and controlled attenuation parameter (CAP) values ($P < 0.05$). Conversely, NAFLD was negatively associated with albumin and adiponectin levels. (**Table.7).**

Table 7. Univariable regression analysis to determine possible risk factors for NAFLD

Variables	Unstandardized Coefficients		OR	Test value	P-value	95.0% Confidence Interval for B	
	B	Std. Error				Lower Bound	Upper Bound
(Constant)	0.295	0.124				0.049	0.54
Age (Years)	0.001	0.002	0.05	0.613	0.541	-0.003	0.006
Male	-0.079	0.079	-0.082	-1.003	0.317	-0.236	0.077
Hb	-0.013	0.009	0.11	1.344	0.181	-0.006	0.031
PLTs	0.003	0.002	0.171	2.114	0.036*	0	0.006
WBCs	0.04	0.026	0.126	1.539	0.126	-0.011	0.092
ALT	0.009	0.001	0.583	8.733	<0.0001*	0.007	0.011
AST	0.019	0.002	0.576	8.567	<0.0001*	0.015	0.024
Alb	-0.441	0.128	-0.273	-3.452	0.001*	-0.694	-0.189
Total Bil	1.96	0.431	0.35	4.543	<0.0001*	1.107	2.812
Direct Bil	0.949	1.318	0.059	0.72	0.473	-1.657	3.554
PT	0.161	0.022	0.509	7.191	<0.0001*	0.117	0.205
INR	2.056	0.279	0.518	7.368	<0.0001*	1.504	2.607
Serum Urea	0.026	0.014	0.154	1.892	0.06	-0.001	0.053
Serum creatinine	0.563	0.28	0.163	2.014	0.046*	0.011	1.116
HOMA IR	0.068	0.006	0.672	11.031	<0.0001*	0.056	0.08

Adiponectin	-0.014	0.003	-0.339	-4.379	<0.0001*	-0.02	-0.008
F. blood glucose	0.021	0.003	0.56	8.229	<0.0001*	0.016	0.027
HbA1C	0.49	0.083	0.435	5.873	<0.0001*	0.325	0.655
Cholesterol	0.004	0	0.53	7.613	<0.0001*	0.003	0.005
triglycerides	0.005	0.001	0.504	7.093	<0.0001*	0.003	0.006
HDL	-0.031	0.004	-0.578	-8.626	<0.0001*	-0.038	-0.024
LDL	0.01	0.001	0.5	7.033	<0.0001*	0.007	0.013
VLDL	0.019	0.003	0.416	5.558	<0.0001*	0.012	0.025
Na	-0.001	0.014	-0.004	-0.052	0.958	-0.029	0.027
K	-0.11	0.091	-0.099	-1.212	0.227	-0.291	0.07
Ca	0.009	0.066	0.011	0.138	0.89	-0.121	0.139
BMI	0.044	0.004	0.673	11.069	<0.0001*	0.036	0.051
WC	0.017	0.003	0.435	5.881	<0.0001*	0.011	0.023
LSM Value	0.135	0.012	0.692	11.671	<0.0001*	0.112	0.158
CAP value	0.017	0.001	0.81	16.794	<0.0001*	0.015	0.02

There were significant positive associations of HbA1c (P = 0.039), cholesterol (P = 0.005), LDL (P = 0.047), BMI (P = 0.019), and CAP value (P < 0.0001) with NAFLD. (**Table. 8).**

Table 8. Multivariable regression analysis to determine possible risk factors for NAFLD

