ORIGINAL RESEARCH

Changes in Serum Irisin Levels and Their Significance in Carotid Atherosclerosis Associated with Obesity

Shaohua Fu, BM; Guisheng Xing, BM

ABSTRACT

Objective • This study aims to comprehensively examine and assess the changes and significance of irisin levels in individuals with carotid atherosclerosis (CAS) who are concurrently dealing with obesity.

Method • A total of 156 CAS patients admitted between March 2020 and March 2022 were categorized into CAS normal weight (C1), CAS overweight (C2), and CAS obesity (C3) groups based on BMI. Simultaneously, 80 healthy adults from the same period comprised the control group (C0). The study conducted a comparative analysis of biochemical indexes, serum irisin levels, and carotid artery ultrasounds across all groups. Correlation analyses and diagnostic assessments for serum irisin in obesity with carotid atherosclerosis were executed using ROC curves.

Results • Statistically significant differences were observed in cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum homocysteine (Hcy), and irisin levels among all groups ($P < .05$). Obese CAS patients exhibited higher levels of TC, TG, LDL-C, and Hcy, with notably lower levels of HDL-C and irisin compared to other groups ($P < .05$). Significant variations in carotid artery ultrasound intima-media thickness (IMT) and Crouse scores were also evident among all groups ($P < .05$). Specifically, IMT and Crouse scores were higher in obese CAS patients compared to other groups ($P < .05$). Correlation analysis results indicated a significant negative correlation between irisin and TC, TG, LDL-C, Hcy, IMT, and Crouse score ($P < .05$). Conversely, irisin exhibited a significant positive correlation with HDL-C levels ($P < .05$). The ROC curve analysis for the diagnostic value of irisin in obesity with CAS revealed an area under the curve of 0.957.

Conclusions • Serum irisin levels are significantly reduced in CAS patients, particularly those with overweight and obese. Additionally, irisin levels are closely associated with patients' blood lipids, homocysteine levels, and the severity of atherosclerosis. (*Altern Ther Health Med.* 2024;30(12):194-199).

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INTRODUCTION

Obesity is a medical condition characterized by excess body fat, often measured by an elevated body mass index (BMI).[1] The prevalence of obesity has increased globally in recent years. According to epidemiological data from the World Health Organization, over 1.2 billion individuals worldwide have an elevated BMI, with more than 300 million being obese, constituting approximately 12% of the population in China.[1,2] Research indicates that obesity stands as an independent risk factor for coronary atherosclerosis (CAS), with a higher incidence of cardiovascular events among obese individuals compared to their normal counterparts.[2]

CAS refers to the narrowing and hardening of coronary arteries, limiting blood flow to the heart muscle, and is a significant cardiovascular condition.[3] Irisin, a peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) dependent muscle factor, is dependent on fibronectin type III domain-containing protein 5 (FNDC5).[3,4] Synthesized and released by skeletal muscle, Irisin functions as a hormone with its expression positively linked to exercise.[4,5] Research affirms that serum Irisin influences fat metabolism and plays a role in insulin resistance.[6] However, the correlation between Irisin levels and obesity, as well as atherosclerosis, remains unestablished.

Therefore, this study aims to fill this gap by analyzing the changes and significance of Irisin levels in individuals with CAS complicated by obesity. Comprehensive clinical data were collected, facilitating an in-depth exploration and analysis of the correlation between Irisin, obesity, and atherosclerosis. Our findings contribute valuable insights for

developing new strategies in the clinical prevention and treatment of obesity and atherosclerosis.

