Am J Transl Res 2026;18(1):729-740
www.ajtr.org /ISSN:1943-8141/AJTR0169341

Original Article

Effect of circulating irisin level on clinical outcome in obesity-associated atherosclerosis

Guisheng Xing, Shaohua Fu

Clinical Laboratory, Ningbo Zhenhai People's Hospital (Ningbo No. 7 Hospital), Ningbo, Zhejiang, China

Received October 15, 2025; Accepted December 17, 2025; Epub January 15, 2026; Published January 30, 2026

Abstract: Aims: To investigate the association between circulating irisin levels, metabolic profiles, and clinical outcomes in obesity-associated atherosclerosis. Methods: This retrospective study included 220 patients admitted to the Department of Cardiology between January 2022 and January 2025, stratified into four groups based on body mass index and atherosclerosis status. Fasting blood samples were analyzed for serum irisin, glucose, HbA1c, insulin (HOMA-IR), and lipid profiles, including small dense LDL. Echocardiography was performed to assess cardiac structure and function, while carotid ultrasonography was used to quantify intima-media thickness and plaque burden; severe atherosclerosis was defined as a plaque area > 30 mm^2. Patients were prospectively followed up for major adverse cardiovascular events (MACE), which were adjudicated by an independent blinded committee. Results: Serum irisin levels were highest in normal-weight controls and progressively decreased across the overweight and atherosclerotic groups ($P < 0.05$). Concomitantly, fasting glucose, HbA1c, and HOMA-IR increased in a stepwise manner, with the most significant derangements observed in overweight patients with atherosclerosis. Dyslipidemia was characterized by elevated total cholesterol, LDL-C, and triglycerides, along with reduced HDL-C, particularly in groups with vascular disease. Cardiac structural remodeling was evident, with progressive increases in left ventricular (LV) mass and left atrial size, reductions in ejection fraction and global longitudinal strain, and worsening diastolic indices (all $P < 0.001$). The incidence of MACE increased from 3.6% in Group A to 45.5% in Group D ($P < 0.001$). Cox regression analysis identified age, BMI, diabetes, and LDL-C as independent risk factors, whereas each 1 ng/mL increase in irisin was associated with a 26% reduction in MACE risk. Logistic regression further revealed that low irisin was the strongest predictor of severe atherosclerosis (OR 0.128, 95% CI 0.058-0.283, $P < 0.001$). Conclusion: Low circulating irisin is an independent predictor of severe atherosclerosis and adverse cardiovascular outcome in patients with obesity-associated vascular disease.

Keywords: Irisin, obesity, atherosclerosis, biomarker, clinical outcomes

Introduction

Obesity has developed into a worldwide pandemic and a major precursor of cardiometabolic disease [1, 2]. Atherosclerosis is among its numerous complications contributing to morbidity and mortality, and serving as a key pathway linking excess adiposity to cardiovascular-related morbidity and mortality [3-5]. Pathophysiologically, obesity interacts with atherosclerosis through mechanisms including, but not limited to, chronic low-grade inflammation, endothelial dysfunction, and dysregulated energy homeostasis [6]. These complex processes underscore the urgent need to develop novel biomarkers that not only elucidate disease biology but also provide prognostic information with regard to clinical outcomes.

Since its discovery as a myokine derived from fibronectin type III domain-containing protein 5 (FNDC5), irisin has garnered significant attention due to its proposed roles in energy expenditure, browning of white adipose tissue, and whole-body metabolism [7]. Circulating irisin levels have been reported to vary in association with obesity, diabetes, and cardiovascular disorders [8-11], but the clinical implications of these variations remain controversial. While some studies indicate that irisin exerts vasoprotective and anti-inflammatory effects [12, 13], others have found contradictory associations between irisin and adverse metabolic con-

https://doi.org/10.62347/AGWY9542

ditions [14, 15]. This inconsistency in findings highlights a knowledge gap regarding the relevance of irisin, to obesity-related atherosclerosis.

Given the dual burdens of obesity and atherosclerosis leading to cardiovascular disease, evaluating irisin as an extracellular bridging molecule represents a promising approach to understanding its contribution to disease pathogenesis and prognosis. The findings regarding the association between serum irisin levels and specific clinical outcomes could enhance mechanistic insights and facilitate risk stratification in this high-risk patient population. Based on this, the current study aimed to explore the impact of circulating irisin levels on clinical outcomes in patients with weight gain-induced atherosclerosis, with the intent of advancing scientific knowledge and informing potential clinical practice.

Materials and methods

Case section

The present study involved a retrospective analysis of clinical records from patients admitted to the Department of Cardiology between January 2022 and January 2025. A total of 224 individuals were initially identified during the screening process. After applying predefined exclusion criteria, 4 patients were excluded - two due to incomplete clinical or imaging data, one due to rehospitalization for an acute condition during the screening period, and one due to pregnancy diagnosed at admission. Thus, 220 eligible patients were included and stratified into four groups based on body mass index (BMI) and atherosclerosis status: (1) normal-weight subjects without atherosclerosis (Group A, n = 55); (2) overweight subjects without atherosclerosis (Group B, n = 54); (3) normal-weight subjects with atherosclerosis (Group C, n = 56); and (4) overweight patients with atherosclerosis (Group D, n = 55).

Since these exclusions were based solely on objective eligibility criteria and implemented prior to group allocation, they were unlikely to introduce substantial selection bias. Normal weight and overweight were defined according to World Health Organization recommendations (BMI: 18.5-24.9 kg/m^2 and 25.0-29.9 kg/m^2, respectively) [16]. Atherosclerosis was diagnosed using evidence from carotid ultrasound, coronary angiography, or computed tomography angiography, which demonstrated intimal thickening, plaque presence, or luminal stenosis [17]. All patients underwent at least one of these imaging modalities; 100% of participants completed carotid ultrasound, while 32% additionally underwent coronary CT angiography and 18% underwent invasive coronary angiography. Imaging interpretations were independently evaluated by two experienced radiologists/sonographers blinded to all clinical and biochemical data, with discrepancies resolved by consensus. Inter-observer agreement for atherosclerosis diagnosis showed excellent consistency (Kappa = 0.87), confirming the reliability of imaging-based classification.