Variables	Unstandardized Coefficients		OR	Test value	P-value	95.0% Confidence Interval for B	
	B	Std. Error				Lower Bound	Upper Bound
(Constant)	-2.771	1.803				-6.342	0.799
Age (Years)	-0.001	0.002	0.999	-0.504	0.615	-0.004	0.002
Male	-0.005	0.049	0.995	-0.103	0.918	-0.102	0.092
Hb	-0.004	0.006	0.996	-0.713	0.477	-0.016	0.007
PLTs	0.0002	0.001	1	-0.036	0.971	-0.002	0.002
WBCs	0.01	0.016	1.0101	0.622	0.535	-0.022	0.042
ALT	0.002	0.002	1.002	1.064	0.289	-0.002	0.006
AST	0.002	0.004	1.002	0.605	0.546	-0.005	0.009
Alb	-0.036	0.093	0.9646	-0.386	0.7	-0.219	0.148
Total Bil	0.304	0.328	1.3553	0.928	0.356	-0.346	0.954
Direct Bil	-0.76	0.838	0.4677	-0.908	0.366	-2.419	0.898
PT	-0.202	0.134	0.8171	-1.51	0.134	-0.468	0.063
INR	1.18	1.692	3.2544	0.697	0.487	-2.171	4.531
Serum Urea	-0.008	0.018	0.992	-0.453	0.651	-0.044	0.028
Serum creatinine	0.359	0.366	1.4319	0.982	0.328	-0.366	1.084
HOMA IR	0.001	0.012	1.001	0.086	0.931	-0.023	0.025
Adiponectin	0.001	0.003	1.001	0.348	0.729	-0.005	0.006
F. blood glucose	-0.001	0.003	0.999	-0.331	0.741	-0.008	0.006
HbA1C	0.17	0.081	1.1853	2.087	0.039*	0.009	0.331

Cholesterol	0.003	0.001	0.997	2.866	0.005*	-0.005	0.001
triglycerides	0	0.001	1	0.153	0.878	-0.002	0.003
HDL	-0.007	0.006	0.993	-1.15	0.253	-0.018	0.005
LDL	0.004	0.002	1.004	2.005	0.047*	0	0.008
VLDL	-0.001	0.004	0.999	-0.213	0.832	-0.008	0.007
Na	0	0.009	1	-0.05	0.96	-0.018	0.017
K	0.021	0.056	1.0212	0.37	0.712	-0.09	0.131
Ca	-0.031	0.04	0.9695	-0.773	0.441	-0.111	0.049
BMI	0.031	0.013	1.0315	2.375	0.019*	0.005	0.057
WC	0.005	0.003	0.995	1.714	0.089	-0.011	0.001
LSM Value	0.013	0.018	1.0131	0.718	0.474	-0.022	0.047
CAP value	0.014	0.002	1.0141	6.669	<0.0001*	0.01	0.018

Discussion

Our study findings revealed no significant differences in age or sex between groups. However, both BMI and WC were significantly elevated in NAFLD cases compared to controls. Specifically, NAFLD cases were obese with higher WC, with all pairwise comparisons showing statistical significance.

Verma et al. (2023) found no significant differences in age, gender, or BMI between cases and controls, aligning with our results. Similarly, **Mathew et al. (2024)** reported comparable ages (~50 years) between NAFLD and non-NAFLD groups, indicating no age-related significance.

However, **Mathew et al. (2024)** reported a higher male prevalence in the NAFLD group (58% vs. 38%) and a strong association with central obesity (79.2% vs. 36% with obese WC), contrasting our findings on sex distribution but supporting the link between increased BMI, WC, and NAFLD.

Our study findings showed no significant differences in hemoglobin or platelet count between groups. However, WBC was significantly higher in NAFLD cases than in controls, with no significant variation across subgroups.

These findings are consistent with **Feng et al. (2016),** who observed stable platelet counts, slightly reduced iron, and mildly elevated WBCs in NAFLD, indicating low-grade inflammation. **Chung et al. (2016)** also reported significantly higher WBCs in NAFLD, supporting this link. However, **Zhong et al. (2021)** found elevated hemoglobin in NAFLD, differing from our results and possibly reflecting a compensatory response to steatosis.

Our study also revealed significantly elevated liver enzymes in NAFLD cases. ALT and AST levels were markedly higher than in controls, with NAFLD values exceeding those in non-NAFLD. Albumin was significantly lower in cases compared to controls. Total bilirubin was higher in NAFLD than in controls. Prothrombin time and INR were significantly prolonged in both NAFLD and non-NAFLD subgroups, without inter-subgroup differences.

All participants were negative for viral hepatitis. These enzyme and function changes are supported by **Liu et al. (2021)** and **Zhong et al. (2021),** who both reported significantly elevated ALT and AST levels in NAFLD patients, consistent with hepatocellular damage that worsens with disease severity.