DATA AND METHODS

Study Design

A cross-sectional design was employed and a total of 156 patients diagnosed with CAS at our hospital between March 2020 and March 2022 were enrolled in this study. The patients were categorized based on their BMI. Specifically, 64 patients with a BMI between 19 kg/m² and BMI < 24 kg/m² constituted the normal weight group (C1 group), while 49 patients with a BMI between 24 kg/m² and BMI < 28 kg/m² were categorized as the overweight group (C2 group). The remaining 43 patients with a BMI ≥ 28 kg/m² were designated as the CAS obesity group (C3 group). Furthermore, a control group (C0 group) comprised 80 healthy adults with a BMI between 19 kg/m² and BMI < 24 kg/m², who underwent medical checkups at our hospital during the same timeframe. Ethical approval for this study was obtained from the Ethics Committee of our hospital, ensuring adherence to ethical standards throughout the research process.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) Color Doppler ultrasonography revealing carotid artery intima thickness ≥1.0mm (carotid intima-media thickness thickened) or ≥1.2mm (carotid plaque formed); (2) Patients aged ≥18 years; (3) Voluntary signing of informed consent by each group of subjects. Exclusion criteria were as follows: (1) Individuals with type 1 or type 2 diabetes. Type 1 diabetes is diagnosed with fasting blood glucose ≥7.0mmol/l or blood glucose ≥11.1mmol/l 2 hours after glucose loading. For type 2 diabetes, normal fasting blood glucose is 3.9-6.1mmol/L, 6.1-7.0mmol/L is considered high fasting blood glucose, and >7.0mmol/L with typical diabetes symptoms; (2) Patients with severe cardiovascular and cerebrovascular diseases, hypertension, hepatorenal insufficiency, or severe hyperthyroidism and hypothyroidism; (3) Individuals with malignant tumors, systemic infections, or autoimmune diseases; and (4) Females during pregnancy or lactation.

Anthropometric and Demographic Assessment

The sex and age of each study group were accurately recorded. Additionally, comprehensive anthropometric measurements were conducted, encompassing height, weight, waist-hip ratio, systolic blood pressure (SBP), and diastolic blood pressure (DBP). These comprehensive assessments serve as fundamental baseline data, offering a holistic understanding of the participants' physical characteristics and cardiovascular health, thereby enhancing the overall significance and depth of the study.

Biochemical Analysis

Fasting venous blood samples were collected from the participants for a comprehensive biochemical analysis. This thorough examination, facilitated by an automatic biochemical analyzer, precisely quantified crucial biochemical indices. These indices include cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and serum homocysteine (Hcy). The results of these analyses provide valuable insights into the participants' lipid profiles and homocysteine levels, thereby contributing significantly to the comprehensive understanding of their cardiovascular health and metabolic status.

Ultrasound Examination

Each study group underwent a comprehensive ultrasound examination using Color Doppler ultrasound technology (GE LOGIQ-7). The examination, conducted with a 10.0MHz frequency probe, positioned patients in a supine position. The probe was carefully placed on the anterior edge of the sternocleidomastoid muscle. The examination involved continuous longitudinal and transverse scans from the origin of the superior fossa artery of the clavicle.

Testing was performed three times at both the left and right common carotid artery bifurcation, three times at the initial 1cm of the internal carotid artery, and three times at the proximal 1cm. Each subject underwent a total of 6 tests on both sides. The mean value of the measurements was calculated, and the carotid intima-media thickness (IMT) was recorded. Furthermore, the total maximum thickness of isolated patches was documented as the Crouse score. This ultrasound assessment serves as a crucial component in evaluating the structural aspects of the carotid arteries and determining the severity of atherosclerotic changes.

Serum Irisin Level Analysis

For the determination of serum Irisin levels, an Enzyme-Linked Immunosorbent Assay (ELISA) was employed as the detection method. The ELISA kit utilized for this analysis was procured from Abcam (ab285295) United States. The detection procedure precisely followed the instructions provided with the kit, with a detection sensitivity of 1ng/ml ensuring accuracy and consistency. The detection sensitivity achieved through this method was 1ng/ml. This careful approach in utilizing ELISA technology with a high-quality detection kit enhances the reliability and precision of the serum Irisin level measurements.

Statistical Analysis

We utilized the Statistical Package for the Social Sciences (SPSS) version 27.0 (International Business Machines, Corp., Armonk, NY, USA). For data processing and analysis. In comparing measurement data across multiple groups, variance analysis was employed, with post-comparisons assessed using the LSD-*t* test. Counting data among various groups underwent analysis through the rank sum test. The comparison of counting data employed the χ^2 test, while correlation analysis utilized the Pearson test. Furthermore, we investigated the correlation factors between obesity and carotid atherosclerosis using univariate and multivariate

Logistic regression analyses. This robust statistical approach enhances the depth and reliability of our findings, contributing to a comprehensive understanding of the interrelationships within our data.

