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Research article

Study on changes in serum irisin level in free-flap transplantation and the correlation of serum irisin level with flap blood flow

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ABSTRACT

Background and aims: The beneficial effect of myokine irisin on ischemia-reperfusion of skin flaps has been rarely reported in clinical studies. This study was designed to determine whether irisin plays a protective role in flap transplantation and identify the factors affecting serum irisin levels.
Materials and methods: We analyzed the changes in serum irisin levels and flap blood flow before and after surgery in 40 patients who underwent skin-flap transplantation. Factors affecting serum irisin levels were analyzed by metabolic parameter measurements.
Results: Preoperative serum irisin levels were positively correlated with blood flow in the skin flap 7 days post-surgery. The increase in serum irisin levels in the first 3 days after surgery positively correlated with flap blood flow. A longer duration of high-intensity exercise, higher skeletal muscle content, lower body mass index, and waist-to-hip ratio were associated with higher irisin levels. Fasting blood glucose and glycosylated hemoglobin levels showed significant negative correlations with serum irisin levels. Several other indicators, including sex, were not associated with serum irisin levels.
Conclusions: Serum irisin levels benefit blood flow recovery during flap transplantation. Better outcomes may be achieved by adjusting the timing and intensity of the exercise and controlling the patient's body size.

1. Introduction

With recent advancements in microsurgical techniques and equipment, the success rate of free-flap transplants has significantly improved, reaching 95%–98.2 % [1,2].However, partial or complete flap necrosis remains a common clinical issue [3]. Among the processes contributing to flap necrosis, ischemia-reperfusion injury (IRI) is inevitable, accompanied by a series of pathophysiological processes [4–6]. Although various hypotheses exist regarding the mechanism of IRI, well-established ones include free oxygen radical production [7], intracellular calcium overload [8], and leukocyte aggregation [9]. These inflammatory mediators lead to the necrosis of vascular endothelial cells [10], destruction of blood vessels, and further tissue damage [2]. Therefore, vascular endothelial cells can be protected against IRI by inhibiting the inflammatory response, reducing oxidative activity, and enhancing antioxidant pathways.

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Irisin, an endogenous peptide fragment formed by the cleavage of fibronectin type III domain-containing protein 5 [11]. Patients' metabolic status, including glucose [12] and lipid metabolism [13], can affect irisin levels. Additionally, pathophysiological changes can induce irisin secretion [14]. Numerous studies have reported that irisin plays a role in various pathophysiological processes that can reduce oxidative stress, improve mitochondrial dysfunction, and inhibit inflammation; therefore, it is believed to prevent IRI [15,16]. In addition, irisin alleviates vascular endothelial cell dysfunction caused by IRI and promotes vascular endothelial cell proliferation to counteract reperfusion injury [17–19]. In our previous animal studies, exogenous irisin injection can improve angiogenesis and the survival rate of multi-territory perforator flaps [16,20]. However, these investigations only involved exogenous interventions and were limited to animal experiments. Therefore, the clinical application of irisin requires further exploration.

This study aimed to identify factors that affect irisin secretion in patients undergoing skin-flap transplantation and assess endogenous irisin's protective effect against IRI. Patients who had undergone free femoral anterolateral flap transplantation were selected. Blood perfusion and serum irisin levels were recorded and analyzed, along with the patient's physiological and metabolic parameters, skeletal muscle content, physical activity levels, and physical conditions. This study provided new ideas and insights for postoperative recovery after flap transplantation and reduced the occurrence of adverse reactions.

2. Materials and methods

2.1. Ethics statement

All patients signed an informed consent form, and the study was approved by the Ethics Committee of Wuxi Ninth People' s Hospital Affiliated to Soochow University (No. LW202037).

2.2. Patients' data

Patients who underwent free anterolateral thigh flap transplantation were enrolled between July 2020 and July 2022 at the Department of Hand Surgery of Wuxi Ninth People' s Hospital Affiliated to Soochow University. The inclusion criteria were as follows: (1) age between 18 and 60 years, (2) skin and soft tissue defects of the upper limb with traumatic or non-traumatic causes, and (3) simple free anterolateral thigh flap transplantation for wound repair (i.e., not combined with other tissue flap transplantation). The exclusion criteria included severe liver and kidney dysfunction, cardiac insufficiency, serious chronic wasting disease, blood disease, rheumatic immune disease, infectious disease, cardiovascular and cerebrovascular disease, respiratory disease, mental disease, and other serious systemic diseases.