Our study further demonstrated significantly higher fasting glucose, HOMA-IR, and HbA1c in NAFLD cases than in controls, indicating pronounced insulin resistance. Adiponectin was significantly reduced in NAFLD. Lipid profile analysis revealed elevated cholesterol, LDL, VLDL, and triglycerides, along with significantly reduced HDL in both NAFLD and non-NAFLD cases versus controls. Sodium and calcium levels showed no significant differences. Potassium was lower in cases compared to controls.

These findings are in harmony with **Liu et al. (2021),** who also noted elevated HOMA-IR, triglycerides, cholesterol, and LDL-C, with significantly lower HDL-C in NAFLD cases. **Zhang et al. (2018)** reported similar HOMA-IR elevation in NAFLD (8.72 ± 3.03) compared to non-NAFLD (4.80 ± 1.86, $P < 0.01$). **Gonullu et al. (2010), Polyzos et al. (2011), and Aleidi et al. (2015),** and all observed significantly lower adiponectin levels in NAFLD, consistent with our results.

Zhong et al. (2021) confirmed these lipid abnormalities, reporting higher triglycerides (1.69 ± 1.17 vs. 0.99 ± 0.65 mmol/L), total cholesterol (5.36 ± 0.97 vs. 5.00 ± 0.92 mmol/L), and LDL-C (3.19 ± 0.84 vs. 2.86 ± 0.79 mmol/L), and lower HDL-C (1.21 ± 0.26 vs. 1.43 ± 0.34 mmol/L) in NAFLD groups ($P < 0.001$).

However, **Kim et al. (2015)** found higher potassium in those with more insulin resistance (4.25 ± 0.48 vs. 4.09 ± 0.44 mEq/L, $P = 0.015$), in contrast to our findings. Overall, our data reinforces the link between NAFLD, insulin resistance, and metabolic dysregulation, largely consistent with the literature.

Our study findings revealed significantly higher liver stiffness in NAFLD cases compared to controls. Advanced fibrosis (F3/F4) was present in 58.18% of NAFLD patients, while all controls showed F0 fibrosis. CAP was highest in NAFLD, followed by non-NAFLD and controls, with a statistically significant difference. Steatosis grading confirmed that 63.64% of NAFLD cases had moderate steatosis (S2) and 27.27% had severe steatosis (S3), while no steatosis was observed in controls.

These findings are supported by **Koehler et al. (2016),** who reported higher liver stiffness in NAFLD patients, and by **Tang et al. (2023),** who found more advanced fibrosis and steatosis in NAFLD compared to non-NAFLD and healthy individuals.

Our study showed that HOMA-IR had strong diagnostic accuracy for NAFLD, with 80% overall accuracy. Liver stiffness measurement also had high AUC and good performance in detecting fibrosis. CAP was the most reliable for steatosis, showing nearly perfect sensitivity and specificity. Conversely, adiponectin performed poorly as a diagnostic marker, with accuracy below 40%.

Zeng et al. (2023) found HOMA-IR >2.0 had high sensitivity (84%) for detecting metabolic risk in NAFLD, supporting our results. Despite adiponectin's low diagnostic value in our study, **Boutari and Mantzoros (2020)** and **Zhang et al. (2019)** highlighted its potential in predicting NAFLD and its progression, especially in older adults.

This study has some limitations, including the relatively small sample size that may limit generalizability. Liver biopsy was not performed, reducing diagnostic precision compared to the gold standard. Also, unmeasured factors like diet, physical activity, and genetics may have influenced the results.

Regression analysis revealed that higher levels of levels of PLTs, ALT, AST, total bilirubin, PT, INR, serum creatinine, HOMA-IR, fasting blood glucose, HbA1c,

cholesterol, triglycerides, LDL, VLDL, BMI, waist circumference (WC), liver stiffness measurement (LSM), and controlled attenuation parameter (CAP) values, highlighting the combined impact of poor glycemic control, dyslipidemia, obesity, and liver fat accumulation on the development of the disease. However, adiponectin was negatively associated with NAFLD.

