TJVS Turkish Journal of Vascular Surgery

Turk J Vasc Surg 2025;34(1):61-9
DOI: 10.9739/tjvs.2025.02.010

Available online at www.turkishjournalofvascularsurgery.org

Original Article

Irisin: An influential perspective on the protective effect of metformin on aortic and cardiac aging in rats

Ozgur Baris[1], Gulcin Gacar[2], Burak Acar[3], Feride Ipek Komsuoglu Celikyurt[4], Yusufhan Yazir[2,5], Tijen Utkan[4]

[1]Kocaeli University, Faculty of Medicine, Department of Cardiovascular Surgery, Kocaeli, Türkiye
[2]Kocaeli University, Institute of Health Sciences, Center for Stem Cell and Gene Therapies Research and Practice, Kocaeli, Türkiye
[3]Kocaeli University, Faculty of Medicine, Department of Cardiology, Kocaeli, Türkiye
[4]Kocaeli University, Faculty of Medicine, Department of Pharmacology, Kocaeli, Türkiye
[5]Kocaeli University, Faculty of Medicine, Department of Histology and Embryology, Kocaeli, Türkiye

Received: February 24, 2025 Accepted: March 07, 2025 Published online: March 20, 2025

Abstract

Aim: Irisin, a polypeptide hormone, demonstrates a protective function in metabolic diseases and has been observed to enhance endothelial functions. It regulates energy metabolism, fat tissue and glucose metabolism. The predictive value of irisin for the effects of metformin on cardiac and aortic aging in an aged rat model was evaluated by quantitative and histological confirmation.

Material and Methods: A total of twenty-four rats were used in our study. The subjects were equally distributed among four intervention groups: young control group (<12 months), young-metformin group (<12 months), old-control group (>24 months), and old-metformin group (>24 months). The effect of senescence on the heart and aortic tissues, inflammatory and oxidative stress markers, and proliferation indicators (Ki-67, PDGFR-β, TNF-α, IL-1β, NF-κB, TGF-β, e-NOS, and SOD-1) were evaluated with RT-PCR analyses quantitatively. Serum sampling was conducted to determine irisin levels. A histomorphological and immunhistochemical analysis of the heart and aorta sections was performed.

Results: Metformin treatment altered irisin levels in young and old rats compared to controls. Irisin concentrations were higher in metformin-treated rats: 2.57 (0.39) versus 1.89 (0.16) in young rats, and 1.79 (0.31) versus 1.19 (0.22) in old rats ($P<0.001$ for both). Differences were noted between young-metformin and old-control groups ($P=0.002$), and old-control and old-metformin groups ($P=0.008$). Aortic tissue showed significant variations among groups in NF-κB ($P=0.024$), TGF-β ($P<0.001$), e-NOS ($P=0.001$), and SOD-1 ($P<0.001$) levels. In cardiac tissue, significant differences were observed for Ki-67 ($P<0.001$), TGF-β ($P=0.001$), e-NOS ($P=0.003$), and PDGFR-β ($P=0.003$) levels.

Conclusion: Irisin level was significantly affected by metformin treatment, especially in young rats. Irisin was positively correlated with platelet-derived growth factor receptor-β and oxidative-proinflammatory markers were down-regulated with metformin treatment. Irisin can be viewed as a guide and confirmatory in anti-aging studies, aortic pathologies, and the elucidation of new drug studies and new pathways to confirm cardiac damage.

Keywords: Iirisin, metformin, oxidative markers, polymerase chain reaction, vascular inflammation

INTRODUCTION

The peptide irisin, derived from the proteolytic cleavage of full-length fibronectin type III domain containing-5 (FNDC5), plays a crucial role in modulating energy metabolism, adipose tissue function, and glucose homeostasis. This hormone exhibits both anti-obesity and anti-diabetic properties [1]. The synthesis of irisin occurs predominantly in cardiac tissue, skeletal muscles, pancreatic beta cells, hepatic tissue, renal tissue, and salivary glands [2].

Moreover, irisin functions as a myokine dependent on

CITATION
Baris O, Gacar G, Acar B, Komsuoglu Celikyurt FI, Yazir Y, Utkan T. Irisin: An influential perspective on the protective effect of metformin on aortic and cardiac aging in rats. Turk J Vasc Surg. 2025;34(1):61-9.

Corresponding Author: Ozgur Baris, Kocaeli University, Faculty of Medicine, Department of Cardiovascular Surgery, Kocaeli, Türkiye
Email: drozgurbaris@gmail.com

peroxisome proliferator-activated receptor-gamma coactivator-1-alpha (PGC-1α). PGC-1α, a highly adaptable transcriptional coactivator, responds to various physiological and nutritional alterations, subsequently modulating glucose and fatty acid metabolism, as well as mitochondrial functionality [3]. Contemporary research has elucidated that aberrant regulation of PGC-1α plays a significant role in the etiology of diabetes [4,5].

Studies have shown that transgenic mice with selective PGC-1α expression demonstrate notable resilience to obesity and metabolic disorders associated with aging [1]. Moreover, irisin has been found to not only alleviate insulin resistance and diabetes but also provide protection against various cardiovascular conditions, encompassing coronary artery disease, atherosclerosis, and congestive heart failure.