2.3. Surgical procedure

The surgery was categorized into three steps: flap design, removal, and transplantation. In all cases, the perforator vessels on the body surface were located using color ultrasound Doppler and computed tomography angiography before surgery according to their relative position to a central point. The dominant perforator branch was determined by comparing hemodynamic conditions. The flap for the anterolateral thigh region was designed based on wound size and shape. The skin and subcutaneous tissue of the flap were superficially elevated to the deep fascia, and the perforator vessels of the flap were identified. In cases with multiple perforator vessels, one or two dominant perforator vessels were selected as nutrient vessels based on flap area and vessel location within the flap. The descending branch of the lateral femoral circumflex artery was exposed, and the perforator vessels were dissected from their origin. Before the flap transplantation, the vessels were ligated and severed. The donor area was either directly sutured or grafted with a full-thickness skin graft. A skin flap covered the wound, with its margin sutured, and the vessels in the blood vessel pedicle were anastomosed to the blood vessels in the recipient area through a subcutaneous tunnel.

2.4. Physical examination

Patient data, including sex, age, cause of injury, and surgical methods, were recorded. The height, weight, waist circumference, and hip circumference were measured. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated using the following formulas: BMI = weight (kg)/height $(m)^2$ and WHR = waist circumference (cm)/hip circumference (cm).

2.5. Physical activity level survey

Previous occupations related to physical labor and the amount of participation in leisure sports were investigated using a questionnaire. The patients were classified and grouped according to their activity intensity and time.

2.5.1. Activity intensity grouping

According to the 1995 Centers for Disease Control and Prevention/American College of Sports Medicine recommendations in the United States [21], combined with the exercise intensity classification proposed by Wong et al. [22],exercise intensity can be categorized into three levels: (1) low intensity when the energy consumption is < 3.0 metabolic equivalents, which corresponds to engaging in sedentary work such as paperwork, taking a walk, or performing other low-energy exercises; (2) medium intensity when the consumption of energy is 3.0–6.0 metabolic equivalents, which corresponds to standing or walking as the primary activity, or

general jogging, swimming, and other sports; and (3) high intensity when the consumption of energy is > 6.0 metabolic equivalents, which corresponds to heavy physical labor such as lifting heavy objects or machinery, strenuous running, ball games, and other sports (1 metabolic equivalent is the metabolic energy consumption at rest, equal to an oxygen intake of 3.5 mL/kg). In this study, the patients were categorized into low-, medium-, and high-intensity groups (intensity groups 1, 2, and 3, respectively) based on activity intensity.

2.5.2. Exercise time grouping

Based on the standards proposed by the Technical Implementation Group of the Survey of Nutrition and Health Status of Chinese Residents [23], high-, medium-, and low-intensity activities were categorized into four groups. The exercise times of the medium- and low-intensity groups were 90–150, 150–300, 300–420, and >420 min/week, respectively, whereas the high-intensity group was categorized into 0–10, 10–60, 60–150, and >150 min/week. This study categorized patients into four groups (time groups 1, 2, 3, and 4), from low to high activity duration.

2.6. Determination of skeletal muscle content

Skeletal muscle content was measured through preoperative tissue composition analysis using a human body component analyzer (InBody 270; InBody, Seoul, Korea).

2.7. Determination of metabolic parameters in blood samples

Fasting venous blood samples were collected preoperatively. An automatic biochemical analyzer was used to determine the total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose (FBG), and glycosylated hemoglobin (HbA_{1c}) levels.

2.8. Determination of serum irisin levels

Fasting venous blood samples were collected before surgery and on postoperative days 1, 3, and 7. After centrifugation, serum was collected and stored at −80 °C. Thawed serum samples were analyzed at room temperature. Serum irisin levels were determined using an enzyme-linked immunosorbent assay (ELISA) and a human irisin ELISA kit (EK-067-29, Phoenix Pharmaceuticals, USA), following the manufacturer's instructions.

2.9. Measurement of skin-flap blood flow

Blood perfusion measurements (in perfusion units [PU]) were taken using a non-contact laser speckle blood flow meter (PeriCam PSI; Perimed, Sweden) before pedicle amputation, immediately after blood circulation, and at 1, 3, and 7 days after surgery.