Inclusion criteria were as follows: adults aged 18-75 years with complete clinical histories, laboratory data, and imaging findings sufficient for diagnosis. Exclusion criteria included: history of malignant tumors; severe hepatic or renal dysfunction; acute or chronic infectious or inflammatory diseases; autoimmune disorders; uncontrolled endocrine disorders other than obesity (e.g., thyroid dysfunction, Cushing syndrome); and major cardiovascular events occurring within the preceding 3 months (including acute myocardial infarction, ischemic stroke, or coronary/peripheral revascularization), these exclusions were implemented to minimize confounding influence by acute-phase metabolic alterations. In addition, patients taking medications known to significantly affect circulating irisin levels (e.g., glucocorticoids or thiazolidinediones) were excluded. Individuals with incomplete clinical or imaging data, those rehospitalized during screening for conditions affecting study endpoints, and pregnant patients. Two investigators independently reviewed all eligible cases to ensure consistency in classification; any unresolved discrepancies resulted in case exclusion.

This study was approved by the Ethics Committee of Ningbo Zhenhai People's Hospital, and all procedures were conducted in accordance with the Declaration of Helsinki.

Data collection

Demographic and baseline clinical characteristics were documented using standardized inter-

views and medical record reviews, including age, sex, body mass index (BMI), smoking status, hypertension, and diabetes mellitus. Venous blood samples were collected after an overnight fast to measure serum irisin, fasting glucose, glycated hemoglobin (HbA1c), and insulin levels. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the standard formula: fasting blood glucose (mmol/L) × fasting insulin (μU/mL)/22.5. Insulin concentrations in this study were reported in μU/mL (equivalent to mU/L), consistent with commonly used clinical laboratory units. This unit definition is explicitly stated to ensure methodological clarity and reproducibility of results.

Serum irisin levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp., Wuhan, China; Catalog No. SEC244Hu; Lot No. 20240108). The assay had a limit of detection (LOD) of 0.12 ng/mL and a limit of quantification (LOQ) of 0.45 ng/mL. Analytical performance was verified prior to sample analysis: intra-assay coefficient of variation (CV) was < 8%, and inter-assay CV was < 10%, consistent with the manufacturer's specifications. Calibration curves were generated using recombinant human irisin standards (0-40 ng/mL), and all samples were measured in duplicate; repeated testing was performed if duplicate measurements differed by > 10%.

Lipid profiles - including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and small dense LDL (sdLDL) were determined using enzymatic assays and nuclear magnetic resonance (NMR) spectroscopy. Quantification of sdLDL was performed using an NMR-based lipoprotein particle analysis platform (LipoScience/LabCorp, Vantera® NMR Analyzer, USA) with proprietary lipid subclass analysis reagents (LipoProfile® reagent set, Lot No. 20240118). In accordance with established NMR lipoprotein subclassification criteria, sdLDL was defined as LDL particles with a mean diameter < 25.5 nm, corresponding to LDL subclasses 3-6 on the LipoProfile® spectrum. All assays were performed following the manufacturer's operating procedures. Analytical performance was verified using internal quality-control materials with known particle-size distributions; intra-assay CV for sdLDL concentration was < 6%, and inter-assay CV was < 8%. Daily calibration was conducted using manufacturer-provided reference standards to ensure consistency and comparability across batches. These procedures ensured that sdLDL measurements were accurate, reproducible, and aligned with widely accepted clinical lipid subclassification standards.

Echocardiographic measurements were performed in accordance with the recommendations of the American Society of Echocardiography. Left ventricular (LV) mass was calculated using the Devereux-modified ASE formula and indexed to body surface area to derive the LV mass index. Left atrial diameter and left atrial volume index were measured using the biplane area - length method. LV ejection fraction (LVEF) was assessed using biplane Simpson's method. Global longitudinal strain (GLS) was quantified via 2-dimensional speckle-tracking echocardiography, based on apical 2-, 3-, and 4-chamber views, with automated tracking and manual adjustment as required; GLS values represented the average strain of 17 myocardial segments. Diastolic function indices - including the E/A ratio, E/e' ratio, and deceleration time - were measured using pulsed-wave and tissue Doppler imaging. Right ventricular (RV) systolic pressure was estimated from tricuspid regurgitation velocity using standard Doppler methods.

Carotid ultrasonography was used to evaluate intima-media thickness and plaque burden, with severe atherosclerosis defined as a plaque area > 30 mm^2. Participants were prospectively followed for the occurrence of major adverse cardiovascular events (MACE), including cardiovascular mortality, non-fatal myocardial infarction, ischemic stroke, unplanned coronary revascularization, and hospitalization for heart failure. All endpoints were adjudicated by an independent committee blinded to clinical events. The median follow-up duration was 32 months (interquartile range: 28-36 months), with a minimum follow-up of 24 months and a maximum of 38 months. Follow-up duration was well balanced across the four groups, with no significant differences (P = 0.41). A total of 6 patients (2.7%) were lost to follow-up, and the loss-to-follow-up rate did not differ significantly between groups (P = 0.78). Baseline characteristics - including age, BMI, atherosclerosis status, glucose and lipid parameters, and serum

irisin levels - were comparable between patients who completed follow-up and those lost to follow-up (all P > 0.10). Thus, loss to follow-up was minimal and unlikely to introduce significant attrition bias.

Outcome measure

The primary outcomes were biochemical and clinical end points, such as serum irisin level, glycometabolic outcomes [fasting glucose, glycated hemoglobin (HbA1c), and HOMA-IR], lipid levels [by total cholesterol, LDL-C, HDL-C, triglycerides, and small dense LDL], echocardiographic ones [left ventricular mass index, left atria size, left ventricular ejection fraction, left ventricular global longitudinal strain]. The secondary outcome was severe atherosclerosis, defined by carotid plaque burden > 30 mm^2 on imaging, which served as the endpoint for logistic regression analyses.