Investigations have revealed that irisin plays a crucial role in attenuating cardiac dysfunction resulting from myocardial ischemia/reperfusion, operating through a mechanism dependent on mitochondrial ubiquitin-ligase [6]. Moreover, scientific evidence indicates that irisin exhibits cardioprotective properties against myocardial infarction by stimulating angiogenesis, while also mitigating atherosclerosis through the prevention of endothelial damage in diabetic mice lacking Apolipoprotein-E [7].

The primary endpoint of this study is examining the association between irisin concentrations and the response to the aortic and cardiac preservative effects of metformin.

MATERIAL AND METHODS

Experimental Model

A total of twenty-four male Wistar-Albino rats (275-325 gr), twelve of which were young (<12 months old) and twelve of which were naturally aged (>24 months), were randomly selected for the experimental study and maintained in a controlled laboratory environment. The standardized conditions included a humidity level of 45%, ambient temperature of 22±2°C, and a 12-hour light cycle (07:00 AM to 07:00 PM). The animals were provided unrestricted access to food and water throughout the study period.

The subjects were equally distributed among four intervention groups: young control group (<12 months), young-metformin group (<12 months), old-control group (>24 months), and old-metformin group (>24 months). The sample size was determined in accordance with the literature, with reference to Arifin WN's study [8], with a maximum number of animals of six.

Every day, 100 mg/kg of metformin was added to the water that rats in the two metformin groups drank, ensuring that they fully received the medication. The gavage method was not used to avoid stress that could affect inflammatory and oxidative stress markers. The cages were placed adjacent to each other to allow the animals to be in communication with each other so that social isolation and feelings of isolation would not cause oxidative stress damage due to unpredictable stress in rats. Metformin administration continued for one month.

The subjects were initially anesthetized with ketamine hydrochloride (Ketalar®; Pfizer, İstanbul, Türkiye), 100 mg/kg, administered intraperitoneally. Subsequently, the subjects were euthanized via the injection of a lethal dose of sodium thiopental. (Pentothal® Sodium; Abbott Laboratories, Italy). After the hearts and the thoracic aortas were explored with a median sternotomy, they were excised and placed in 0.9% saline for tissue examinations. The effect of senescence on the heart and aortic tissues, inflammatory and oxidative stress markers, and proliferation indicators were interpreted by the Real-Time Polymerase Chain Reaction (RT-PCR) technique, and serum sampling was enforced to determine irisin levels.

Irisin ELISA Assay

Blood samples were extracted from terminally anesthetized rats for Irisin quantification. Serum preparation involved centrifugation at 1,000 g for 15 minutes at 4°C, followed by storage at -80°C pending analysis. A commercial rat irisin enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, cat. no. E6281Ra, Yangpu Dist, Shanghai, China) was employed to assess protein levels, adhering to the manufacturer's guidelines. Absorbance measurements were conducted at 450 nm using a microtiter ELISA reader (VersaMax Molecular Devices, Sunnyvale, CA, USA). The ELISA results were reported in pg/mL.

Real-Time PCR Gene Expression

The RT-PCR method was employed to evaluate markers, including Ki67, superoxide dismutase type 1 (SOD-1), tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), platelet-derived growth factor receptor-β (PDGFR-β), Nuclear factor-κB (NF-κB), transforming growth factor-beta (TGF-β), and endothelial nitric oxide synthase (e-NOS). The acquired tissues underwent a washing procedure with phosphate-buffered saline (PBS) to eliminate residual blood and clots, prior to RNA extraction. RNA isolation was performed using the GeneJET RNA Purification kit (Thermo Scientific, Dreieich, Germany). Mechanical disruption of the tissues occurred in a 1.5 mL centrifuge tube containing the provided buffer solution. Following centrifugation at 8000g for five minutes to remove cellular and tissue debris, the resulting supernatant was mixed with 99% ethanol in a 3:1 ratio and subsequently transferred to the supplied RNA purification column.

RNA samples were washed three times with washing solution. To elute RNA fragments from the column, 50 µL of elution solution (10 mM Tris-HCl buffer, pH 7.5) was added, and total RNA was obtained after centrifugation at 13000G for two

minutes. The purity and quantity of the samples were measured using a Picodrop device (Picodrop Limited, Saffron Walden, Cambridgeshire, United Kingdom).

The Maxima H Minus First Strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA) was employed for cDNA synthesis, with strict adherence to the manufacturer's instructions. As specified in the protocol, the cDNA synthesis process was executed using a standard PCR device (Takara, Tokyo, Japan) using 2 µg of RNA sample, 5 µM oligo-dT primers, RNase inhibitor (1X), and reverse transcriptase enzyme (50 mU/µL). The synthesis process included denaturation and priming at 25°C for five minutes, reaction at 46°C for 20 minutes, and inactivation at 95°C for one minute. For gene expression analysis, the Bright Green 2x qPCR master solution containing SYBR dye (Abcam, Cambridge, MA, USA) was used.

The amplification of synthesized cDNA samples (100 ng) was conducted using an RT-PCR machine (LightCycler480-II, Roche, Rotkreuz, Switzerland) with gene-specific primers (30 nM) in the master mixture. For this study, the ActB gene functioned as the reference gene. The RT-PCR machine was programmed with the following temperature settings: 95°C for 10 minutes to activate enzymes, 95°C for 15 seconds to denature DNA, and 60°C for one minute for annealing and extension, with a total of 45 cycles. Upon completion, the machine output was analyzed to determine the Cp value. Subsequently, the ΔΔCp value was normalized against the reference gene and calculated in relation to the control group. Results were presented as x-fold increases or decreases compared to the control group.