The blood flow recovery rate (i.e., the percentage increase in blood perfusion on day 7 after surgery compared with blood perfusion immediately after surgery) was calculated using the following formula.

$$\text{Blood flow recovery rate} = \frac{(\text{7 days after surgery (PU)} - \text{immediate blood circulation (PU)})}{\text{immediate blood circulation (PU)}} \times 100\%$$

2.10. Statistical analysis

Continuous variables are expressed as mean ± standard deviation. A two-sample *t*-test was used to compare and analyze the differences between the two groups. One-way analysis of variance was used to compare differences between multiple groups, and post hoc analysis (least significant difference [LSD]) was used for multiple comparisons. Correlations between two variables were analyzed using Pearson's correlation coefficient. Differences were considered statistically significant at $p < .05$. SPSS (version 22.0; SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

3. Results

3.1. General data

Forty patients were enrolled, including 30 men and 10 women, aged 18–58 years, with an average age of 35.9 ± 11.2 years. Among these patients, there were 21 avulsion, 12 compression, four friction injuries, and three heat compression cases, respectively. Additionally, there were 33 cases of soft tissue defects of the hand and wrist and seven cases of forearm defects, with a wound area of 6.0 cm × 10 cm–17 × 28 cm. All the patients underwent free anterolateral femoral flap grafting for wound repair. Flaps were pedicled using the descending branch of the lateral circumflex femoral artery and carried one to two perforator vessels. The radial artery served as the recipient artery in all cases, and the recipient veins were the cephalic vein and subordinate branch or the cephalic vein and radial artery with accompanying veins. The mean operative time was 243.2 ± 40.5 min, and the mean hot ischemia time of the flap was 41.1 ± 12.4 min.

According to the activity intensity, the patients were categorized into three groups: 11, 17, and 13 patients in the low-, medium-,

and high-intensity groups, respectively. The patients were further classified into four groups based on the exercise time, ranging from short to long: five patients in group 1, 17 in group 2, 10 in group 3, and eight in group 4.

All enrolled patients' mean BMI and WHR were 22.8 ± 4.8 kg/m^2 and .84 ± .07, respectively. The average skeletal muscle content was 25.32 ± 4.82 kg. The average TG, TC, total cholesterol, and HbA_{1c} levels were 1.16 ± .44 mmol/L, 4.16 ± .92 mmol/L, 1.49 ± .93 mmol/L, 1.41 ± .35 mmol/L, 5.38 ± 1.11 mmol/L, and 5.28 % ± .89 %, respectively.

3.2. Increase in blood perfusion of the flap after surgery

The skin blood perfusion was relatively low (35.88 ± 20.30 PU) before flap pedicle division; however, it showed a significant increase (47.25 ± 15.42 PU) after blood circulation was restored (Table 1). The blood perfusion rate continued to increase within 7 days after surgery, and the final recovery rate at 7 days after surgery was 51.7 % ± 50.7 %.

3.3. Increase in serum irisin level after surgery

The mean serum irisin level before surgery was 2791.13 ± 560.52 ng/mL, which was relatively low. Increased serum irisin levels were observed on postoperative days 1, 3, and 7. The highest increase in the rate compared to the level before surgery was observed on day 3 (Table 2).

3.4. Relationship between serum irisin level and blood perfusion of the flap

Pearson correlation analysis (Fig. 1A) showed that the preoperative serum irisin level positively correlated with the recovery rate of blood perfusion of the flap on day 7 after surgery, and the correlation was moderate and significant ($r = .440$, $p = .004$). Furthermore, as shown in Fig. 1B, the flap's blood flow recovery rate positively correlated with an increase in serum irisin levels on postoperative day 1, with a moderate and significant correlation ($r = .461$, $p = .003$). Similarly, Fig. 1C shows that on day 3 after surgery, the flap's blood flow recovery rate positively correlated with an increase in serum irisin levels, exhibiting moderate and significant correlation ($r = .417$, $p = .007$). However, on day 7 after surgery, there was no significant correlation between the flap's blood flow recovery rate and the increased rate of serum irisin levels ($r = -.131$, $p = .420$) (Fig. 1D).