Sample size calculation

The sample size for this study was determined *a priori* based on the expected difference in the primary clinical outcome - the cumulative incidence of major adverse cardiovascular events (MACE) - between patients with lower versus higher circulating irisin levels. Based on previous data from similar high-risk cardiovascular cohorts, we assumed a 3-year MACE incidence of approximately 35% in the low-irisin group versus 15% in the high-irisin group. With a two-sided α of 0.05 and a β of 0.20 (80% statistical power), a chi-square test for comparing two proportions indicated that a minimum of 196 patients was required. To account for an anticipated loss to follow-up of up to 10%, we planned to enroll at least 220 patients, which is consistent with the final analyzed cohort (n = 220). This overall sample size - with approximately 54-56 patients in each BMI/atherosclerosis stratum - also provides adequate power (approximately 70-80%) to detect clinically meaningful effect sizes (e.g., hazard ratios of 0.55-0.60 or odds ratios ≥ 2.0) in key prespecified subgroups, such as age < 60 years versus ≥ 60 years and diabetic versus non-diabetic patients, for diagnostic and prognostic efficacy analyses. Subgroup differences of smaller magnitudes were interpreted as exploratory.

Statistical methods

Continuous variables were summarized as mean ± standard deviation (SD) or median (interquartile range, IQR), and comparisons across groups were performed using one-way analysis of variance (ANOVA) or Kruskal-Wallis tests with appropriate post hoc adjustments. Categorical variables were analyzed using the χ^2 test or Fisher's exact test, as appropriate. Independent predictors of MACE were identified using Cox proportional hazards regression, while predictors of severe atherosclerosis (plaque burden > 30 mm^2) were determined using multivariable logistic regression. Results were expressed as hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (CIs). All statistical analyses were performed using SPSS and R software. A two-sided *p*-value < 0.05 was considered significant.

Results

Comparison of baseline characteristics

Age differed significantly across groups, with overweight participants with atherosclerosis being the oldest (65.84 ± 11.29 years vs. 51.11 ± 7.83 years in normal-weight participants without atherosclerosis; P < 0.001). The sex distribution was comparable between groups (P = 0.445). Both BMI and waist circumference were substantially higher in overweight than in normal-weight individuals (BMI: 28.44 ± 2.09 vs. 22.37 ± 1.55 kg/m^2; waist circumference: 95.31 ± 6.43 vs. 81.00 ± 5.59 cm; both P < 0.001). The prevalence of hypertension increased progressively from 20.0% in normal-weight participants without atherosclerosis to 61.8% in overweight participants with atherosclerosis (P < 0.001). Diabetes mellitus was more common among atherosclerosis participants, reaching 38.2% in overweight participants with atherosclerosis compared with 12.7% in normal-weight participants without atherosclerosis (P = 0.007). In contrast, smoking history and family history of cardiovascular disease did not differ significantly between groups (P = 0.300 and P = 0.491, respectively) (**Table 1**).

Comparison of glycometabolic parameters

Serum irisin levels (**Figure 1A**) were highest in Group A and progressively decreased in Groups B, C, and D, with significant differences detected (P < 0.05). Fasting glucose concentrations (**Figure 1B**) were markedly elevated in Groups B-D relative to Group A (P < 0.05), with Group D

Table 1. Comparison of baseline characteristics

Variable	Group A (n = 55)	Group B (n = 54)	Group C (n = 56)	Group D (n = 55)	F/χ^2	*p*-value
Age (years)	50.25 ± 13.76	51.11 ± 7.83	59.91 ± 11.55	65.84 ± 11.29	23.756	< 0.001
Male sex, n (%)	27 (49.1)	29 (53.7)	33 (58.9)	35 (63.6)	2.671	0.445
BMI (kg/m^2)	22.37 ± 1.55	26.92 ± 1.53	23.19 ± 1.12	28.44 ± 2.09	137.437	< 0.001
Waist circumference (cm)	81.00 ± 5.59	92.27 ± 8.33	82.77 ± 6.82	95.31 ± 6.43	57.718	< 0.001
Systolic BP (mmHg)	122.82 ± 10.59	129.30 ± 12.35	135.34 ± 13.82	138.76 ± 14.72	16.115	< 0.001
Diastolic BP (mmHg)	75.65 ± 8.39	80.35 ± 8.03	82.61 ± 8.46	87.47 ± 10.03	17.275	< 0.001
Hypertension, n (%)	11 (20.0)	16 (29.6)	28 (50.0)	34 (61.8)	24.718	< 0.001
Diabetes mellitus, n (%)	7 (12.7)	10 (18.5)	18 (32.1)	21 (38.2)	12.080	0.007
Smoking history, n (%)	12 (21.8)	14 (25.9)	19 (33.9)	20 (36.4)	3.663	0.300
Family history of CVD, n (%)	8 (14.5)	10 (18.5)	13 (23.2)	14 (25.5)	2.412	0.491

Note: BMI: Body mass index; BP: Blood Pressure; CVD: Cardiovascular Disease.

