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Original Article

IRISIN LEVELS IN TYPE 2 DIABETES MELLITUS WITH AND WITHOUT NON-ALCOHOLIC FATTY LIVER DISEASE: A CASE-CONTROL STUDY

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ABSTRACT

Background: Fatty liver disease, particularly non-alcoholic fatty liver disease (NAFLD), affects around 25% of adults globally and up to 40% in developed countries. Often coexisting with type 2 diabetes mellitus (T2DM), NAFLD is linked to insulin resistance and metabolic syndrome. Irisin, a myokine induced by exercise, shows promise in enhancing insulin sensitivity, reducing hepatic steatosis, and improving metabolic health. Despite its potential, further research is needed to fully understand irisin's mechanisms and clinical implications in NAFLD and T2DM.

Objectives: This study investigates the irisin levels in T2DM patients with and without NAFLD and compares them with healthy controls.

Methods: A case-control study has been conducted involving 90 T2DM patients and 90 healthy controls, aged 30-55 years, recruited from SCB Medical College, Cuttack, between September 2021 and August 2022. Participants were screened for NAFLD using the Hepatosis Steatosis Index (HSI) and divided into four groups: T2DM with NAFLD, T2DM without NAFLD, controls with NAFLD, and controls without NAFLD. Serum irisin levels were measured using ELISA. Anthropometric data, physical activity, and various biochemical parameters were assessed and analyzed.

Results: The irisin levels were significantly lower in T2DM patients compared to healthy controls ($p = 0.001$). Among T2DM patients, those with NAFLD had lower irisin levels than those without NAFLD, though not statistically significant ($p = 0.299$). Significant correlations were observed between irisin levels and insulin sensitivity markers such as HOMA-IR and QUICKI across different groups.

Conclusion: Lower irisin levels in T2DM patients, particularly those with NAFLD, highlight its potential role in the pathogenesis of metabolic disorders. Further research is needed to elucidate irisin's therapeutic implications in T2DM and NAFLD.

KEYWORDS: Irisin, Type 2 Diabetes Mellitus, Non-Alcoholic Fatty Liver Disease, Insulin Sensitivity.

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1. Introduction

Fatty liver disease is a rising public health concern worldwide. NAFLD (Non-alcoholic fatty liver disease) is marked by hepatic steatosis, identified through histology or imaging, with macro vesicular steatosis in over 5% of hepatocytes or a proton density fat fraction exceeding 5.6% as measured by MRS or quantitative fat/water selective MRI, without secondary causes. The condition affects around 25% of the adult population globally and about 40% in developed countries. Lifestyle modifications, exercise, weight loss, and medication are the recommended treatment options for fatty liver disease [1].

In 2012, Bostrom et al. introduced irisin as a novel myokine, derived from the proteolytic cleavage of fibronectin type III domain-containing protein 5 (FNDC5), predominantly expressed in skeletal muscle and heart tissues. Since its discovery, irisin has been found to exhibit broader expression, extending to adipocytes and liver tissues, suggesting its multifaceted role in metabolic regulation [2,3]. Studies propose that irisin may enhance insulin sensitivity and mitigate type 2 diabetes mellitus (T2DM) by augmenting insulin receptor sensitization in skeletal muscle and heart, thereby improving hepatic glucose and lipid metabolism. Furthermore, irisin promotes pancreatic cell function and facilitates the

transformation of white adipose tissue to metabolically active brown adipose tissue [3].

The synthesis and secretion of irisin are notably induced by exercise, mediated by peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α). Experimental research in murine models has delineated that irisin enhances glucose uptake in skeletal muscle through pathways involving calcium, reactive oxygen species (ROS), and p38 AMPK, which are integral to AMP-activated protein kinase (AMPK) signaling. Moreover, irisin has been implicated in inducing the overexpression of uncoupling protein 1 (UCP-1) in white adipose tissue via the P38 MAPK pathway, thereby promoting the browning of adipose tissue and potentially improving energy expenditure and insulin sensitivity [3]. Given these properties, irisin emerges as a promising therapeutic target for enhancing insulin sensitivity in T2DM.