3.5. Relationship between irisin level and sex or age

No statistically significant difference in preoperative irisin levels was observed between the male and female patients ($p = .973$), as shown in Fig. 2A. Fig. 2B shows that the preoperative irisin level was negatively correlated with age ($r = -.433$, $p = .005$) and the irisin level showed a decreasing trend with increasing age.

3.6. Relationship between irisin level and physical activity

A correlation was observed between different physical activity intensities and irisin levels before surgery. Post hoc LSD analysis for multiple comparisons showed statistical differences between intensity groups 1 and 2 and also between intensity groups 1 and 3 ($p = .005$ and $p < .0001$, respectively); however, there was no statistical difference between intensity groups 2 and 3 ($p = .125$) (Fig. 3A).

The effect of different durations of physical activity on preoperative irisin levels was relatively small. A statistical difference was observed only between groups 2 and 4 ($p = .028$), and no statistical difference was observed between the other groups (Fig. 3B).

3.7. Relationship between irisin level and physical fitness or metabolic status

Pearson's correlation analysis showed that the preoperative serum irisin level was negatively correlated with BMI and WHR, with a moderate degree of significance (irisin *vs.* BMI: $r = -.451$, $p = .003$; irisin *vs.* WHR: $r = -.313$, $p = .049$), as shown in Fig. 4A and B. Additionally, preoperative serum irisin levels were positively correlated with skeletal muscle content (Fig. 4C), and the correlation was moderate and significant ($r = .543$, $p = .0001$).

Serum irisin levels were negatively correlated with FBG and HbA_{1c} levels, and the correlation was moderate and significant (irisin *vs.* FBG: $r = -.453$, $p = .003$; irisin *vs.* HbA_{1c}: $r = -.580$, $p < .001$) (Fig. 5A and B). No significant correlation was observed between serum irisin levels and TG, TC, LDL-C, or HDL-C levels (irisin *vs.* TG: $r = -.252$, $p = .117$; irisin *vs.* Tc: $r = -.225$, $p = .162$; irisin *vs.* LDL-C: $r = .174$, $p = .283$; irisin *vs.* HDL-C: $r = -.060$, $p = .712$) (Fig. 5C–F).

Table 1
Blood perfusion and blood flow recovery rate of flap at different time points.

Time Point	Pedicle division	Blood Filling	Day 1		Day 3		Day 7	
	BP (PU)	BP (PU)	BP (PU)	RR (%)	BP(PU)	RR (%)	BP(PU)	RR (%)
FlapBlood Flow	35.88 ± 20.30	47.25 ± 15.42	59.15 ± 15.08	20.09 ± 24.28	64.46 ± 18.22	32.29 ± 36.00	68.86 ± 17.18	51.7 ± 50.7

BP: Blood perfusion; RR: Recovery Rate; PU: Perfusion Unit.

Table 2
The concentration the growth rate of irisin on different time points as compared with the preoperative rate.

Time Point	Preparation	Day 1		Day 3		Day 7	
	Con.(ng/mL)	Con.(ng/mL)	IR(%)	Con.(ng/mL)	IR(%)	Con.(ng/mL)	IR(%)
Serum Irisin	2791.13 ± 560.52	2985.81 ± 479.45	12.34 ± 34.90	3367.05 ± 504.73	25.49 ± 30.94	3193.91 ± 534.99	19.29 ± 32.16

Con.: Concentration; IR: Increasement Rate.