Histological Study

Following euthanasia of the rodents in accordance with ethical protocols, the excised cardiac and thoracic aortic tissues were promptly rinsed with physiological saline solution and subsequently fixed in 10% neutral buffered formalin for 72 hours in preparation for light microscopy analysis. The tissues were dehydrated by passing through concentrated alcohol solutions (70%, 90%, 96%, 100%) sequentially. The tissue samples underwent a clearing process in xylene for two 10-minute intervals, followed by overnight incubation in paraffin within a 60°C oven. Subsequently, the samples were embedded in cassettes using an appropriate device. Paraffin blocks, sectioned to approximately 4 µm thickness, were subjected to hematoxylin and eosin (H-E) staining. Examination and imaging of the sections were conducted using a LEICA DM 1000 light microscope (Leica Microsystems GmbH, Wetzlar, Germany).

Immunohistochemical Staining for Analysis of Oxidative and Inflammatory Markers

To evaluate the expression of SOD-1, e-NOS, and TNF-α in aorta sections and cardiomyocytes, tissues were collected and fixed in formalin (10%) for three days for histochemical and immunofluorescence staining. After the fixed tissue samples were washed in water, they were incubated in 70%-80%-90% and 100% ethanol for 30 min each, for dehydration. The specimens underwent a series of preparatory steps for histological examination. Initially, they were immersed in absolute ethanol for 30 minutes, followed by a 1-hour xylene treatment. Subsequently, the samples were transferred to liquefied paraffin at 60°C and maintained at this temperature in an oven for 1 hour. The prepared specimens were then positioned in metal molds and encased in paraffin. Using a microtome, 5 µm thick sections were cut from the solidified paraffin blocks and mounted on slides. To prepare for immunofluorescence staining, the slides were heated in an oven at 70°C for 1 hour. The paraffin was then removed from the sections by exposing the slides to xylene for a duration of 5 minutes. Then, the slides were kept in a 100%–90%–80%–70% ethanol series for 5 min. The slides were washed with pure water for rehydration. For antigen retrieval, the sections were subjected to a 20-minute boiling process in 50 mM trisodium citrate solution (pH 6.0). Immunofluorescence staining commenced with a 20-minute incubation of cells in the appropriate blocking serum. The membrane was then exposed to the primary antibody for 16 hours at 4°C. Following this, the samples underwent PBS washing and were subsequently labeled via a 90-minute incubation with fluorophore-conjugated secondary antibodies at room temperature. After thorough washing to eliminate unbound antibodies, the nuclear stain was overlaid with DAPI-containing mounting medium (Mounting Medium with DAPI, Santa Cruz). Visualization and examination of the samples were conducted using a Leica DMI 8 Confocal Microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany).

Statistical Analysis

Statistical analysis was conducted using SPSS Statistics, version 20 (IBM Inc., Armonk, NY, USA). The Kolmogorov-Smirnov test was employed to assess the normal distribution of continuous variables. For descriptive statistics, continuous variables were expressed as medians (interquartile range, IQR), while categorical variables were presented as frequencies and percentages. To evaluate differences in mean irisin levels following metformin administration, one-way ANOVA was performed, succeeded by a post hoc Tukey test. The relationship between serum irisin level and aortic or cardiac inflammatory markers was examined using Spearman correlation analysis. A two-tailed P value of <0.05 was considered statistically significant.

Approved Ethics Council Information

In adherence to the ARRIVE Guidelines, the experimental protocol employed in this investigation was meticulously executed. The experimental protocol received approval from the Kocaeli University-Local Ethics Committee for Animal Studies (Protocol Number: KOÜ HADYEK- 4/1-2020; approval date: June 25, 2020).

RESULTS

Irisin ELISA Assay

After one month of treatment, there was a difference in irisin levels between metformin-treated and control rats (Table 1). The level of irisin was elevated in both the young and old rats, compared to control rats [2.57 (0.39) vs. 1.89 (0.16); 1.79 (0.31) vs. 1.19 (0.22); P<0.001, respectively]. The level of irisin was higher in young rats treated with metformin. Post-hoc statistical evaluation elucidated significant distinctions among the experimental groups. Notably, the young cohort receiving metformin demonstrated a statistically significant difference when juxtaposed with the old control group (P=0.002). Additionally, a substantial variance was identified between the old control and old metformin groups (P=0.008).

Findings of RT-PCR Analyses

Aortic Tissue Results: Table 2a presents the mean values of inflammatory and oxidative stress markers in aortic tissue across the groups. Analysis revealed no significant variations in Ki-67, PDGFR-β, TNF-α, and IL-1β among the four groups. Nevertheless, one-way ANOVA demonstrated statistically significant inter-group differences for NF-κB (P=0.024), TGF-β (P<0.001), e-NOS (P=0.001), and SOD-1 (P<0.001) levels (Table 2a). The assessment of statistical disparities between aortic tissue groups was conducted employing post hoc analyses, specifically the Tukey HSD test for multiple comparisons. Statistically significant results of multiple comparisons are shown in Table 2b.