RESULTS

Comparison of Clinical Data in Each Group

Within each group, no significant differences were observed in terms of gender and age ($P > .05$). However, statistically significant differences were evident in key anthropometric and cardiovascular parameters, including BMI, waist-hip ratio, DBP, and SBP, across all groups ($P < .05$) as detailed in Table 1. This analysis establishes a baseline uniformity in gender and age distribution while highlighting noteworthy distinctions in critical clinical metrics among the study groups, providing a comprehensive overview of the participants' health profiles.

Comparison of Biochemical Indexes and Irisin Levels in Each Group

Statistically significant differences were observed in the levels of TC, TG, HDL-C, LDL-C, Hcy, and Irisin among all groups ($P < .05$). Notably, CAS patients with obesity exhibited higher levels of TC, TG, LDL-C, and Hcy compared to other groups ($P < .05$), while the levels of HDL-C and Irisin were significantly lower than those in other groups ($P < .05$), as detailed in Table 2. These findings emphasize the distinctive biochemical profiles in CAS patients with obesity, shedding light on the intricate relationship between these variables in the context of cardiovascular health.

Carotid Artery Ultrasound Results in Each Group

Significant variations were noted in carotid artery ultrasound results, specifically in IMT and Crouse scores, across all groups ($P < .05$), as described in Table 3. Significantly elevated IMT and Crouse scores were observed in obese patients with CAS compared to the other groups ($P < .05$). These findings emphasize the distinctive ultrasound markers of atherosclerosis severity in the context of obesity, providing valuable insights into the vascular health of the study participants.

Correlation Analysis

The correlation analysis returned noteworthy results. Irisin exhibited a significant negative correlation with TC, TG, LDL-C, Hcy, IMT, and Crouse scores ($P < .05$). Conversely, Irisin displayed a significant positive correlation with HDL-C levels ($P < .05$), as illustrated in Table 4 and Figure. 1. These findings emphasize the complex relationship between Irisin and various lipid and atherosclerotic markers, shedding light on its potential role in modulating cardiovascular health.

ROC Curve Analysis for the Diagnostic Value of Irisin in Obesity Complicated with Carotid Atherosclerosis

The diagnostic efficacy of Irisin in obesity with CAS was carefully evaluated through ROC curve analysis. The results showed a highly significant AUC of 0.957 ($P = .000$, 95% CI: 0.931~0.982), affirming its robust discriminatory power. The optimal cutoff value was determined to be 34.485μg/ml, as illustrated in Figure 2. These findings emphasize the potential

Table 1. Comparison of General Data in Each Group of Subjects

Clinical Data	Group C0 (n=80)	Group C1 (n=64)	Group C2 (n=49)	Group C3 (n=43)	F/χ^2	*P* value
Gender (male/female)	40/40	36/28	26/23	23/20	0.562	.905
Age	55.96±9.88	55.48±9.38	56.72±11.21	56.14±10.73	0.138	.937
BMI (kg/m^2, ±s)	21.44±2.04	21.13±1.28	25.63±1.29^{ab}	30.03±2.12^{abc}	301.814	.000
Waist-to-hip ratio (±s)	0.84±0.09	0.83±0.07	0.88±0.05^{ab}	0.92±0.06^{abc}	17.687	.000
DBP (mmHg, ±s)	75.68±9.84	80.20±7.48^{a}	84.57±9.22^{ab}	88.37±10.25^{abc}	20.720	.000
SBP (mmHg, ±s)	121.83±13.52	123.94±10.97	128.41±11.19^{ab}	137.58±14.24^{abc}	16.126	.000

Note: Values are presented as mean ± standard deviation ($\bar{x} \pm s$). Significant differences ($P < 0.05$) are denoted by different superscript letters (a, b, c) within the BMI and Waist-to-hip ratio columns, indicating statistical variations between groups.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 2. Comparison of Biochemical Indicators and Serum Irisin Levels (±s)