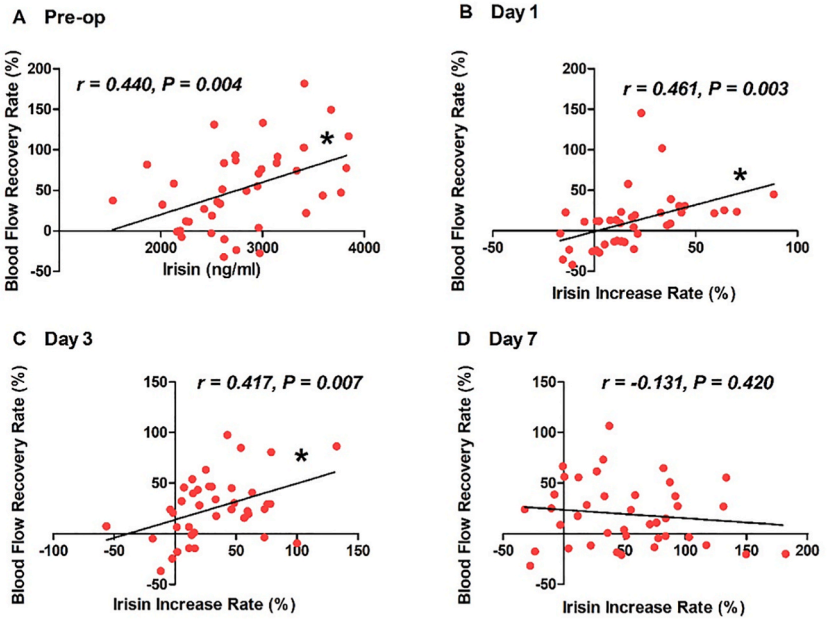

Fig. 1. Correlation analysis of serum irisin level/increase rate of serum irisin level and the recovery rate of the blood perfusion of the flap. A. Preoperative serum irisin level was positively correlated with blood flow recovery rate on day 7 after surgery. B, C. The increased rate of serum irisin level on days 1 and 3 was positively correlated with the increased rate of the blood perfusion of the flap. D. No significant correlation was observed between the increased rate of serum irisin level and the recovery rate of the blood perfusion of the flap on day 7 after surgery. * Represents a significant correlation ($p < .05$).

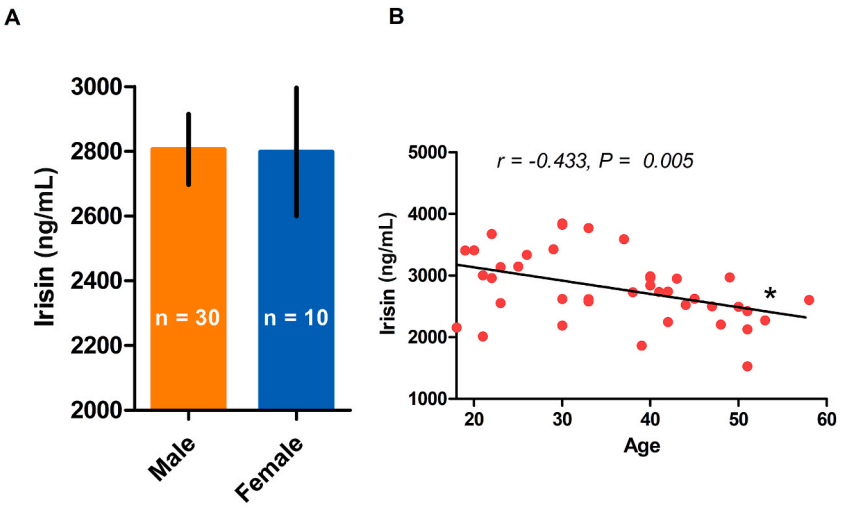

Fig. 2. Relationship between irisin level and sex or age. A. No statistical difference in irisin level was observed between male and female patients. B. A negative correlation was observed between age and irisin level. * Represents a significant correlation ($p < .05$).

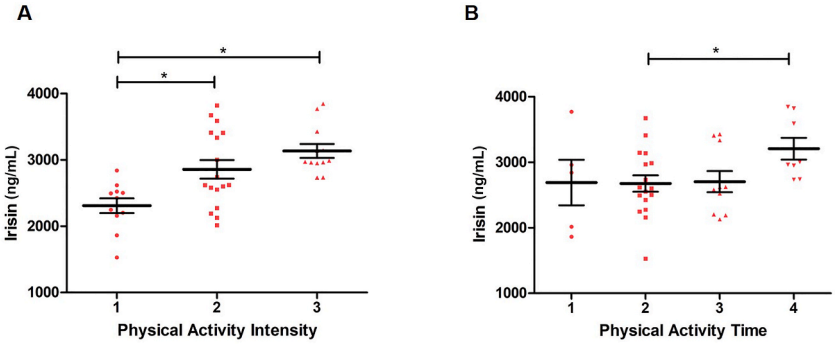

Fig. 3. Relationship between serum irisin level and the intensity or duration of physical activity. A, B. Serum irisin levels in groups with different activity intensities (from low to high: groups 1, 2, and 3) and activity times (from low to high: groups 1, 2, 3, and 4), respectively. * Represents the multiple comparisons in the post-hoc least significant difference analysis, with a statistical difference between the two groups ($p < .05$).