Cardiac Tissue Results: Table 3a presents the mean values of inflammatory and oxidative stress markers in cardiac tissue across the groups. Analysis revealed no significant variations in NF-κB, SOD-1, TNF-α and IL-1β among the four groups. Nevertheless, one-way ANOVA demonstrated statistically significant inter-group differences for Ki-67 (P<0.001), TGF-β (P=0.001), e-NOS (P=0.003), and PDGFR-β (P=0.003) levels (Table 3a). The assessment of statistical disparities between cardiac tissue groups was conducted employing post hoc analyses, specifically the Tukey HSD test for multiple comparisons. Statistically significant results of multiple comparisons are shown in Table 3b.

Finally, the analysis revealed a significant inverse relationship (r=-0.579; P=0.019) between irisin and TGF-β concentrations in aortic tissue. Conversely, a notable positive association was established between irisin and PDGFR-β levels in both cardiac and aortic tissues (r=0.585; P=0.017 and r=0.523; P=0.037, respectively), as illustrated in Table 4.

Cardiac Histological and Immunohistochemical Analyses

Normal cardiac morphology was observed with muscle fibers and intercalar discs in the young control (A) and young + metformin (B) groups. In the old control group (C), damage caused by inflammatory cells (arrowhead) together with degenerated cardiomyocytes with irregularly shaped myofibrillar sequence (arrow) was evident, while in the old + metformin group (D), degenerated cardiomyocytes were significantly reduced and myofibrillar alignment was improved, showing much less intensity of degeneration with regular-shaped cardiomyocytes (*), similar to Group A (Figure 1).

In the immunohistochemical analyses of cardiomyocytes, it was observed that SOD-1 response decreased in the old control (OC) group compared to the young control (YC) group. It was also determined that e-NOS and TNF-α expression decreased in the old + metformin (OM) group (Figure 1).

Aortic Histological and Immunohistochemical Analyses

In the results of H&E-stained aortic tissues, Normal morphology of the tunica intima (TI), tunica media (TM), tunica adventitia (TA), and aortic layers was observed in young control (A) and young + metformin (B) groups. Edema and irregular elastic lamellae (arrow), together with thickening of the tunica media and disruption of endothelial integrity (*) were observed in the old control group (C). The intimal irregularity due to aging observed in group C was significantly reduced in the old metformin group (D). A recovery close to normal morphology was observed in group D (Figure 2).

In aortic tissues, when e-NOS and TNF-α expression was examined, it was observed that oxidative and inflammatory activity, which was significantly increased in the OC group compared to that in the YC and young + metformin (YM) groups, was clearly decreased in the OM group (Figure 2).

Histological examinations revealed that the drug-treated groups exhibited cellular structures similar to those observed in the young control group. Notably, the old drug group demonstrated significant morphological improvements, with cells closely resembling those in the young control group. These findings provide evidence for metformin's anti-aging effects on aged cells.

Table 1. Mean serum irisin levels of the study subjects and controls

Study group	Serum irisin (pg/mL)	P value
All groups		**0.002**
Young-control	1.89±0.16	
Young+metformin	2.57±0.39	
Old-control	1.19±0.22	
Old+metformin	1.79±0.31	
Post hoc analysis with Tukey - HSD		
Young-control vs young+metformin		0.061
Young-control vs old-control		0.296
Young-control vs old+metformin		0.196
Young+metformin vs old-control		**0.002**
Young+metformin vs old+metformin		0.888
Old-control vs old+metformin		**0.008**

DOI: 10.9739/tjvs.2025.02.010

Table 2a. Real-time PCR gene expression: aortic tissue results

	Young control	Young + metformin	Old control	Old + metformin	P value
NF-κB (mean±SD)	1.01±0.19	1.08±0.26	1.26±0.6	0.39±0.19	**0.024**
TGF-β (mean±SD)	1.00±0.12	0.68±0.11	0.85±0.2	0.25±0.16	**0.001**
e-NOS (mean±SD)	1.02±0.23	2.32±0.5	2.85±0.89	0.87±0.37	**0.001**
Ki-67 (mean±SD)	1.01±0.12	0.84±0.17	0.84±0.06	0.93±0.1	0.220
PDGFR-β (mean±SD)	1.01±0.13	1.85±0.17	1.03±0.8	1.53±0.4	0.066
SOD-1 (mean±SD)	1.05±0.39	0.28±0.13	0.21±0.11	0.12±0.07	**0.001**
TNF-α (mean±SD)	1.01±0.21	1.24±0.94	1.23±0.56	1.09±0.83	0.957
IL-1β: (mean±SD)	1.02±0.22	1.38±0.75	2.14±1.11	1.31±0.57	0.224

e-NOS: endothelial nitric oxide synthase, IL-1β: interleukin-1β, Ki-67: a protein known as a proliferation marker, NF-κB: nuclear factor-κB, PDGFR-β: platelet-derived growth factor receptor-β, SOD-1: superoxide dismutase-1, TGF-β: transforming growth factor-β, TNF-α: tumor necrosis factor-α