Indicators	Group C0 (n=80)	Group C1 (n=64)	Group C2 (n=49)	Group C3 (n=43)	*F*	*P* value
TC (mmol/L)	4.78±0.84	4.91±0.95	5.37±0.80^{ab}	5.88±0.79^{abc}	18.588	.000
TG (mmol/L)	1.37±0.41	1.40±0.39	2.03±0.52^{ab}	3.28±0.75^{abc}	157.682	.000
HDL-C (mmol/L)	1.21±0.17	1.07±0.15^{a}	1.01±0.17^{a}	0.92±0.13^{abc}	36.161	.000
LDL-C (mmol/L)	2.73±0.55	2.93±0.47^{a}	3.35±0.60^{ab}	3.74±0.58^{abc}	43.410	.000
Hcy (μmol/L)	11.74±2.83	16.48±3.20^{a}	17.03±3.42^{a}	20.58±3.54^{abc}	78.673	.000
Irisin (μg/ml)	63.38±10.27	55.93±8.37^{a}	39.78±7.56^{ab}	28.05±8.33^{abc}	178.232	.000

Note: Values are presented as mean ± standard deviation ($\bar{x} \pm s$). Significant differences ($P < .05$) are denoted by different superscript letters (a, b, c) within each indicator column, indicating statistical variations between groups.

Abbreviations: TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Hcy, homocysteine.

Table 3. Results of Carotid Ultrasound Examination (mm, $\bar{x} \pm s$)

Indicators	Group C0 (n=80)	Group C1 (n=64)	Group C2 (n=49)	Group C3 (n=43)	*F*	*P* value
IMT	0.64±0.15	1.19±0.13^{a}	1.29±0.15^{ab}	1.35±0.16^{abc}	338.465	.000
Crouse Score	1.08±0.26	3.59±0.41^{a}	3.94±0.55^{ab}	4.33±0.49^{abc}	823.498	.000

Note: Values are presented as mean ± standard deviation ($\bar{x} \pm s$). Significant differences ($P < .05$) are denoted by different superscript letters (a, b, c) within each indicator column, indicating statistical variations between groups.

Abbreviation: IMT, carotid intima-media thickness.

Table 4. Correlation Analysis between Irisin and Other Indicators

Indicator	Value of Statistics	TC	TG	HDL-C	LDL-C	Hcy	IMT	Crouse Score
Irisin	r	-0.416	-0.626	0.435	-0.491	-0.523	-0.613	-0.671
	P value	.000	.000	.000	.000	.000	.000	.000

Note: Correlation coefficients (r) indicate the strength and direction of the correlation, while *P*-values signify the statistical significance of the correlation. All correlations are significant at $P < .05$.

Abbreviations: TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Hcy, homocysteine; IMT, carotid intima-media thickness.

Figure 1. Correlation Analysis between Irisin and Other Indicators. A: Irisin was negatively correlated with TC (total cholesterol), r = -0.416, *P* = .000; B: Irisin was negatively correlated with TG (triglycerides), r = -0.626, *P* = .000; C: Irisin was positively correlated with HDL-C (high-density lipoprotein cholesterol), r = 0.435, *P* = .000; D: Irisin was negatively correlated with LDL-C (low-density lipoprotein cholesterol), r = -0.491, *P* = .000; E: Irisin and Hcy (homocysteine) were negatively correlated, r = -0.523, *P* = .000; F: Irisin and IMT (carotid intima-media thickness) were negatively correlated, r = -0.613, *P* = .000; G: Irisin was negatively correlated with Crouse score, r = -0.671, *P* = .000. The correlation coefficients (r) indicate the strength and direction of the correlation, while *P*-values signify the statistical significance of the correlation. All correlations are significant at *P* < .05.