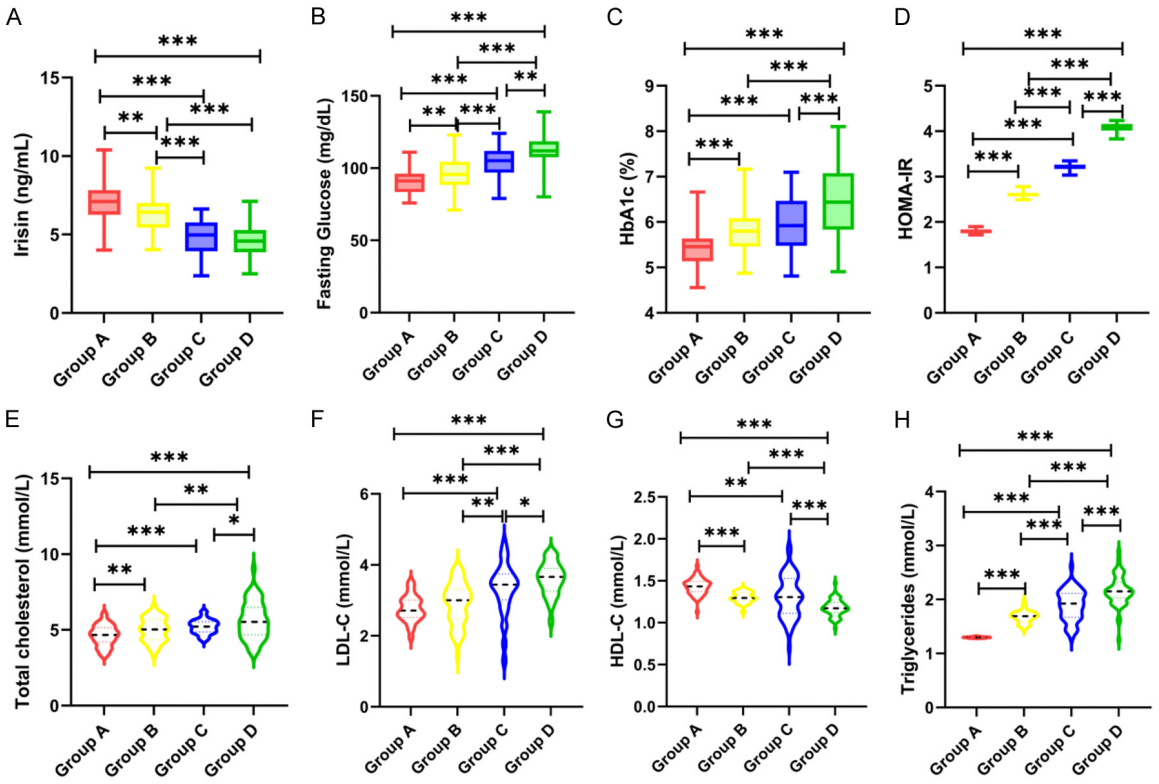

Figure 1. Comparison of glycometabolic and blood lipid parameters between four groups. (A) Serum irisin levels, (B) fasting glucose, (C) HbA1c, (D) HOMA-IR, (E) Total cholesterol, (F) LDL-C, (G) HDL-C, and (H) Triglycerides. Compare with group A or group B or group C, **P < 0.01, ***P < 0.001. Note: HbA1c: Hemoglobin A1c; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol.

exhibiting the greatest increase (P < 0.05). Similarly, HbA1c levels (**Figure 1C**) were significantly higher in Groups B, C, and D compared to Group A (P < 0.001), with the most pronounced elevation in Group D (P < 0.05). Moreover, HOMA-IR (**Figure 1D**), an indicator of insulin resistance, was significantly increased in Groups B-D compared to Group A (P < 0.05), with Group D again showing the highest values (P < 0.05).

Comparison of blood lipid

Total cholesterol (TC) levels (**Figure 1E**) were significantly higher in Groups B, C, and D compared to Group A (P < 0.05), with Group D show-

Table 2. Comparison of cardiac structure and function

Parameter	Group A (n = 55)	Group B (n = 54)	Group C (n = 56)	Group D (n = 55)	F/χ^2	*p*-value
LV mass index (g/m^2)	98.18 ± 13.25	97.78 ± 16.28	113.57 ± 23.34	125.57 ± 16.86	30.839	< 0.001
Left atrial diameter (mm)	34.34 ± 4.24	36.13 ± 3.93	38.51 ± 3.80	38.83 ± 5.80	12.106	< 0.001
Left atrial volume index (mL/m^2)	26.50 ± 6.11	32.81 ± 6.95	37.60 ± 6.46	37.24 ± 7.82	31.326	< 0.001
LVEF (%)	63.93 ± 3.41	61.95 ± 4.49	60.86 ± 4.95	58.65 ± 6.33	11.045	< 0.001
GLS (%)	-18.96 ± 2.36	-17.76 ± 2.26	-17.55 ± 2.20	-16.73 ± 2.29	9.012	< 0.001
E/A ratio	1.28 ± 0.06	1.14 ± 0.07	1.01 ± 0.08	0.96 ± 0.07	231.701	< 0.001
E/e' ratio	9.13 ± 2.50	10.38 ± 2.54	12.70 ± 2.49	13.74 ± 4.13	27.137	< 0.001
Deceleration time (ms)	189.07 ± 22.25	200.55 ± 26.48	217.26 ± 33.25	224.70 ± 31.11	17.433	< 0.001
RV systolic pressure (mmHg)	27.63 ± 5.04	29.18 ± 5.05	31.36 ± 5.78	34.43 ± 6.08	15.728	< 0.001

Note: LV: Left Ventricular; LVEF: Left Ventricular Ejection Fraction; GLS: Global Longitudinal Strain; E/A: Early (E) to Atrial (A) Filling Velocity; RV: Right Ventricle.

Table 3. Incidence of MACE

Outcome (n, %)	Group A (n = 55)	Group B (n = 54)	Group C (n = 56)	Group D (n = 55)	χ^2	*p*-value
Cardiovascular death	1 (1.8)	2 (3.7)	5 (8.9)	10 (18.2)	11.769	0.008
Non-fatal myocardial infarction	1 (1.8)	2 (3.7)	6 (10.7)	8 (14.5)	8.206	0.042
Non-fatal stroke	0 (0.0)	1 (1.9)	4 (7.1)	6 (10.9)	8.606	0.035
Heart failure hospitalization	0 (0.0)	1 (1.9)	4 (7.1)	6 (10.9)	8.606	0.035
Unplanned coronary revascularization	0 (0.0)	2 (3.7)	7 (12.5)	10 (18.2)	14.275	0.003
Composite MACE (any event above)	2 (3.6)	5 (9.3)	17 (30.4)	40 (72.7)	78.384	< 0.001

Note: MACE: Major Adverse Cardiovascular Events.

ing the highest values (P < 0.001). Similarly, LDL-C concentrations (**Figure 1F**) were markedly elevated in Groups B, C, and D compared to Group A (P < 0.05), with Group D again reaching the highest level (P < 0.05). In contrast, HDL-C (**Figure 1G**) was significantly reduced in Groups B, C, and D compared to Group A (P < 0.05), with the lowest concentration observed in Group C (P < 0.05). For triglycerides (TG) (**Figure 1H**), Groups B, C, and D exhibited significantly higher levels compared to Group A (P < 0.05), with Group C showing the greatest elevation (P < 0.05).