Our study can be supported by **Jung and Choi (2014)** findings where multivariable regression analysis showed that elevated HbA1C, cholesterol, LDL and BMI levels are significantly linked to a higher risk of NAFLD, emphasizing the combined effects of impaired glycemic control, dyslipidemia, obesity, and in disease development.

Yadav et al., (2013), supported our study by reporting that adiponectin negatively correlated with markers of metabolic dysfunction in NAFLD cases including BMI, waist circumference and HOMA-IR.

Our study aligns with **Zeng et al. (2023)** study, who reported a strong positive association between elevated HOMA-IR levels and the prevalence of NAFLD. Additionally, **Zhang et al. (2019)** reported that low adiponectin levels are a associated with the progression to NAFLD among middle-aged and elderly subjects.

This study has some limitations, including the relatively small sample size that may limit generalizability. Liver biopsy was not performed, reducing diagnostic precision compared to the gold standard. Also, unmeasured factors like diet, physical activity, and genetics may have influenced the results.

Conclusion

Insulin-resistant, non-diabetic, non-alcoholic individuals with NAFLD showed marked elevations in ALT, AST, fasting glucose, HOMA-IR, HbA1c, lipid levels, and liver stiffness, along with significantly reduced adiponectin, compared to non-NAFLD and healthy controls.

References

- **Aleidi S, Issa A, Bustanji H, Khalil M, Bustanji Y. (2015).** Adiponectin serum levels correlate with insulin resistance in type 2 diabetic patients. Saudi Pharmaceutical Journal, 23(3): 250–256.
- **Boutari C, Mantzoros CS. (2020).** Adiponectin and leptin in the diagnosis and therapy of NAFLD. Metabolism-Clinical and Experimental, 103(1): e154028.
- **Chung GE, Yim JY, Kim D, Kwak MS, Yang JI, Chung SJ, et al. (2016).** Associations between White Blood Cell Count and the Development of Incidental Nonalcoholic Fatty Liver Disease. Gastroenterology Research and Practice, 2016(1): e7653689.
- **Feng YM, Zhao D, Zhang N, Yu CG, Zhang Q, Thijs L, Staessen JA. (2016).** Insulin resistance in relation to lipids and inflammation in type-2 diabetic patients and non-diabetic people. PLoS One, 11(4): e0153171.
- **Gonullu G, Kahraman H, Bedir A, Bektas A, Yücel I. (2010).** Association between adiponectin, resistin, insulin resistance, and colorectal tumors. International Journal of Colorectal Disease, 25(2): 205–212.
- **Gutierrez-Buey G, Núñez-Córdoba JM, Llavero-Valero M, Gargallo J, Salvador J, Escalada J. (2017).** Is HOMA-IR a potential screening test for non-alcoholic fatty liver disease in adults with type 2 diabetes? European journal of internal medicine, 41(1): 74-78.
- **Jung UJ, Choi MS. (2014).** Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance,

dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences (Int. J. Mol. Sci), 15(4): 6184-6223.