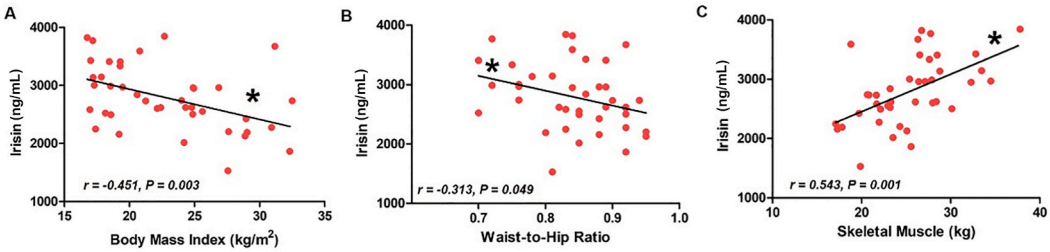

Fig. 4. Relationship between serum irisin level and body mass index (BMI), waist-hip ratio (WHR), or skeletal muscle content. A, B. Serum irisin level was negatively correlated with BMI and WHR. C. Serum irisin level was positively correlated with skeletal muscle content. * Represents a significant correlation ($p < .05$).

4. Discussion

Serum irisin is important for promoting blood flow after flap transplantation. In this study, the serum irisin level before surgery was positively correlated with the blood flow recovery rate on postoperative day 7. Increased serum irisin levels positively correlated with blood flow recovery rate on days 1 and 3 after surgery. Necrosis after flap transplantation is believed to be the primary cause of IRI [8]. Some studies have indicated that irisin protects against IRI by improving vascular epithelial cell function and proliferation. Kozusko et al. found that in patients with acute lung injury caused by IR [24], irisin improved lipopolysaccharide-induced alveolar epithelial barrier dysfunction by activating the adenosine monophosphate-activated protein kinase/silent information regulator 1pathway by suppressing inflammation and apoptosis. Bi et al. also found that irisin can alleviate liver IR injury by protecting mitochondria [25]. This implies that the increase in blood flow may result from the irisin-induced repair of vascular injury. However, whether this protective effect results from a reduction in IR injury remains to be investigated. In addition, it should be noted that the blood flow of the flap was low before the pedicle was removed from the donor area. However, the blood flow was higher in the recipient area than in the donor area after the transplantation, which may be related to the different pressures of the supplying artery and the different perfusion blood flow velocities.

Correlations were also observed between serum irisin levels, skeletal muscle mass, and age, whereas no obvious correlation was observed between serum irisin levels and sex. BMI and WHR negatively correlated with irisin levels. The intensity and duration of physical activity increased serum irisin levels. The factors affecting irisin secretion have also been investigated. Several studies have reported that various factors regulate irisin [14,26]. As irisin is secreted from the skeletal muscle, we further considered why some of the evaluated factors influenced irisin while others did not. This may be because individuals with lower BMI and WHR usually have higher skeletal muscle content [27,28]. Similarly, higher exercise intensity and longer exercise time benefit muscle growth [29]. Aging is typically accompanied by muscle loss [30].

Among the FBG, HBA_{1c}, TC, TG, LDL-C, and HDL-C levels, only the FBG and HBA_{1c}exhibited a significant negative correlation with serum irisin levels. Irisin can improve glucose and lipid metabolism by protecting pancreatic beta cells, reducing peripheral tissue insulin resistance, and increasing insulin sensitivity [31]. A previous study also indicated that irisin levels are negatively correlated with the prevalence of diabetes [32]. However, the reason for the differences in FBG, HBA_{1c}, TC, TG, LDL-C, and HDL-C regarding their correlation with serum irisin levels remains unclear and requires further investigation.

The limitations of this study included the relatively small sample size, a short-term observation period, single-center evaluation, and lack of follow-up. The association of age, skeletal muscle, gender, WHR ratio and BMI were not investigated in this study.