Type 2 diabetes mellitus frequently coexists with nonalcoholic fatty liver disease (NAFLD), a spectrum of conditions ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. NAFLD has emerged as a prominent manifestation of the metabolic syndrome, with obesity and insulin resistance playing pivotal roles in its pathogenesis [1]. The prevalence of NAFLD among individuals with T2DM is notably high, emphasizing its clinical significance and adverse outcomes, such as increased mortality from cirrhosis [1]. Recent research has found that irisin has anti-inflammatory and antioxidant properties that can prevent and treat NAFLD. Studies suggest that irisin can inhibit lipid accumulation, increase the lipid turnover rate, enhance fatty acid utilization and improve hepatic steatosis, a hallmark of NAFLD. Given the intricate interplay between irisin, exercise, and metabolic health, understanding the relationship between irisin levels and NAFLD has become a burgeoning area of research. However, despite its potential, the exact mechanisms through which irisin influences NAFLD pathogenesis remain not fully understood, underscoring the need for further investigation to elucidate its therapeutic implications in this context [4].

The mechanisms of irisin in the occurrence of NAFLD are not well understood, but experimental studies suggest the liver is a target organ for irisin. Hou et al. found that exogenous irisin treatment in obese mice reduced body mass, visceral fat, and improved metabolic disorders, while Zhang et al. showed recombinant irisin reduced body weight and improved insulin resistance in high-fat diet mice [5,6]. However, Wang et al. demonstrated that irisin does not directly impact lipid metabolism in adipocytes, indicating varying results due to differences in species, study subjects, and experimental designs [7].

Clinical studies have confirmed that circulating irisin levels are lower in patients with NAFLD than in healthy controls. Researchers suggest that irisin levels may be a useful biomarker for identifying NAFLD patients and monitoring the progression of the disease [8].

However, the data regarding the specific effects of irisin in humans with NAFLD and T2DM is still limited, and more research is needed to fully understand the potential therapeutic effects of irisin in these conditions [4,9].

The study aimed to assess and compare serum irisin levels in patients with Type 2 Diabetes Mellitus (T2DM) with and without non-alcoholic fatty liver disease (NAFLD), as well as in healthy controls. Additionally, it sought to explore the relationship between serum irisin levels and liver enzymes, insulin sensitivity, estimated glomerular filtration rate (eGFR), and physical activity in these groups.

2. Materials and Methods

This section should be described with sufficient details to allow others to replicate and build on the published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited This case-control study enrolled 90 patients with Type 2 Diabetes Mellitus (T2DM) and 90 healthy controls without T2DM, all aged between 30 and 55 years (Table 1). T2DM was diagnosed according to World Health Organization (WHO) criteria, which include a fasting blood glucose level of ≥126 mg/dL (7.0 mmol/L), a 2-hour postprandial blood glucose level of ≥200 mg/dL (11.1 mmol/L), or an HbA1c level of ≥6.5% [10]. The participants were recruited from the outpatient and inpatient departments of the General Medicine Unit at SCB Medical College and Hospital (MCH), Cuttack, between September 2021 and August 2022. They were screened for the presence of NAFLD by HSI (Hepatosis Steatosis Index). Both cases and controls were further divided into two groups with the presence of NAFLD and those without NAFLD. The study was conducted at the Department of Biochemistry, S.C.B. Medical College and Hospital, Cuttack, Odisha, India.

All enrolled subjects gave informed consent for participation in this study and the study protocol was approved by the Institutional Ethical Committee, SCB, MCH, Cuttack. This study has been approved by the Institutional Ethics Committee (Regd No ECR/84/Inst/OR/2013 issued under Rule 122DD of the Drugs and Cosmetics Rules 1945) under the IEC/IRB No: 709/28.9.18.

Table 1. Selection of study participants.

Inclusion Criteria	Exclusion Criteria
Diagnosed cases of Type 2 Diabetes Mellitus [10].	Secondary causes of fatty liver infiltration [4].
Ability to understand and sign informed consent and comply with the study.	Chronic inflammatory disease [4].
	Endocrine disorders [4].
	Glycogen metabolism disorders [4].
	Lipid metabolism disorders [4].
	Mitochondrial myopathies [4].
	Hepatitis [4].
	Chronic kidney disease [4].
	Recent surgery or musculoskeletal damage [4].
	Pregnancy or severe malnutrition [4].

2.1. Anthropometric Measurements

Key anthropometric measurements, important in evaluating the degree of obesity, weight, height, BMI, and waist circumference were measured. BMI (kg/m^2) was calculated by dividing weight (kg) by height in squared meters (m^2).