Table 2b. Post Hoc tests of aortic tissues/ multiple comparisons with Tukey HSD

Marker	Groups	P value
NF-κB	OC-***OM***	**0.021**
TGF-β	YC-***YM***	**0.043**
	YC-***OM***	**0.000**
	YM-OM	**0.008**
	OC-***OM***	**0.001**
e-NOS	YC-***YM***	**0.028**
	YC-OC	**0.003**
	YM-OM	**0.015**
	OC-***OM***	**0.001**
SOD-1	YC-***YM***	**0.001**
	YC-OC	**0.001**
	YC-***OM***	**0.000**

YC: young control, YM: young + metformin, OC: old control, OM: old + metformin (Drug groups are indicated in bold and italic characters)

Table 3a. Real-time PCR gene expression of cardiac tissue

	Young control	Young + metformin	Old control	Old + metformin	P value
NF-κB (mean±SD)	1.17±0.7	2.1±0.97	1.77±2.15	2.14±1.44	0.759
TGF-β (mean±SD)	1.04±0.32	5.57±1.88	6.12±1.46	3.43±1.12	**0.001**
e-NOS (mean±SD)	1.01±0.14	2.5±0.73	2.6±0.32	3.61±0.48	**0.001**
Ki-67 (mean±SD)	1.07±0.43	1.84±0.80	7.13±3.64	1.88±0.91	**0.003**
PDGFR-β (mean±SD)	1.04±0.31	15.42±4.56	0.63±0.55	9.02±8.89	**0.003**
SOD-1 (mean±SD)	1.23±0.83	1.22±0.80	1.77±1.32	0.33±0.21	0.196
TNF-α (mean±SD)	1.66±1.70	1.88±1.42	2.18±2.00	5.27±5.71	0.385
IL-1β: (mean±SD)	1.16±0.69	1.39±1.26	1.71±1.19	1.73±1.13	0.855

e-NOS: endothelial nitric oxide synthase, IL-1β: interleukin-1β, Ki-67: a protein known as a proliferation marker, NF-κB: nuclear factor-κB, PDGFR-β: platelet-derived growth factor receptor-β, SOD-1: superoxide dismutase-1, TGF-β: transforming growth factor-β, TNF-α: tumor necrosis factor-α

DOI: 10.9739/tjvs.2025.02.010

Table 3b. Post Hoc tests of cardiac tissues/ multiple comparisons with Tukey HSD

Marker	Groups	P value
Ki-67	YC-***YM***	**0.004**
	YC-OC	**0.002**
	YC-***OM***	**0.000**
	YM-OM	**0.026**
TGF-β	YC-***YM***	**0.002**
	YC-OC	**0.001**
e-NOS	YC-OC	**0.004**
	YM-OC	**0.010**
	OC-***OM***	**0.011**
PDGFR-β	YC-***YM***	**0.007**
	YM-OC	**0.006**

YC: young control, YM: young + metformin, OC: old control, OM: old + metformin (Drug groups are indicated in bold and italic characters)

Table 4. Spearman correlation analysis of inflammatory markers and serum irisin levels

Parameters	Irisin			
	Cardiac tissue		Aortic tissue	
	r value	P value	r value	P value
SOD-1	-.015	0.957	-.309	0.244
TNF-α	-.365	0.165	-.297	0.263
IL-1β	-.115	0.672	-.190	0.481
e-NOS	-.441	0.087	-.322	0.223
PDGFR-β	**.585**	**0.017**	**.523**	**0.037**
NF-κB	-.322	0.896	-.411	0.113
TGF-β	.061	0.822	**-.579**	**0.019**
Ki-67	-.158	0.559	-.065	0.810

e-NOS: endothelial nitric oxide synthase, IL-1β: interleukin-1β, Ki-67: a protein known as a proliferation marker, NF-κB: nuclear factor-κB, PDGFR-β: platelet-derived growth factor receptor-β, SOD-1: superoxide dismutase-1, TGF-β: transforming growth factor-β, TNF-α: tumor necrosis factor-α

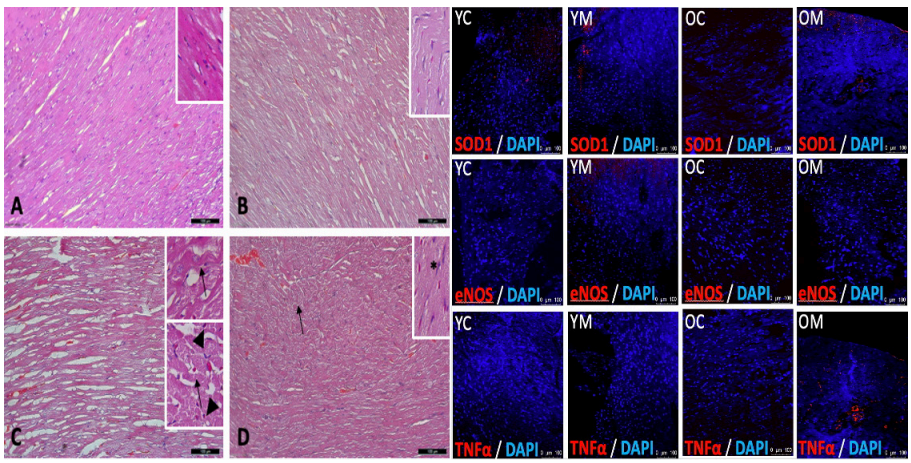

Figure 1. Photomicrographs of H&E-stained cardiac tissues and immunhistochemical findings; H&E: Photomicrograph showing cardiomyocytes from young control (**A**), young + metformin (**B**), old control (**C**) and old + metformin (**D**) groups; Scale: 100 µm; IHC: Photomicrograph showing SOD-1, e-NOS and TNF-α expression in cardiomyocytes from young control (**YC**), young + metformin (**YM**), old control (**OC**) and old + metformin (**OM**) groups. Scale: 100 µm