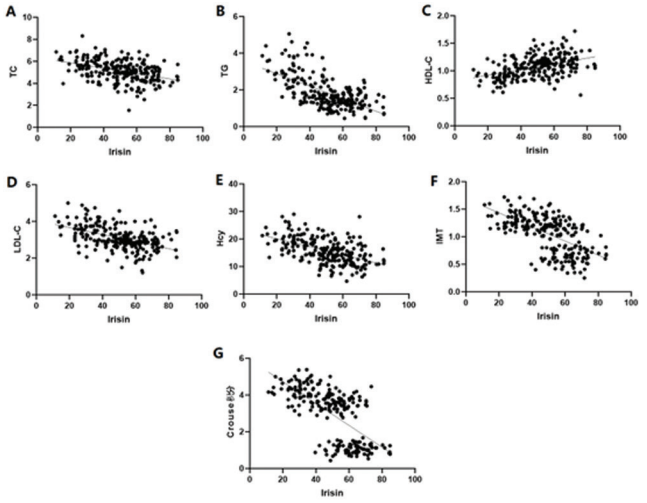
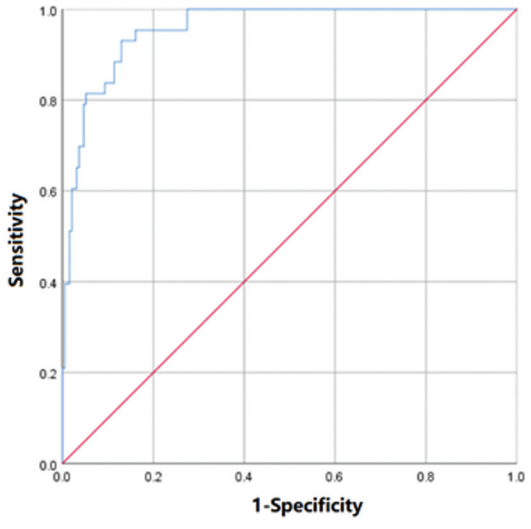


Figure 2. Diagnostic Value of Irisin for Obesity with CAS Analyzed by ROC Curve



Note: The ROC curve illustrates the diagnostic performance of Irisin for obesity with carotid atherosclerosis (CAS). The area under the curve (AUC) was 0.957 (*P* = .000, 95% CI: 0.931~0.982), and the optimal truncation value was 34.485 μg/ml.

of Irisin as a valuable diagnostic biomarker for identifying obesity in combination with carotid atherosclerosis, emphasizing its clinical relevance.

DISCUSSION

The prevalence of obesity and overweight individuals is on the rise, mirroring the gradual improvement in living standards. In the Western context, the ratio of obese to normal individuals stands at approximately 2:1. In China, while the obesity ratio is not as pronounced as in the West, it still registers a significant 8:1 ratio.[7] This increase in obesity rates is contributing to a rise in metabolic diseases and target organ damage.

Research indicates that obesity is a complex chronic condition, manifested by the accumulation of visceral and subcutaneous fat, ultimately predisposing individuals to cardiovascular diseases.[8,9] As living standards improve globally, understanding and addressing the multifaceted implications of obesity becomes paramount for public health management. Furthermore, obesity has been established as an independent risk factor for cardiovascular diseases.[10]

Atherosclerosis, the foundational pathological process in most cardiovascular events, manifests through endothelial dysfunction and arterial wall thickening.[11,12] Research affirms that the progression of atherosclerosis is intricately tied to age, with chronic inflammatory conditions like obesity and diabetes exacerbating its development.[13,14] This association underscores the importance of addressing obesity not only as a standalone health concern but also as a contributor to the broader spectrum of cardiovascular risks, emphasizing the need for comprehensive preventive measures.

Moreover, numerous mechanisms, including abnormal lipid metabolism, insulin resistance, inflammation, endothelial dysfunction, adipocytokine imbalance, and inflammatory activation, are recognized as fundamental contributors to the association between obesity and atherosclerosis.[15,16] In recent years, the rising prevalence of obesity has matched with a significant increase in the incidence and mortality rates of atherosclerosis. This correlation highlights the intricate physiological factors linking obesity and atherosclerosis, emphasizing the urgent need for targeted interventions to mitigate the escalating impact on public health. Hence, the quest for novel molecular targets in the realms of obesity and atherosclerosis has emerged as a prominent focus of research.

With the advancement of Irisin research, researchers are placing growing importance on its role in regulating energy metabolism and improving insulin resistance.[17] Currently, Chinese and international studies assert a close association between Irisin and type 2 diabetes. Furthermore, the prevailing consensus suggests that Irisin levels are lower in individuals with type 2 diabetes compared to a healthy control group, positioning it as a protective factor against type 2 diabetes.[18,19] This evolving understanding emphasizes Irisin's potential significance in unraveling pathways for intervention and treatment strategies in the context of metabolic disorders.

Research carried out by scholars on Irisin in elderly individuals with type 2 diabetes has demonstrated that elevated Irisin levels correlate with a reduced risk of hypertension, diabetes, and obesity in the regression model, even after adjusting for BMI, blood glucose, and blood pressure.[20] In animal experiments, transcriptase determined *FNDC5*-related genes, revealing higher expression of the *FNDC5* gene in healthy mice compared to artificially induced type 2 diabetes mice. This inference suggests that the impact of Irisin on diabetes is associated with safeguarding B-cell function and alleviating insulin resistance.[21] These findings contribute valuable insights into the potential protective role of Irisin in mitigating the multifaceted risks associated with type 2 diabetes in the elderly.