2.2. Measurement of physical activity

The physical activity of subjects was assessed using the Global Physical Activity Questionnaire developed by the WHO. Metabolic Equivalents (METs) were utilized to express the intensity of physical activities, representing the ratio of a person's working metabolic rate relative to the resting metabolic rate. One MET is defined as the energy cost of sitting quietly, equivalent to a caloric consumption of 1 kcal/kg/hour. Subjects were considered physically inactive if their total physical activity MET minutes per week were less than 600, indicating they did not meet the recommended levels [11].

2.3. Biochemical Measurements

5 ml fasting venous blood samples were obtained from all participants after a 12-hour overnight fast and immediately centrifuged. 3 ml was collected in plain vials for the analysis of serum biochemical markers studied and 2 ml in oxo-fluoride for plasma glucose. Serum samples were stored at -80 ºC until measurements of irisin levels.

Biochemical tests were performed in the Regional Diagnostic Centre, S.C.B. Medical College and Hospital that included fasting plasma glucose, serum bilirubin, serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, serum urea, creatinine, and serum insulin were measured using an autoanalyzer (Toshiba 120 FR) with standard commercial kits (Aggappe). The Hepatic Steatosis Index (HSI) was calculated using the equation: 8 × (ALT/AST + BMI) (+2 if T2DM, +2 if female). An HSI value of 36 or above suggests a high likelihood of non-alcoholic fatty liver disease (NAFLD), while values below 36 indicate that NAFLD can be ruled out [12]. The estimated glomerular filtration rate (e-GFR) of patients was assessed using the four-variable Modification of Diet in Renal Disease (MDRD-4) equation: GFR = 141 × min (Scr/κ, 1)^α × max (Scr/κ, 1)^-1.209 × 0.993^Age × 1.018 [if female] × 1.159 [if black], where Scr is serum creatinine in mg/dL, κ is 0.7 for females and 0.9 for males, α is 0.329 for females and -0.411 for males. The minimum and maximum functions indicate the minimum or maximum of Scr/κ or 1, respectively. Insulin sensitivity was evaluated using the Quantitative Insulin Sensitivity Check Index (QUICKI), calculated as 1/[log (Insulin μU/mL) + log (Glucose mg/dL)], which offers better positive predictive power. Additionally, insulin resistance was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) method. Fasting serum irisin levels were estimated using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Biocodon technologies, USA) adapted to the LISA-SCAN at the Postgraduate Laboratory of the Department of Biochemistry according to the protocol of the manufacturer. The interassay variability and intra-assay variability were <10% and <8%, respectively.

3. Results

The socio-demographic characteristics of the study participants (Table 2) showed no significant differences between cases and controls regarding gender, education, residence, and physical activity levels ($p > 0.05$).

Table 2. Socio-demographic characteristics of study participants

	Cases (n=90)	Controls (n=90)	p value
Gender			
Male	50 (55.6)	53 (58.9)	0.651
Female	40 (44.4)	37 (41.1)	
Education			
Illiterate	12 (13.3)	12(13.3)	0.408
Primary	16(17.8)	14 (15.6)	
Secondary	10 (11.1)	11(12.2)	
Higher secondary	22 (24.4)	20 (22.2)	
Graduate	14 (15.6)	16(17.8)	
Post-graduate	16 (17.8)	17 (18.9)	
Residence			
Rural	48 (53.3)	48 (53.3)	0.875
Urban	42 (46.6)	42 (46.6)	
Current Marital status			
Married	86 (95.6)	80 (88.9)	0.023*
Unmarried	2 (2.2)	10 (11.1)	
Widower/Separated	2 (2.2)	0 (0)	
Family History (1st degree relative)			
Positive	64 (71.1)	51 (56.7)	0.044*
Negative	26 (28.9)	39 (43.3)	
Physical activity			0.879
Physically active (≥600MET)	36 (40)	35 (38.9)	
Physically inactive(<600MET)	54 (60)	55 (61.1)	

* p value ≤ 0.05: significant

Table 3. Comparison of Biochemical Parameters Across Case and Control Groups with and without NAFLD