DOI: 10.9739/tjvs.2025.02.010

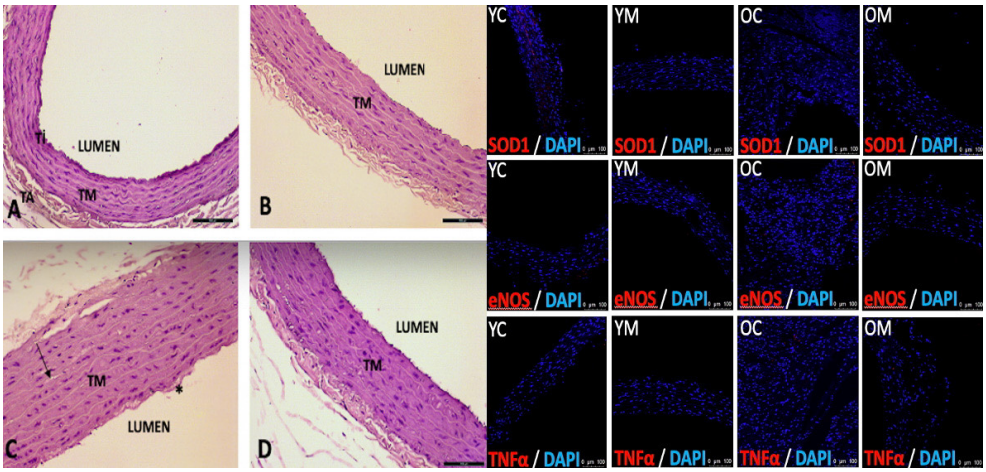

Figure 2. Photomicrographs of H&E-stained aortic tissues and immunhistochemical findings; H&E: Photomicrograph showing aortic tissues from young control (**A**), young + metformin (**B**), old control (**C**) and old + metformin (**D**) groups; Scale: 100 µm; IHC: Photomicrograph showing SOD-1, e-NOS and TNF-α expression in aortic tissues from young control (**YC**), young + metformin (**YM**), old control (**OC**) and old + metformin (**OM**) groups; Scale: 100 µm

DISCUSSION

The primary finding of this investigation was that metformin appears to stimulate irisin secretion in both young and aged rats. Irisin showed a positive correlation with PDGFR-β in both cardiac tissue and aortic tissue. However, only in aortic tissue, it exhibited a negative correlation with TGF-β. Furthermore, following a four-week treatment period with metformin, significant alterations in inflammatory and oxidative markers were observed in both aortic and cardiac tissues. Irisin, first described in 2012 as exercise-induced myokine, behaves like an insulin-sensitizing hormone in mice [1]. The irisin amino acid sequence is almost identical among most mammalian species. The cleavage of FNDC5 in a PGC-1α-dependent manner causes irisin secretion [4]. PGC-1α is a transcriptional coactivator and is important in the pathogenesis of type 2 diabetes, closely related to sedentary lifestyle, obesity, and irisin activated via PGC-1α [4,5]. Recent research has demonstrated a correlation between type 2 diabetes and decreased levels of circulating irisin in patients exhibiting PGC-1α expression [9]. Diabetic animal models showed that overexpression of irisin provides energy expenditure and lower fasting glucose levels in mice. Genetically modified mice with enhanced PGC-1α expression in muscle tissue exhibit resistance to diabetes and obesity associated with aging. Skeletal muscle-derived myokines, which are secreted proteins, impact various tissues in relation to changes in energy expenditure [1]. Irisin also stimulates β-cell proliferation in vivo in mice [10], inhibiting gluconeogenesis and increasing membrane-associated glucose transport protein-4 on the cytoplasmatic membrane [11]. Another reported study using obese mice showed that irisin caused up-regulation of betatrophin. As the primary therapeutic intervention for type 2 diabetes mellitus, particularly in obese patients, metformin, a biguanide class anti-diabetic agent, is widely prescribed [12]. Research conducted by Li et al. examined the impact of intramuscular metformin and glibenclamide administration on blood irisin levels, comparing diabetic mice with normal, wild-type controls. Their study revealed an upregulation of FNDC5 protein and mRNA expression in skeletal muscle, subsequently promoting irisin secretion [13]. In a separate investigation, Liu et al. observed that exenatide therapy led to increased irisin concentrations in individuals with type 2 diabetes. Notably, irisin levels were inversely correlated with fasting blood glucose and glycated hemoglobin measurements [14].

The irisin response in young rats was more pronounced than in elderly rats; however, this difference did not reach significance. Notably, even in elderly rats, metformin induced a significant elevation in irisin levels. The subgroup analysis revealed that young rats receiving metformin exhibited higher irisin levels than the elderly control group.