Comparison of cardiac structure and function

LV mass index increased stepwise from Group A (98.18 ± 13.25 g/m^2) to Group D (125.57 ± 16.86 g/m^2; P < 0.001). Similarly, left atrial diameter and left atrial volume index showed significant enlargement with advancing group status (all P < 0.001), rising from 34.34 ± 4.24 mm and 26.50 ± 6.11 mL/m^2 in Group A to 38.83 ± 5.80 mm and 37.24 ± 7.82 mL/m^2 in Group D, respectively. Regarding systolic function, LVEF declined progressively from 63.93 ± 3.41% in Group A to 58.65 ± 6.33% in Group D (P < 0.001), accompanied by a parallel reduction in global longitudinal strain (GLS) from -18.96 ± 2.36% to -16.73 ± 2.29% (P < 0.001). Marked impairments in diastolic function were also evident, as reflected by a decreasing E/A ratio (1.28 ± 0.06 to 0.96 ± 0.07), an increasing E/e' ratio (9.13 ± 2.50 to 13.74 ± 4.13), and prolonged deceleration time (189.07 ± 22.25 ms to 224.70 ± 31.11 ms) across the groups (all P < 0.001). Furthermore, right ventricular (RV) systolic pressure increased significantly from 27.63 ± 5.04 mmHg in Group A to 34.43 ± 6.08 mmHg in Group D (P < 0.001) (**Table 2**).

Incidence of MACE

As shown in **Table 3**, the composite MACE rate was lowest in Group A (3.6%) and increased progressively through Group B (9.3%) and Group C (30.4%), reaching the highest incidence in Group D (72.7%; P < 0.001). Among individual endpoints, cardiovascular death occurred most frequently in Group D (18.2%), compared with 1.8%, 3.7%, and 8.9% in Groups A, B, and C, respectively. Similarly, non-fatal myocardial infarction showed a stepwise increase from 1.8% in Group A to 14.5% in

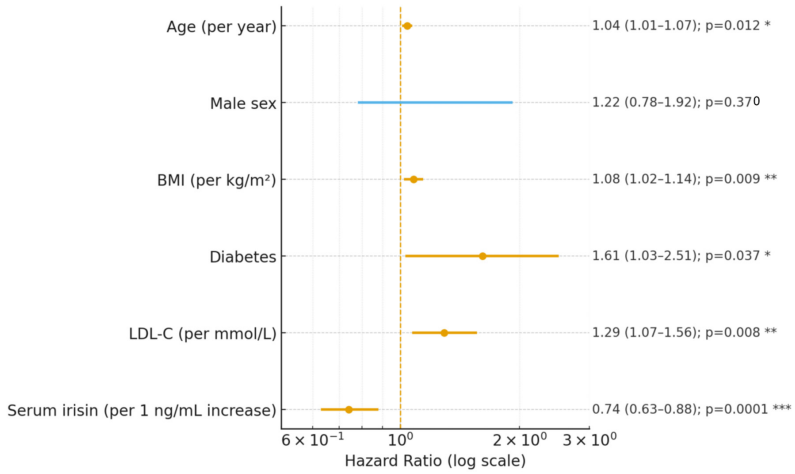

Figure 2. Cox regression for predictors of major adverse cardiovascular events (MACE). Note: MACE: Major Adverse Cardiovascular Events.

Table 4. Logistic regression: predictors of severe atherosclerosis (Plaque Burden > 30 mm²)

Variable	OR (95% CI)	*p* Value
Low Irisin (< 6 ng/mL)	0.128 (0.058-0.283)	< 0.001
Small dense LDL (%)	0.854 (0.810-0.901)	< 0.001
Smoking	1.942 (1.007-3.746)	0.048
Low Adiponectin	9.901 (4.209-23.289)	< 0.001

Note: LDL: Low-Density Lipoprotein Cholesterol.

Group D. Unplanned coronary revascularization also increased markedly, from 0% in Group A to 18.2% in Group D. Notably, Group D had the highest rates of non-fatal stroke (10.9%) and heart failure hospitalization (10.9%), whereas these events were rare or absent in Groups A and B. These findings underscore the synergistic effect of obesity and atherosclerosis on adverse cardiovascular outcome and highlight the markedly elevated risks of overweight patients with established atherosclerotic disease.

Cox regression for predictors of MACE

In the multivariable Cox proportional hazards model, we adjusted for a comprehensive set of traditional cardiovascular risk factors, including age, sex, BMI, smoking status, hypertension, diabetes mellitus, and LDL-C levels. All variables were included using a full-variable inclusion approach rather than stepwise or automated selection, in accordance with the prespecified analysis plan. Assessment of multicollinearity showed that all covariates had Variance Inflation Factor (VIF) values < 2.1, indicating no significant multicollinearity. After full adjustment, advancing age remained significantly associated with increased MACE risk (HR 1.04 per year, 95% CI 1.01-1.07; P = 0.012). Higher BMI was similarly associated with elevated risk (HR 1.08 per kg/m², 95% CI 1.02-1.14; P = 0.009), and diabetes mellitus also independently predicted adverse outcome (HR 1.61, 95% CI 1.03-2.51; P = 0.037). LDL-C levels were a significant predictor of MACE (HR 1.29 per mmol/L, 95% CI 1.07-1.56; P = 0.008), whereas male sex, smoking, and hypertension were not independently associated with risk after mutual adjustment. Importantly, serum irisin remained a strong and independent protective factor, with each 1 ng/mL increase associated with a 26% reduction in MACE risk (HR 0.74, 95% CI 0.63-0.88; P = 0.0001) (**Figure 2**). These findings confirm the prognostic value of circulating irisin beyond established cardiovascular risk factors.

Logistic regression: predictors of severe atherosclerosis (plaque burden > 30 mm²)

Low circulating irisin levels (< 6 ng/mL) showed a strong inverse association with severe atherosclerosis, with a markedly reduced odds ratio (OR 0.128, 95% CI 0.058-0.283; P < 0.001). The percentage of small dense LDL was also significantly associated with plaque burden (OR 0.854, 95% CI 0.810-0.901; P < 0.001), highlighting the contribution of lipoprotein subfraction patterns. Traditional risk factors further contributed to atherosclerotic severity, with smoking showing a significant association (OR 1.942, 95% CI 1.007-3.746; P = 0.048). Among metabolic parameters, low adiponectin levels exhibited the strongest positive association with severe atherosclerosis (OR 9.901, 95% CI 4.209-23.289; P < 0.001) (**Table 4**). Collectively, these findings indicate