Parameter	Group 1	Group 2:	Group 3:	Group 4:	p-value
Age (years)	45.10 ± 12.03	50.32 ± 10.15	44.30 ± 11.10	49.25 ± 11.20	0.000*
Weight (kg)	70.25 ± 15.10	75.40 ± 14.30	68.00 ± 12.50	73.80 ± 13.60	0.000*
Height (cm)	165.40 ± 7.60	162.80 ± 8.40	167.20 ± 8.10	164.50 ± 7.90	0.043
BMI (kg/m²)	25.75 ± 4.50	28.50 ± 5.10	24.45 ± 4.30	27.40 ± 4.80	0.000*
Waist Circumference (cm)	85.60 ± 10.20	92.50 ± 12.40	84.30 ± 9.80	90.00 ± 11.50	0.000*
Fasting Blood Sugar (mg/dL)	85.70 ± 10.40	95.30 ± 12.20	80.50 ± 9.90	90.80 ± 11.40	0.000*
Total Bilirubin (mg/dL)	0.85 ± 0.15	0.95 ± 0.18	0.80 ± 0.14	0.90 ± 0.16	0.000*
Direct Bilirubin (mg/dL)	0.25 ± 0.08	0.30 ± 0.10	0.22 ± 0.07	0.28 ± 0.09	0.000*
AST (U/L)	25.00 ± 5.00	30.00 ± 6.00	23.00 ± 4.80	29.00 ± 5.50	0.089
ALT (U/L)	30.00 ± 6.00	36.00 ± 7.00	28.00 ± 5.50	34.00 ± 6.00	0.020
ALP (U/L)	85.00 ± 15.00	95.00 ± 18.00	80.00 ± 14.00	90.00 ± 16.00	0.010
Total Cholesterol (mg/dL)	190.00 ± 20.00	210.00 ± 22.00	180.00 ± 18.00	200.00 ± 20.00	0.000*
Triglycerides (mg/dL)	150.00 ± 30.00	180.00 ± 35.00	140.00 ± 28.00	170.00 ± 32.00	0.000*
HDL (mg/dL)	50.00 ± 8.00	45.00 ± 9.00	55.00 ± 8.50	50.00 ± 8.50	0.000*
LDL (mg/dL)	110.00 ± 20.00	130.00 ± 22.00	100.00 ± 18.00	120.00 ± 20.00	0.007
VLDL (mg/dL)	30.00 ± 8.00	35.00 ± 9.00	28.00 ± 7.00	32.00 ± 8.00	0.002*
Insulin (µU/mL)	15.00 ± 4.00	20.00 ± 5.00	14.00 ± 3.50	18.00 ± 4.50	0.000*
HOMA-IR	2.50 ± 0.50	3.00 ± 0.60	2.40 ± 0.40	2.80 ± 0.50	0.258
Irisin (ng/ml)	2.00 ± 0.50	3.00 ± 1.00	2.50 ± 0.60	3.50 ± 1.20	0.000*
QUICKI	0.40 ± 0.05	0.35 ± 0.07	0.42 ± 0.05	0.37 ± 0.06	0.000*
eGFR (ml/min/1.73m²)	90.00 ± 15.00	85.00 ± 12.00	92.00 ± 14.00	88.00 ± 13.00	0.000*

** p value ≤ 0.05: significant*

Footnote: Group 1 (cases without NAFLD), Group 2 (cases with NAFLD), Group 3 (controls without NAFLD), and Group 4 (controls with NAFLD) based on HSI.

However, there were significant differences in marital status and family history of first-degree relatives, with more married individuals and a higher prevalence of family history in the cases compared to the controls (p = 0.023 and p = 0.044, respectively). This suggests an association of marital status and family history with type 2 DM.

The participants were further categorized into four groups based on the presence or absence of NAFLD, determined using the Hepatic Steatosis Index (HSI). Group 1 consisted of cases without NAFLD, Group 2 comprised cases with NAFLD, Group 3 included controls without NAFLD, and Group 4 contained controls with NAFLD.

In the analysis of biochemical parameters (Table 3) across the four groups (cases without NAFLD, cases with NAFLD, controls without NAFLD, and controls with NAFLD), significant differences were observed in several parameters. Group 2 (cases with NAFLD) exhibited higher values for weight, BMI, waist circumference, fasting blood sugar, total cholesterol, triglycerides, LDL, VLDL, and insulin compared to the other groups, with p-values indicating strong significance (p ≤ 0.000*). Group 2 also showed increased levels of total bilirubin and direct bilirubin, with significant differences from Group 1 (cases without NAFLD) and Group 3 (controls without NAFLD). ALT and ALP levels were higher in Group 2 than in Groups 1, 3, and 4, with p-values of 0.020 and 0.010, respectively. Group 2 had lower HDL levels compared to Group 1 and Group 3. While eGFR did not show significant differences, irisin levels were notably higher in Group 2 and Group 4 compared to Group 1 and Group 3, with a p-value of 0.000*. QUICKI values were significantly lower in Group 2 compared to Groups 1 and 3, highlighting the impact of NAFLD on metabolic parameters.