- **Kim HW, Lee DH, Lee SA, Koh G. (2015).** A relationship between serum potassium concentration and insulin resistance in patients with type 2 diabetes mellitus. International Urology and Nephrology, 47(1): 991–999.
- **Koehler EM, Plompen EP, Schouten JN, Hansen BE, Darwish Murad S, Taimr P, et al. (2016).** Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: the Rotterdam study. Hepatology, 63(1): 138–147.
- **Lee SH, Park SY, Choi CS. (2022).** Insulin resistance: from mechanisms to therapeutic strategies. Diabetes and Metabolism Journal, 46(1): 15–37.
- **Liu C, Shao M, Lu L, Zhao C, Qiu L, Liu Z. (2021).** Obesity, insulin resistance and their interaction on liver enzymes. PLoS One, 16(4): e0249299.
- **Loomba R, Friedman SL, Shulman GI. (2021).** Mechanisms and disease consequences of nonalcoholic fatty liver disease. Cell, 184(10): 2537–2564.
- **Mathew M, Pope ZC, Schreiner PJ, Jacobs DR Jr, VanWagner LB, Terry JG, et al. (2024).** Non-alcoholic fatty liver modifies associations of body mass index and waist circumference with cardiometabolic risk: The CARDIA study. Obesity Science and Practice, 10(2): e751.
- **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner R (1985).** Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. diabetologia, 28(1): 412-419.
- **Padda J, Khalid K, Khedr A, Tasnim F, Al-Ewaidat OA, Cooper AC, et al. (2021).** Non-alcoholic fatty liver disease and its association with diabetes mellitus. Cureus, 13(8), e17321.
- **Piccinni R, Rodrigues SG, Montani M, Murgia G, Delgado MG, Casu S, et al. (2020).** Controlled attenuation parameter reflects steatosis in compensated advanced chronic liver disease. *Liver International*, *40*(5): 1151-1158.
- **Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. (2011).** Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism, 60(3): 313–326.
- **Qiu, Y. Y., Zhang, J., Zeng, F. Y., and Zhu, Y. Z. (2023).** Roles of the peroxisome proliferator-activated receptors (PPARs) in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Pharmacological Research, 192, 106786.
- **Serra JT, Mueller J, Teng H, Elshaarawy O, Mueller S (2020).** Prospective comparison of transient elastography using two different devices: performance of FibroScan and FibroTouch. Hepatic Medicine: Evidence and Research, 1(1): 41-48.
- **Tanase DM, Gosav EM, Costea CF, Ciocoiu M, Lacatusu CM, Maranduca MA, et al. (2020).** The intricate relationship between type 2 diabetes mellitus (T2DM), insulin resistance (IR), and nonalcoholic fatty liver disease (NAFLD). Journal of Diabetes Research, 2020(1): 3920196.
- **Tang A, Ng CH, Phang PH, Chan KE, Chin YH, Fu CE, et al. (2023).** Comparative burden of metabolic dysfunction in lean NAFLD vs non-lean NAFLD – A systematic review and meta-analysis. Clinical Gastroenterology and Hepatology, 21(7): 1750–1760.e12.

- **Verma S, Verma M, Khari S. (2023).** Association of hematological parameters (mean platelet volume and red cell distribution width) with nonalcoholic fatty liver disease. APIK Journal of Internal Medicine, 11(1): 37–44.
- **Yadav A, Kataria MA, Saini V, Yadav A. (2013).** Role of leptin and adiponectin in insulin resistance. Clinica acta, 41(7): 80-84.
- **Yang B, Gong M, Zhu X, Luo Y, Li R, Meng H, Wang Y. (2025).** Correlation between liver fibrosis in non-alcoholic fatty liver disease and insulin resistance indicators: a cross-sectional study from NHANES 2017–2020. Frontiers in Endocrinology, 16(1): 1514093.
- **Zeng P, Cai X, Yu X, Huang L, Chen X. (2023).** HOMA-IR is an effective biomarker of non-alcoholic fatty liver disease in non-diabetic population. Journal of International Medical Research, 51(10): 3000605231204462.
- **Zeng, P., Cai, X., Yu, X., Huang, L., and Chen, X. (2023).** HOMA-IR is an effective biomarker of non-alcoholic fatty liver disease in non-diabetic population. Journal of International Medical Research (J Int Med Re). 51(10): 1-20.
- **Zhang H, Niu Y, Gu H, Lu S, Zhang W, Li X, et al. (2019).** Low serum adiponectin is a predictor of progressing to nonalcoholic fatty liver disease. Journal of Clinical Laboratory Analysis, 33(3): e22709.
- **Zhang Z, Wang J, Wang H. (2018).** Correlation of blood glucose, serum chemerin and insulin resistance with NAFLD in patients with type 2 diabetes mellitus. Experimental and Therapeutic Medicine, 15(3): 2936–2940.
- **Zhao, X., An, X., Yang, C., Sun, W., Ji, H., and Lian, F. (2023).** The crucial role and mechanism of insulin resistance in metabolic disease. Frontiers in endocrinology, 14, 1149239.
- **Zhong F, Guan L, Lin H, Zhao M, Qin Y, Li Q, et al. (2021).** Red blood cell count: an unrecognized risk factor for nonalcoholic fatty liver disease. Frontiers in Endocrinology, 12(1): e760981.