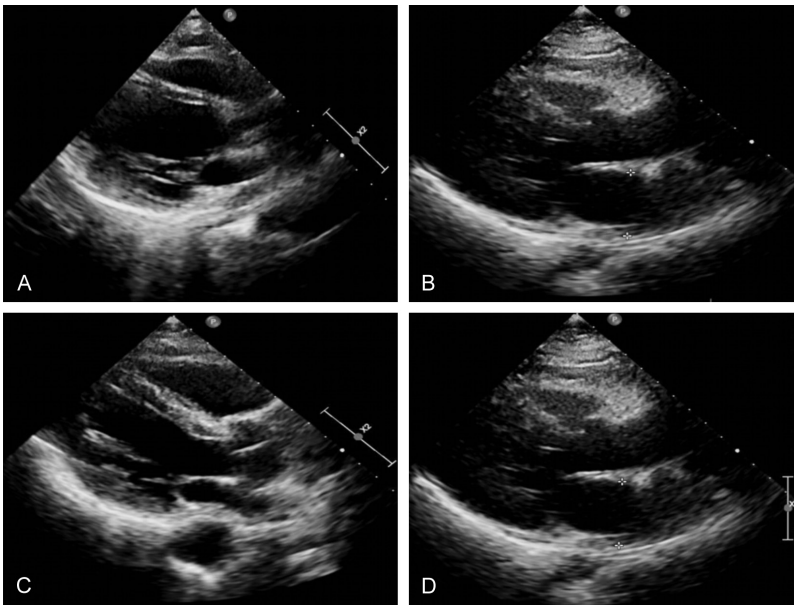

Figure 3. Representative echocardiographic images of study groups. (A) Normal-weight subjects without atherosclerosis; (B) Overweight subjects without atherosclerosis; (C) Normal-weight subjects with atherosclerosis; and (D) overweight patients with atherosclerosis.

that both metabolic dysregulation (low irisin, low adiponectin, and small dense LDL) and lifestyle factors (smoking) synergistically predict the presence of advanced atherosclerotic lesions, suggesting a pivotal role of irisin deficiency in the pathophysiology of obesity-associated vascular disease.

Representative echocardiographic images

Figure 3 presents representative echocardiographic images from each study group. Panel (A) shows an echocardiogram of normal-weight subjects without atherosclerosis, demonstrating a normal LV structure. Panel (B) depicts overweight subjects without atherosclerosis, where mild structural changes are observed. Panel (C) illustrates an echocardiogram of normal-weight subjects with atherosclerosis, showing increased LV mass and chamber enlargement typical of early atherosclerotic changes. Lastly, Panel (D) presents overweight patients with atherosclerosis, displaying the most pronounced LV structural remodeling, accompanied by significant chamber enlargement and reduced systolic function. These images highlight the progressive nature of cardiac structural changes across the groups, with the most severe alterations observed in those with both obesity and atherosclerosis.

Discussion

In this prospective observational study of patients stratified by body weight and the presence of atherosclerosis, we provided strong evidence that circulating irisin levels were closely correlated with glycometabolic dysregulation, unfavorable lipid profiles, cardiac structural and functional remodeling, and the development of atherosclerotic burden. In particular, low irisin levels were consistently associated with elevated fasting glucose, insulin resistance, dyslipidemia, impaired endothelial function, and increased carotid intima-media thickness. Notably, logistic regression analysis identified low irisin concentration as the strongest independent predictor of severe carotid plaque burden, while Cox regression confirmed that irisin serves as a protective factor against MACE even after adjusting for traditional risk factors. These results build on previous observations by integrating metabolic, vascular, and clinical outcome data, supporting the notion that irisin is more than just a metabolic regulator - it represents a possible intrinsic biomarker and therapeutic target in obesity-related atherosclerosis.

Our findings demonstrate a strong correlation between irisin levels and glycometabolic health. Serum irisin concentrations showed a gradual decline from normal-weight, non-atherosclerotic individuals without vascular disease to overweight patients with pre-existing vascular disease, which was accompanied by progressive elevations in fasting glucose, HbA1c, and HOMA-IR levels. These results are consistent with previous studies showing that irisin enhances glucose uptake and insulin sensitivity via activation of AMP-activated protein kinase (AMPK) and induction of GLUT4 translocation to the skeletal muscle membrane [18-

20]. Reduced irisin levels in patients with type 2 diabetes have also been reported to correlate with increased insulin resistance and poor glycemic control [21]. The progressive decrease in irisin across our study groups suggests that obesity combined with atherosclerosis may suppress this myokine, possibly due to chronic low-grade inflammation, mitochondrial dysfunction, and impaired endocrine function of skeletal muscle. These observations indicate that irisin deficiency acts as a mechanistic link between metabolic imbalance and vascular injury, and that maintenance of muscle-derived endocrine homeostasis may play a protective role in the prevention of cardiometabolic diseases.

Beyond glucose metabolism, our study confirms a robust association between irisin and lipid regulation. Decreased serum irisin concentrations were associated with higher levels of total cholesterol, LDL-C, and triglycerides, as well as reduced HDL-C levels in patients. Although previous experimental studies have demonstrated that irisin modulates lipid metabolism - for instance, by promoting cholesterol efflux via upregulation of ABCA1/ABCG1 expression and regulating lipolysis through the p38 MAPK and ERK signaling pathways - our study did not directly assess these molecular mechanisms [22]. Therefore, these pathways are discussed here as biologically plausible explanations supported by prior basic research, rather than as causal evidence derived from our cohort. Our results reflect observational associations, and any mechanistic interpretations should be regarded as hypothesis-generating rather than confirmatory. Lower circulating irisin levels have also been linked to a higher prevalence of dyslipidemia in both diabetic and obese patients [23]. Notably, our logistic regression analysis identified small dense LDL as an independent predictor of plaque burden severity, ranking second only to irisin deficiency. Small dense LDL particles are more atherogenic because they have a greater propensity to penetrate arterial walls, are more susceptible to oxidation, and possess a longer plasma half-life. The strong correlation between low irisin levels and elevated small dense LDL levels suggests that irisin may be involved in lipoprotein remodeling, and that irisin deficiency may create a permissive environment for the formation of atherogenic lipoprotein particles.