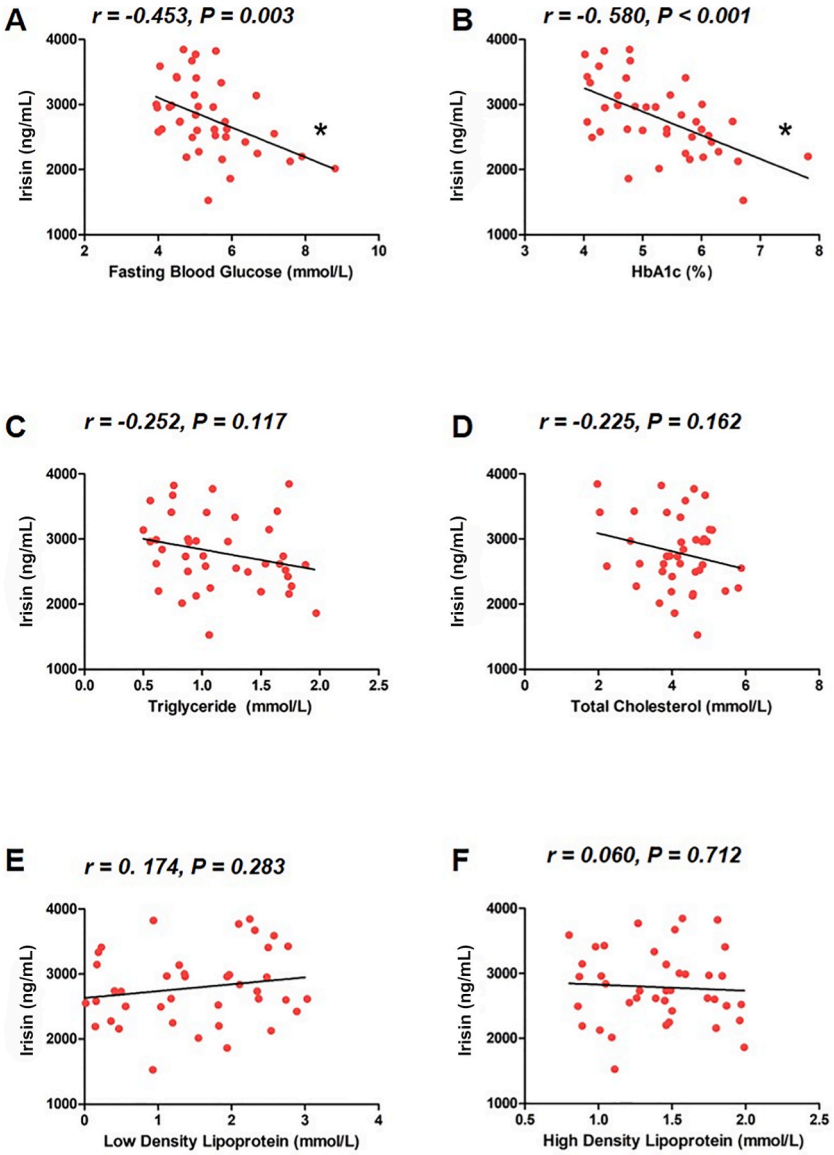

Fig. 5. Relationship between serum irisin level and various metabolic parameters. A, B. Serum irisin level was negatively correlated with fasting blood glucose (FBG) and glycated hemoglobin (HbA_{1c}). C–F. Serum irisin level had no significant correlation with triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). * Represents a significant correlation ($p < .05$).

5. Conclusions

Irisin promotes blood flow after flap transplantation. Benefits can be acquired from high preoperative irisin levels, increased skeletal muscle content, and good metabolic status in high-intensity and long-duration exercise patients. This study sets the foundation for further research on the role of exogenous irisin in skin flap IR injury and provides good insights for clinical practice.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki.This study was conducted with approval from the Ethics Committee of Wuxi Ninth People’ s Hospital affiliated to Soochow University (LW202037).A written informed consent was obtained from all participants.

CRediT authorship contribution statement

Xianyao Tao: Formal analysis, Data curation, Conceptualization. **Xiaoyun Pan:** Writing – review & editing, Formal analysis, Data curation. **Gang Zhao:** Software, Formal analysis, Data curation. **Yongjun Rui:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

IRIIschemia-reperfusion injury.
BMIBody mass index.
WHRWaist-to-hip ratio.
TCTotal cholesterol.
TGTriglyceride.
LDL-CLow-density lipoprotein cholesterol.
HDL-CHigh-density lipoprotein cholesterol.
FBGFasting blood glucose.
HbA1cGlycosylated hemoglobin.
ELISAEnzyme-linked immunosorbent assay.
PUPerfusion units.
LSDLeast significant difference.

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