Serum irisin was positively correlated with PDGFR-β in both aortic and cardiac tissue. Previous studies have shown the blood pressure-lowering effect of irisin in hypertensive rats [15]. In addition to its metabolic and cardiac effects, its blood antihypertensive effect has recently drawn attention. Research conducted by Altaweel et al. on rats administered escalating doses of irisin revealed that this hormone significantly impacts the structure of the rat thoracic aorta wall. The study observed an increase in intima-medial thickness, smooth muscle cell (SMC) nuclei count, and the number of elastic lamellae in the tunica media [16]. The researchers proposed that irisin may have influenced growth factors affecting the thoracic aorta walls and stimulated SMCs within the aortic layers. Platelet-derived growth factors (PDGFs) play a crucial role in regulating blood vessel cell growth and are involved in cellular proliferation, angiogenesis, inflammation, and fibrogenic processes.

Consequently, imbalanced PDGF levels can impact diabetes mellitus and its associated complications [17]. The current investigation demonstrated a positive correlation between irisin levels and PDGFR levels in both aortic and cardiac tissues.

The pathogenic and protective roles of TGF-β in various disorders remain a topic of scientific discourse, with conflicting evidence presented in the literature [18,19]. This study revealed an inverse relationship between irisin and TGF-β concentrations in aortic tissue, while no such correlation was observed in cardiac tissue. Transforming growth factor-1beta (TGF-1β1), a recognized pro-fibrogenic cytokine, plays a crucial role in the production of extracellular matrix and synthesis of collagen [20]. Notably, TGF-1β demonstrated protective properties against the expansion of abdominal aortic aneurysms in rat models [21,22]. Furthermore, research by Angelov et al. indicated that systemic neutralization of TGF-β led to a significant increase in the occurrence of abdominal aortic aneurysmal disease in angiotensin-II-infused mice, while the thoracic aorta remained unaffected [23].

Research conducted by Dong et al. revealed that irisin mitigates TGF-β1-induced inflammatory cytokine expression in hepatic cells, suggesting its potential to substantially impede the advancement of hepatic fibrosis [24]. Additionally, a study by Peng et al. elucidated that irisin possesses the ability to interact with TGF-β receptor II (TβRII), thereby antagonizing its effects [25]. These findings collectively indicate the possibility that irisin may counteract TGF-β1-induced differentiation processes. The results of these studies suggest that TGF-β affects different organs through different mechanisms, perhaps with beneficial effects in some organs and simultaneous deleterious effects in others.

In addition to these anti-inflammatory effects, irisin has also been shown to be protective in the cardiovascular system. It has been shown that obese patients with atrial fibrillation have high blood leptin levels, whereas ADA, TNF-α and irisin levels are low, thus indicating that low irisin levels increase the risk of arrhythmia [26]. Numerous in vivo and in vitro studies have shown that administration of exogenous irisin attenuates endothelial damage, inflammatory damage, and oxidative stress, suggesting that vascular aging is slowed by irisin [27], and irisin is an important anti-aging mediator. Recent studies have shown that irisin has vascular anti-aging effects [28].

The anti-aging properties of metformin appear to be linked to elevated irisin levels, as evidenced by its multifaceted effects. Metformin has been shown to inhibit TGF-beta-induced collagen synthesis through AMPK activation [29], decrease hepatic glucose production via the duodenal AMPK-dependent pathway [30], and attenuate enhanced autophagy and cellular senescence by reversing age-associated metabolic and cellular changes, as well as inflammation [31]. These findings suggest a correlation between metformin's anti-aging effects and increased irisin concentrations.

Our study has some limitations. An even larger sample size would have been better in the cross-sectional study. A causality study design would have revealed a more detailed relationship between irisin and tissue-level markers.

CONCLUSION

In conclusion, the results of our investigation reveal that metformin administration facilitates irisin secretion into the circulatory system of both young and aged rats. In addition, irisin was positively correlated with PDGFR-β and was negatively correlated with aortic tissue TGF-β expression. Irisin can be considered a predictor directly proportional to the protective effect of metformin against oxidative damage and inflammation seen in aortic and heart tissues due to aging.

Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee of Experimental Animal Studies of Kocaeli University (Acceptance Protocol No: KOÜ HADYEK: 4/1-2020; date: June 25, 2020).

Patient Consent for Publication: Since this was an experimental animal study, "Patient Consent for Publication" was not required.

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

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

Acknowledgement: The authors extend their sincere appreciation to Mr. Jeremy Huw Jones for his contributions in reviewing and providing feedback on the native English revision. All authors sincerely express their gratitude to Prof. Dr. Canan Baydemir, who worked hard on the statistical analysis of this original study.

REFERENCES

1. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481:463-68.
2. Aydin S. Three new players in energy regulation: preptin, adropin and irisin. Peptides. 2014;56:94-110.
3. Charos AE, Reed BD, Raha D, Szekely AM, Weissman SM, Snyder M. A highly integrated and complex PPARGC1A transcription factor binding network in HepG2 cells. Genome Res. 2012;22:1668-79.

4. Soyal S, Krempler F, Oberkofler H, Patsch W. PGC-1α: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. Diabetologia. 2006;49:1477-88. Erratum in: Diabetologia. 2006;49:2225.

5. Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ. 2006;30:145-51.

6. Lu L, Ma J, Tang J, Liu Y, Zheng Q, Chen S, et al. Irisin attenuates myocardial ischemia/reperfusion-induced cardiac dysfunction by regulating ER-mitochondria interaction through a mitochondrial ubiquitin ligase-dependent mechanism. Clin Transl Med. 2020;10:e166.