Our study also identified a second key finding: reduced irisin levels were consistently associated with unfavorable cardiac and vascular structural and functional remodeling. Patients with low irisin exhibited increased left ventricular mass index and left atrial size, reduced ejection fraction and global longitudinal strain, and impaired diastolic parameters - accompanied by clear evidence of endothelial dysfunction, including lower flow-mediated dilation and higher endothelin-1 concentrations. These clinical observations are supported by preclinical studies showing that exogenous irisin enhances endothelial function via activation of endothelial nitric oxide synthase (eNOS) and reduction of oxidative stress [24], and that irisin attenuates cardiac hypertrophy and fibrosis through modulation of the MAPK or TGF-β1/Smad pathways [25]. Collectively, mechanistic evidence and our clinical data converge to indicate that irisin deficiency may drive maladaptive cardiac and vascular remodeling, ultimately contributing to poorer cardiovascular outcomes.

Our logistic regression analysis provided novel evidence that low irisin is the strongest independent predictor of severe atherosclerosis - surpassing conventional risk factors such as smoking and low adiponectin levels. This finding aligns with recent studies linking reduced irisin levels to coronary artery calcification and carotid plaque vulnerability [26-29]. Irisin may inhibit plaque formation and progression through multiple mechanisms: it suppresses vascular smooth muscle cell proliferation, prevents macrophage foam cell formation, inhibits inflammatory cytokine production, and enhances autophagic clearance of cholesterol-rich lipid debris. Notably, its integrative role - whereby irisin deficiency interacts with environmental risk factors (smoking and dyslipidemia) to predict severe plaque Burden - holds clinical relevance. These results suggest that measuring circulating irisin may offer incremental predictive value for identifying patients at the highest risk of developing advanced atherosclerotic disease.

Several limitations of the study should be acknowledged. First, the study was conducted at a single center in Ningbo. Although the cohort was well-characterized, it may not fully represent patient populations from other regions. Baseline characteristics - such as obesity prevalence, dietary patterns, socioeconom-

ic status, and atherosclerosis severity - may vary across hospitals and geographic areas, particularly between coastal and inland regions or urban and rural populations in China. Second, the study population consisted exclusively of East Asian (Chinese) patients, limiting the generalizability of our findings to other ethnic groups. Prior evidence indicates that circulating irisin levels, body fat distribution, lipid metabolism, and susceptibility to cardiometabolic disease may differ across ethnicities; therefore, caution is warranted when extrapolating our results to non-Asian populations. Third, the retrospective, observational nature of the study precludes causal inference, and residual confounding cannot be entirely excluded despite multivariable adjustment. Fourth, although ELISA remains the most widely used method for irisin quantification, variations between assay platforms may affect absolute measurement values. Fifth, although the follow-up duration was adequate to capture medium-term cardiovascular outcomes, longer follow-up is needed to evaluate the long-term prognostic significance of irisin. Lastly, unmeasured factors - including physical activity levels, dietary intake, socioeconomic influences, and genetic polymorphisms - may have affected both circulating irisin concentrations and cardiovascular risk, and could not be fully accounted for in this analysis.

In conclusion, low circulating irisin levels are a robust predictor of adverse metabolic outcomes, vascular remodeling, severe atherosclerosis, and increased incidence of major adverse cardiovascular events in patients with obesity-associated atherosclerosis. These results establish irisin deficiency as a convergent mechanism linking metabolic impairment and vascular disease, thereby advancing our understanding of the pathophysiology of cardiometabolic risk. Strategies to measure irisin may enhance the specificity of patient stratification, facilitating the development of tailored therapeutic approaches aimed at augmenting irisin signaling and reducing the burden of cardiovascular disease in obese patients. Future research should validate these findings through interventional trials to determine whether targeting irisin pathways can indeed improve outcome.

Disclosure of conflict of interest

None.

Abbreviations

ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; AMPK, AMP-activated protein kinase; ASE, American Society of Echocardiography; BMI, Body mass index; BP, Blood pressure; CABG, Coronary artery bypass grafting; CI, Confidence interval; CT, Computed tomography; CV, Coefficient of variation; CVD, Cardiovascular disease; eNOS, Endothelial nitric oxide synthase; ELISA, Enzyme-linked immunosorbent assay; FNDC5, Fibronectin type III domain-containing protein 5; GLS, Global longitudinal strain; HbA1c, Hemoglobin A1c; HDL-C, High-density lipoprotein cholesterol; HF, Heart failure; HOMA-IR, Homeostasis model assessment of insulin resistance; HR, Hazard ratio; IMT, Intima-media thickness; IQR, Interquartile range; LDL-C, Low-density lipoprotein cholesterol; LOD, Limit of detection; LOQ, Limit of quantification; LV, Left ventricle/left ventricular; LVEF, Left ventricular ejection fraction; LVMI, Left ventricular mass index; MACE, Major adverse cardiovascular events; MAPK, Mitogen-activated protein kinase; MI, Myocardial infarction; NMR, Nuclear magnetic resonance; OR, Odds ratio; PCI, Percutaneous coronary intervention; RV, Right ventricle/right ventricular; SD, Standard deviation; sdLDL, Small dense low-density lipoprotein; SPSS, Statistical Package for the Social Sciences; TC, Total cholesterol; TG, Triglycerides; TGF-β1, Transforming growth factor-β1; TRV, Tricuspid regurgitation velocity; VIF, Variance inflation factor.

Address correspondence to: Shaohua Fu, Clinical Laboratory, Ningbo Zhenhai People's Hospital (Ningbo No. 7 Hospital), No. 718, Nan'er West Road, Luotuo Street, Zhenhai District, Ningbo, Zhejiang, China. Tel: +86-0574-86655021; E-mail: fshaohua0521@126.com

References

[1] Valenzuela PL, Carrera-Bastos P, Castillo-García A, Lieberman DE, Santos-Lozano A and Lucia A. Obesity and the risk of cardiometabolic diseases. Nat Rev Cardiol 2023; 20: 475-494.

[2] Miranda JJ, Barrientos-Gutiérrez T, Corvalan C, Hyder AA, Lazo-Porras M, Oni T and Wells JCK. Understanding the rise of cardiometabolic diseases in low- and middle-income countries. Nat Med 2019; 25: 1667-1679.