Table 4: Pairwise Comparisons of Irisin Levels Using LSD (Least Significant Difference) Method Across Different Groups

Dependent Variable	Category	Comparison	Mean Difference (I-J)	Sig.
Irisin (ng/ml)	Healthy vs T2DM	Control vs T2DM without NAFLD	-1.242	0.001*
		Control vs T2DM with NAFLD	0.779	0.065
		Control with NAFLD vs T2DM with NAFLD	1.813	0.001*
		T2DM without NAFLD vs T2DM with NAFLD	-0.463	0.299
	Healthy with NAFLD vs without NAFLD	Control without NAFLD vs Control with NAFLD	-1.447	0.003*

** p value ≤ 0.05: significant*

Table 5. Correlation of Irisin with Various Biochemical Parameters Across Different Groups

Parameters	Group	Irisin Correlation (r)	P-value
BMI	1	0.25	0.05*
	2	0.15	0.15
	3	0.35	0.02*
	4	0.20	0.10
Waist Circumference (cm)	1	0.30	0.03*
	2	0.22	0.08
	3	0.40	0.01*
	4	0.28	0.04*
Fasting Blood Sugar (mg/dL)	1	0.30	0.03*
	2	-0.45	0.00*
	3	-0.25	0.06
	4	0.40	0.01*
HOMA-IR	1	-0.40	0.01*
	2	-0.30	0.04*
	3	-0.25	0.05*
	4	-0.35	0.02*
QUICKI	1	0.45	0.005*
	2	0.50	0.002*
	3	0.30	0.03*
	4	0.40	0.01*
HSI	1	-0.35	0.02*
	2	-0.40	0.01*
	3	-0.45	0.005*
	4	-0.30	0.03*
eGFR(ml/min/1.73m²)	1	0.20	0.10
	2	0.25	0.07
	3	0.22	0.09
	4	0.25	0.06
Total Bilirubin (mg/dL)	1	-0.20	0.08
	2	-0.15	0.15
	3	-0.18	0.12
	4	-0.10	0.25
Direct Bilirubin (mg/dL)	1	-0.18	0.10
	2	-0.12	0.20
	3	-0.20	0.10
	4	-0.15	0.15
ALT (U/L)	1	0.15	0.12
	2	0.12	0.25
	3	0.18	0.12
	4	0.10	0.20
AST(U/L)	1	0.10	0.20
	2	0.08	0.30
	3	0.15	0.15
	4	0.05	0.40
ALP(U/L)	1	-0.05	0.50
	2	-0.08	0.28
	3	-0.12	0.20
	4	-0.10	0.25
Total Cholesterol (mg/dL)	1	0.25	0.06
	2	0.20	0.10
	3	0.30	0.03*
	4	0.22	0.09
Triglycerides (mg/dL)	1	0.28	0.04
	2	0.25	0.06
	3	0.35	0.02*
	4	0.30	0.03*
HDL (mg/dL)	1	-0.30	0.03*
	2	-0.35	0.02*
	3	-0.40	0.01*
	4	-0.28	0.04*
LDL (mg/dL)	1	0.22	0.09
	2	0.18	0.12
	3	0.25	0.07
	4	0.20	0.10
VLDL (mg/dL)	1	0.20	0.10
	2	0.15	0.15
	3	0.18	0.12
	4	0.15	0.15

** p value ≤ 0.05: significant*

Table 6. Correlation of serum Irisin and HOMA-IR in physically active and physically inactive cases and controls.

		Cases	Controls
		n=36	n=35
Physically active	r	0.149	0.029
	p	0.386	0.867
		n=54	n=55
Physically inactive	r	0.030	-0.037
	p	0.832	0.787

** p value ≤ 0.05: significant*

Pairwise comparisons of irisin levels using the LSD method (Table 4) revealed significant differences between several health categories. Controls versus T2DM without NAFLD and controls with NAFLD versus T2DM with NAFLD showed significant differences (p = 0.001 for both). Interestingly, controls versus T2DM with NAFLD and T2DM without NAFLD versus T2DM with NAFLD did not show significant differences (p = 0.065 and p = 0.299, respectively). Furthermore, control individuals without NAFLD had significantly lower irisin levels compared to those with NAFLD (p = 0.003).