7. Lu J, Xiang G, Liu M, Mei W, Xiang L, Dong J. Irisin protects against endothelial injury and ameliorates atherosclerosis in apolipoprotein E-Null diabetic mice. Atherosclerosis. 2015;243:438-48.

8. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci. 2017;24:101-5.

9. Liu JJ, Wong MDS, Toy WC, Tan CSH, Liu S, Ng XW, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications. 2013;27:365-9.

10. Natalicchio A, Marrano N, Biondi G, Spagnuolo R, Labarbuta R, Porreca I, et al. The myokine irisin is released in response to saturated fatty acids and promotes pancreatic β-cell survival and insulin secretion. Diabetes. 2017;66:2849-56.

11. Liu TY, Shi CX, Gao R, Sun HJ, Xiong XQ, Ding L, et al. Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes. Clin Sci (Lond). 2015;129:839-50.

12. Scheen AJ, Paquot N. Metformin revisited: a critical review of the benefit-risk balance in at-risk patients with type 2 diabetes. Diabetes Metab. 2013;39:179-90.

13. Li DJ, Huang F, Lu WJ, Jiang GJ, Deng YP, Shen FM. Metformin promotes irisin release from murine skeletal muscle independently of AMP-activated protein kinase activation. Acta Physiol (Oxf). 2015;213:711-21.

14. Liu J, Hu Y, Zhang H, Xu Y, Wang G. Exenatide treatment increases serum irisin levels in patients with obesity and newly diagnosed type 2 diabetes. J Diabetes Complications. 2016;30:1555-9.

15. Fu J, Han Y, Wang J, Liu Y, Zheng S, Zhou L, et al. Irisin lowers blood pressure by improvement of endothelial dysfunction via AMPK-Akt-eNOS-NO pathway in the spontaneously hypertensive rat. J Am Heart Assoc. 2016;5:e003433.

16. Altaweel R, Shatarat A, Badran D, Abu Tarboush NM. The effects of irisin on the rat thoracic aorta: a histological study. Folia Morphol (Warsz). 2022;81:923-30.

17. Shen S, Wang F, Fernandez A, Hu W. Role of platelet-derived growth factor in type II diabetes mellitus and its complications. Diab Vasc Dis Res. 2020;17:1479164120942119.

18. Lin F, Yang X. TGF-β signaling in aortic aneurysm: another round of controversy. J Genet Genomics. 2010;37:583-91.

19. Chen X, Lu H, Rateri DL, Cassis LA, Daugherty A. Conundrum of angiotensin II and TGF-β interactions in aortic aneurysms. Curr Opin Pharmacol. 2013;13:180-5.

20. Dewidar B, Meyer C, Dooley S, Meindl-Beinker AN. TGF-β in hepatic stellate cell activation and liver fibrogenesis-updated 2019. Cells. 2019;8:1419.

21. Dai J, Michineau S, Franck G, Desgranges P, Becquemin JP, Gervais M, et al. Long term stabilization of expanding aortic aneurysms by a short course of cyclosporine A through transforming growth factor-beta induction. PLoS One. 2011;6:e28903.

22. Wang Y, Ait-Oufella H, Herbin O, Bonnin P, Ramkhelawon B, Taleb S, et al. TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. J Clin Invest. 2010;120:422-32.

23. Angelov SN, Hu JH, Wei H, Airhart N, Shi M, Dichek DA. TGF-β signaling protects the thoracic and abdominal aorta from angiotensin II-induced pathology by distinct mechanisms. Arterioscler Thromb Vasc Biol. 2017;37:2102-13.

24. Dong HN, Park SY, Le CT, Choi DH, Cho EH. Irisin regulates the functions of hepatic stellate cells. Endocrinol and Metab. 2020;35:647-55.

25. Peng H, Wang Q, Lou T, Qin J, Jung S, Shetty V, et al. Myokine mediated muscle-kidney crosstalk suppresses metabolic reprogramming and fibrosis in damaged kidneys. Nat Commun. 2017;8:1493.

26. Anaszewicz M, Wawrzeńczyk A, Czerniak B, Banaś W, Socha E, Lis K, et al. Leptin, adiponectin, tumor necrosis factor α, and irisin concentrations as factors linking obesity with the risk of atrial fibrillation among inpatients with cardiovascular diseases. Kardiol Pol. 2019;77:1055-61.

27. Cheng ZB, Huang L, Xiao X, Sun JX, Zou ZK, Jiang JF, et al. Irisin in atherosclerosis. Clin Chim Acta. 2021;522:158-66.

28. Wang Y, Wang M, Wang Y. Irisin: a potentially fresh insight into the molecular mechanisms underlying vascular aging. Aging Dis. 2023;15:2491-506.

29. Lu J, Shi J, Li M, Gui B, Fu R, Yao G, et al. Activation of AMPK by metformin inhibits TGF-β-induced collagen production in mouse renal fibroblasts. Life Sci. 2015;127:59-65.

30. Duca FA, Côté CD, Rasmussen BA, Zadeh-Tahmasebi M, Rutter GA, Filippi BM, et al. Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. Nat Med. 2015;21:506-11. Erratum in: Nat Med. 2016;22:217.

31. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. Cell Metab. 2016;23:1060-5.