[3] Libby P. The changing landscape of atherosclerosis. Nature 2021; 592: 524-533.
[4] Herrington W, Lacey B, Sherliker P, Armitage J and Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. Circ Res 2016; 118: 535-546.
[5] Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A and Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med 2014; 5: 927-946.
[6] Henning RJ. Obesity and obesity-induced inflammatory disease contribute to atherosclerosis: a review of the pathophysiology and treatment of obesity. Am J Cardiovasc Dis 2021; 11: 504-529.
[7] Daudon M, Bigot Y, Dupont J and Price CA. Irisin and the fibronectin type III domain-containing family: structure, signaling and role in female reproduction. Reproduction 2022; 164: R1-R9.
[8] Parkin RA and Murray AJ. The therapeutic potential of irisin to mitigate the risk of metabolic syndrome in postmenopausal women. Front Reprod Health 2024; 6: 1355922.
[9] Paoletti I and Coccurello R. Irisin: a multifaceted hormone bridging exercise and disease pathophysiology. Int J Mol Sci 2024; 25: 13480.
[10] Khorasani ZM, Bagheri RK, Yaghoubi MA, Chobkar S, Aghaee MA, Abbaszadegan MR and Sahebkar A. The association between serum irisin levels and cardiovascular disease in diabetic patients. Diabetes Metab Syndr 2019; 13: 786-790.
[11] Fu J, Li F, Tang Y, Cai L, Zeng C, Yang Y and Yang J. The emerging role of irisin in cardiovascular diseases. J Am Heart Assoc 2021; 10: e022453.
[12] Wang Y, Tian M, Tan J, Pei X, Lu C, Xin Y, Deng S, Zhao F, Gao Y and Gong Y. Irisin ameliorates neuroinflammation and neuronal apoptosis through integrin αVβ5/AMPK signaling pathway after intracerebral hemorrhage in mice. J Neuroinflammation 2022; 19: 82.
[13] Wang Y, Wang M and Wang Y. Irisin: a potentially fresh insight into the molecular mechanisms underlying vascular aging. Aging Dis 2023; 15: 2491-2506.
[14] Jang HB, Kim HJ, Kang JH, Park SI, Park KH and Lee HJ. Association of circulating irisin levels with metabolic and metabolite profiles of Korean adolescents. Metabolism 2017; 73: 100-108.
[15] Zhang R, Fu T, Zhao X, Qiu Y, Hu X, Shi H and Yin X. Association of circulating irisin levels with adiposity and glucose metabolic profiles in a middle-aged Chinese population: a cross-sectional study. Diabetes Metab Syndr Obes 2020; 13: 4105-4112.
[16] Traczyk I, Kucharska A, Sińska BI, Panczyk M, Wronka L, Raciborski F, Szostak-Węgierek D and Samoliński B. Every second adult inhabitant of Poland (aged 18-64) is overweight - results of representative cross-sectional studies conducted in 2017-2020. Ann Agric Environ Med 2023; 30: 322-330.
[17] Mushenkova NV, Summerhill VI, Zhang D, Romanenko EB, Grechko AV and Orekhov AN. Current advances in the diagnostic imaging of atherosclerosis: insights into the pathophysiology of vulnerable plaque. Int J Mol Sci 2020; 21: 2992.
[18] Lee HJ, Lee JO, Kim N, Kim JK, Kim HI, Lee YW, Kim SJ, Choi JI, Oh Y, Kim JH, Suyeon-Hwang, Park SH and Kim HS. Irisin, a novel myokine, regulates glucose uptake in skeletal muscle cells via AMPK. Mol Endocrinol 2015; 29: 873-881.
[19] Xin C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, Lee Y, Ye J, Lian K, Xu C, Zhang L, Wang Q, Liu Y and Tao L. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. Int J Obes (Lond) 2016; 40: 443-451.
[20] Yano N, Zhao YT and Zhao TC. The physiological role of irisin in the regulation of muscle glucose homeostasis. Endocrines 2021; 2: 266-283.
[21] Lin H, Yuan Y, Tian S, Han J, Huang R, Guo D, Wang J, An K and Wang S. In addition to poor glycemic control, a high level of irisin in the plasma portends early cognitive deficits clinically in Chinese patients with type 2 diabetes mellitus. Front Endocrinol (Lausanne) 2019; 10: 634.
[22] Li H, Shen J, Wu T, Kuang J, Liu Q, Cheng S, Pu S, Chen L, Li R, Li Y, Zou M, Zhang Z, Jiang W, Qu A and He J. Irisin is controlled by farnesoid x receptor and regulates cholesterol homeostasis. Front Pharmacol 2019; 10: 548.
[23] Liu R, Zhang Q, Peng N, Xu S, Zhang M, Hu Y, Chen Z, Tang K, He X, Li Y and Shi L. Inverse correlation between serum irisin and cardiovascular risk factors among Chinese overweight/obese population. BMC Cardiovasc Disord 2021; 21: 570.
[24] Zhu D, Wang H, Zhang J, Zhang X, Xin C, Zhang F, Lee Y, Zhang L, Lian K, Yan W, Ma X, Liu Y and Tao L. Irisin improves endothelial function in type 2 diabetes through reducing oxidative/nitrative stresses. J Mol Cell Cardiol 2015; 87: 138-147.
[25] Wu Y, Luo J, Song X, Gu W, Wang S, Hao S, Dong Z and Ning Z. Irisin attenuates angiotensin II-induced atrial fibrillation and atrial fibrosis via LOXL2 and TGFβ1/Smad2/3 signaling pathways. Iran J Basic Med Sci 2023; 26: 717-724.

[26] Fu S and Xing G. Changes in serum irisin levels and their significance in carotid atherosclerosis associated with obesity. Altern Ther Health Med 2024; 30: 194-199.
[27] Hisamatsu T, Miura K, Arima H, Fujiyoshi A, Kadota A, Kadowaki S, Zaid M, Miyagawa N, Satoh A, Kunimura A, Horie M and Ueshima H. Relationship of serum irisin levels to prevalence and progression of coronary artery calcification: a prospective, population-based study. Int J Cardiol 2018; 267: 177-182.
[28] Pan JA, Zhang H, Yu Q, Zhang JF, Wang CQ, Gu J and Chen K. Association of circulating irisin levels and the characteristics and prognosis of coronary artery disease. Am J Med Sci 2021; 362: 63-71.
[29] Hirayama K, Ishii H, Kikuchi R, Suzuki S, Aoki T, Harada K, Sumi T, Negishi Y, Shibata Y, Tatami Y, Tanaka A and Murohara T. Clinical impact of circulating irisin on classified coronary plaque characteristics. J Appl Lab Med 2018; 3: 79-88.