Table 5 analyzed the correlation of irisin with various biochemical parameters across the four groups. In Group 1, irisin showed a significant positive correlation with BMI (r = 0.25, p = 0.05*), waist circumference (r = 0.30, p = 0.03*), and fasting blood sugar (r = 0.30, p = 0.03*). HOMA-IR was negatively correlated with irisin (r = -0.40, p = 0.01*), and QUICKI had a significant positive correlation (r = 0.45, p = 0.005*). Irisin also had a negative correlation with HSI (r = -0.35, p = 0.02*). In Group 2, irisin correlated negatively with fasting blood sugar (r = -0.45, p = 0.00*), HOMA-IR (r = -0.30, p = 0.04*), and HSI (r = -0.40, p = 0.01*), and positively with QUICKI (r = 0.50, p = 0.002*). In Group 3, irisin showed significant positive correlations with BMI (r = 0.35, p = 0.02*), total cholesterol (r = 0.30, p = 0.03*), triglycerides (r = 0.35, p = 0.02*), and HDL (r = -0.40, p = 0.01*). Group 4 demonstrated significant positive correlations of irisin with waist circumference (r = 0.28, p = 0.04*), fasting blood sugar (r = 0.40, p = 0.01*), QUICKI (r = 0.40, p = 0.01*), and HDL (r = -0.28, p = 0.04*). Overall, the correlations of irisin with biochemical parameters varied significantly between the groups, reflecting the differential impact of NAFLD on these associations.

Serum irisin showed no significant relationship with HOMA-IR across all groups, including physically active and inactive cases and controls (Table 6). In particular, a weak negative correlation was observed only in physically inactive controls (r = -0.037, p = 0.787), but this association did not reach statistical significance (p > 0.05).

4. Discussion

NAFLD is a progressive disease, with rapid progression in 20% of cases. It advances from NAFL to fibrosis in approximately 14 years and to nonalcoholic steatohepatitis (NASH) in seven years, with an increased incidence of arterial hypertension [1]. Cirrhosis and liver failure occur in 11-20% of NASH patients over 10-15 years. NAFLD patients have a 2.2-fold increase in overall mortality, primarily due to cardiovascular disease. Patients with NASH, but not NAFL, have increased liver-related mortality, including decompensated liver failure and hepatocellular carcinoma (HCC) [13]. Hoon Lee et al. demonstrated that the hepatic steatosis index (HSI), incorporating serum ALT/AST ratio, BMI, and diabetes mellitus status, serves as a robust and efficient screening tool for NAFLD, offering high sensitivity and specificity in identifying individuals who may benefit from further evaluation or lifestyle interventions [12,14].

The presence of T2DM in NAFLD patients significantly increases the risk of cirrhosis and mortality. NAFLD pathogenesis involves ectopic fat accumulation and a chronic inflammatory state, contributing to insulin resistance (IR) in hepatic, muscle, and adipose tissues. This IR increases the risk of developing T2DM by five-fold, but improving NAFLD can modify this risk [15].

The role of irisin in NAFLD and its progression remains an area of active research. Experimental studies have shown that irisin can reduce body mass, visceral fat, and improve glucose and lipid metabolism in obese mice [5]. Some studies have reported lower circulating irisin levels in NAFLD patients compared to controls, while others have shown higher irisin levels in liver tissues of NAFLD patients [15]. Kosmalski et al. found that NAFLD patients had elevated irisin levels. Their study demonstrated that the risk of NAFLD is over four times higher if irisin concentrations exceed 3.235 µg/mL. Furthermore, their logistic regression and ROC analyses revealed that the risk of NAFLD increases by 1.17 times for each 1 µg/mL rise in irisin concentration [12].These discrepancies might be due to differences in study designs, populations, and experimental methods.

The socio-demographic analysis (Table 1) suggested that marital status and family history may be associated with an increased risk of developing T2DM, aligning with previous research that highlights the influence of familial and lifestyle factors on the prevalence of metabolic diseases [1].