Furthermore, scholars have established a negative correlation between Irisin and insulin resistance. In obese patients with elevated serum Irisin levels, there is a demonstrated reduction in insulin resistance. When insulin sensitivity decreases in the body, compensatory release of Irisin from skeletal muscle and adipose tissue occurs, thereby enhancing insulin sensitivity.[22,23] Aydin et al.[24] discovered that, beyond skeletal muscle, *FNDC5* is expressed, and Irisin is secreted into the bloodstream in myocardia, adipose tissue, and brain tissue, with the myocardial expression of Irisin being the most prominent. These findings reveal the potential of Irisin in modulating insulin sensitivity and highlight its diverse expression across various tissues in the body.

Additionally, studies have indicated that the exogenous administration of Irisin can safeguard the vascular endothelium, mitigating damage.[25] Currently, there is no reported research on the association between Irisin and CAS accompanied by obesity. This scarcity underscores the existing research gap and urges further exploration into the potential protective effects of Irisin on the vascular endothelium, especially in the context of CAS coexisting with obesity.

The findings of this study revealed that serum Irisin levels in groups C1, C2, and C3 were significantly lower compared to the control group. Moreover, there was a notable decrease in serum Irisin levels with the increase in BMI. This trend aligns with the findings of previous scholarly research and our initial hypothesis,[26] underscoring the correlation between CAS and obesity with the levels of serum Irisin.

Additionally, the study detected statistically significant differences in TC, TG, HDL-C, LDL-C, Hcy, and carotid ultrasound among all groups. These results contribute valuable insights into the complex relationship between serum Irisin levels and key metabolic and cardiovascular markers, reinforcing the multifaceted connection between CAS, obesity, and Irisin.

Our findings suggest that obesity and atherosclerosis not only induce changes in blood lipid and Hcy levels but also contribute to the aggravation of CAS. Obesity emerges as an important factor influencing the progression of carotid atherosclerosis, aligning with findings from previous scholarly reports. Correlation analysis results revealed that Irisin exhibited a negative correlation with TC, TG, LDL-C, Hcy, IMT, and Crouse scores while demonstrating a positive correlation with HDL-C levels.

These outcomes imply that the Irisin level is intricately linked to the lipid profile of patients, Hcy levels, and the extent of vascular damage in CAS. Hcy is generated through the metabolism of methionine and cysteine in the body, and abnormal fluctuations in its levels can accelerate atherosclerosis, heightening the risk of coronary heart disease and adverse cardiovascular events. These findings emphasize the potential role of Irisin as a marker reflecting not only lipid and metabolic status but also the vascular health of individuals with carotid atherosclerosis.

Study Limitations

While our study provides valuable insights, it is essential to acknowledge certain limitations. Firstly, the cross-sectional nature of the study restricts our ability to establish causation, emphasizing the need for longitudinal investigations to better explain temporal relationships. Additionally, the sample size may limit the generalizability of our findings. Furthermore, the lack of data on lifestyle factors, such as diet and physical activity, hinders a comprehensive understanding of their potential impact on the observed associations. Despite these limitations, our study contributes to the existing body of knowledge on Irisin's role in metabolic and cardiovascular health, providing a foundation for future research to address these limitations and further explore the intricate connections between Irisin, obesity, and CAS.

CONCLUSION

In conclusion, our study highlights the significant reduction in serum Irisin levels among patients with carotid atherosclerosis, particularly in those with overweight and obesity. These findings highlight a close association between Irisin and key cardiovascular indicators, including blood lipids and homocysteine, as well as the severity of CAS. The observed decrease in Irisin levels suggests its potential role as a biomarker in assessing the metabolic and vascular status of individuals with CAS. These insights contribute to a deeper understanding of the intricate connections between Irisin and cardiovascular health, paving the way for further research and potential clinical applications in the management of CAS and related metabolic conditions.

COMPETING INTERESTS

The authors report no conflict of interest.

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None.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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