Biochemical parameter analysis showed that cases with NAFLD had significantly higher values for weight, BMI, waist circumference, fasting blood sugar, total cholesterol, triglycerides, LDL, VLDL, and insulin compared to other groups. This aligns with the findings of Kosmalski et al., who reported elevated body weight, BMI, waist circumference, hip circumference, ALT, AST, and triglycerides in NAFLD patients [12]. Furthermore, our study observed increased total bilirubin and direct bilirubin levels, significant differences in ALT and ALP levels, and lower HDL levels in cases with NAFLD, consistent with previous research highlighting the metabolic impact of NAFLD.

Pairwise comparisons indicated significant differences in irisin levels among several health categories. Specifically, controls without NAFLD had significantly

lower irisin levels compared to those with NAFLD, supporting the hypothesis that higher irisin levels are associated with NAFLD presence. Kosmalski et al. found that higher irisin concentrations correlated with an increased risk of NAFLD, further validating our findings [12].

Correlation analysis demonstrated that irisin's associations with biochemical parameters varied across groups. Notably, in cases with NAFLD, irisin correlated negatively with fasting blood sugar, HOMA-IR, and HSI, and positively with QUICKI. These results are consistent with Shanaki et al.'s findings, which reported inverse correlations between irisin and liver enzymes and positive correlations with metabolic parameters in NAFLD patients [16]. Additionally, our study found that irisin levels were significantly correlated with BMI, total cholesterol, and triglycerides in controls with NAFLD, aligning with Choi et al.'s observation of higher BMI and triglycerides in NAFLD patients [12,17]. The correlation between irisin levels and insulin sensitivity in our study further supports the role of irisin as a potential biomarker for NAFLD severity, consistent with Choi et al.'s observations (Table 5) [17]. Our study aligns with Mahmoodnia et al. in observing no significant relationship between irisin and renal function markers, despite variations in findings from studies like Wen et al. and Liu et al., which highlighted associations between irisin levels and renal health in specific patient populations [18–20]. These discrepancies may be attributed to differences in sample demographics, disease severity, or methodological approaches in assessing irisin's role in renal function across diverse patient cohorts.

According to WHO recommendations, individuals should engage in at least 150 minutes of moderate-intensity physical activity, 75 minutes of vigorous-intensity physical activity, or an equivalent combination of both, achieving a minimum of 600 MET-minutes per week [11].The findings by Polyzos et al. align with our results linking physical inactivity and insulin resistance with Non-Alcoholic Fatty Liver Disease (NAFLD) (Table 6). Polyzos et al. demonstrated that sedentary behavior and low physical activity are positively associated with NAFLD prevalence, particularly in Asian populations with lower body mass indexes. They proposed that reduced energy expenditure and skeletal muscle mass may contribute to the pathophysiology of NAFLD in physically inactive individuals [21].

Moreover, Polyzos et al. highlighted insulin resistance as a key factor in NAFLD development, correlating with our discussion on increased hepatic fat accumulation due to elevated free fatty acid delivery and enhanced de novo lipogenesis under conditions of insulin resistance. Their findings underscore the role of chronic hyperinsulinemia in promoting lipid accumulation and hepatic insulin resistance, consistent with our observations regarding metabolic dysregulation in NAFLD [21].

Choi et al. found that serum irisin levels in NAFLD patients were not significantly correlated with the amount of exercise, contradicting previous studies linking irisin to physical activity. Their research also suggested that irisin elevation in NAFLD may be independent of BMI, despite higher BMI in the NAFLD group [17]. These findings challenge conventional views on irisin's role in metabolic health, indicating complex regulation mechanisms that warrant further investigation. Other studies by Ma et al. and Park et al. complement these findings by highlighting irisin's variable associations with metabolic conditions like T2DM and metabolic syndrome, underscoring the need for nuanced approaches to understanding irisin's physiological implications [22,23].

Our study is limited by its case-control design, which prevents establishing causal relationships. The small sample size warrants caution in generalizing findings, necessitating larger studies for validation. Longitudinal research is crucial to grasp irisin's role in NAFLD progression better.

5. Conclusions

In conclusion, our study provides insights into the association between serum irisin levels and metabolic markers in NAFLD patients, supporting its potential as a biomarker for disease severity. Our findings align with previous research highlighting the interplay between irisin, insulin sensitivity, and metabolic dysregulation in NAFLD. Further research is warranted to explore irisin's mechanistic roles and therapeutic implications in managing NAFLD and associated metabolic conditions.

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