Canadian Journal of Physiology and Pharmacology

Irisin relaxes rat thoracic aorta: MEK1/2 signaling pathway, KV channels, SKCa channels, and BKCa channels are involved in irisin-induced vasodilation

Journal:	<i>Canadian Journal of Physiology and Pharmacology</i>
Manuscript ID	cjpp-2021-0500.R1
Manuscript Type:	Article
Date Submitted by the Author:	28-Oct-2021
Complete List of Authors:	Demirel, Sadettin; Bursa Uludag University, Medicine School, Physiology Department Sahinturk, Serdar; Bursa Uludag University, Medicine School, Physiology Department Isbil, Naciye; Bursa Uludag University, Medicine School, Physiology Department Ozyener, Fadil; Bursa Uludag University, Medicine School, Physiology Department
Is the invited manuscript for consideration in a Special Issue:	Not applicable (regular submission)
Keyword:	Irisin, rat thoracic aorta, vasodilation, mitogen-activated protein kinase kinase, potassium channels

SCHOLARONE™
Manuscripts

Irisin relaxes rat thoracic aorta: MEK1/2 signaling pathway, K_v channels, SK_{Ca} channels, and BK_{Ca} channels are involved in irisin-induced vasodilation

Sadettin Demirel^{a*}, Serdar Sahinturk^a, Naciye Isbil^a, Fadil Ozyener^a

a. *Department of Physiology, Faculty of Medicine, Bursa Uludag University, 16059, Bursa/Turkey*

***. Corresponding Author:**

Sadettin Demirel

Department of Physiology, Faculty of Medicine,
Bursa Uludag University,
16059, Bursa/Turkey
Phone: +90 224 295 40 22
E-mail: sdemirel@uludag.edu.tr
ORCID: 0000-0002-3629-5344

Serdar Sahinturk

Department of Physiology, Faculty of Medicine,
Bursa Uludag University,
16059, Bursa/Turkey
ORCID: 0000-0002-7612-0055

Naciye Isbil

Department of Physiology, Faculty of Medicine,
Bursa Uludag University,
16059, Bursa/Turkey
ORCID: 0000-0002-8792-2555

Fadil Ozyener

Department of Physiology, Faculty of Medicine,
Bursa Uludag University,
16059, Bursa/Turkey
ORCID: 0000-0002-4606-6596

ABSTRACT

In this study, it was aimed to investigate the effects of irisin on vascular smooth muscle contractility in rat thoracic aorta, and the hypothesis that mitogen-activated protein kinase kinase (MEK1/2) signaling pathway, voltage-gated potassium (K_V) channels, small-conductance calcium-activated potassium (SK_{Ca}) channels, and large-conductance calcium-activated potassium (BK_{Ca}) channels may have roles in these effects. Isometric contraction-relaxation responses of isolated thoracic aorta rings were measured with an organ bath model. The steady contraction was induced with 10^{-5} M phenylephrine (PHE), and then the concentration-dependent responses of irisin (10^{-9} - 10^{-6} M) were examined in endothelium-intact and -denuded rat thoracic aortas. Also, the effects of irisin incubations on PHE-mediated contraction and acetylcholine (ACh)-mediated relaxation were studied. Irisin exerted the vasorelaxant effects in both endothelium-intact and -denuded aortic rings at concentrations of 10^{-8} , 10^{-7} , and 10^{-6} M compared to the control groups ($p < 0.001$). Besides, pre-incubation of aortic rings with irisin (10 nM, 100 nM, or 1 μ M for 30 min) augmented ACh-mediated (10^{-9} - 10^{-5}) vasodilation in PHE-precontracted thoracic aorta segments but did not modulate PHE-mediated (10^{-9} - 10^{-5}) contraction. In addition, MEK1/2 inhibitor U0126, K_V channel blocker XE-991, SK_{Ca} channel blocker apamin, and BK_{Ca} channel blocker tetraethylammonium (TEA) incubations significantly inhibited the irisin-induced relaxation responses. In conclusion, the first physiological findings were obtained regarding the functional relaxing effects of irisin in rat thoracic aorta. The findings demonstrated that irisin induces relaxation responses in endothelium-intact and/or endothelium-denuded aortic rings in a concentration-dependent manner. Furthermore, this study is the first to report that irisin-induced relaxation responses are related to the activity of the MEK1/2 pathway, K_V channels, and calcium-activated K^+ (SK_{Ca} and BK_{Ca}) channels.

1 **Keywords:** Irisin, rat thoracic aorta, vasodilation, mitogen-activated protein kinase kinase,
2 potassium channels

3 **1. Introduction**

4 Irisin, a newly identified hormone containing 112 amino acids, which is secreted mainly by
5 skeletal muscle cells during exercise, is a cleavage product of fibronectin type III domain-
6 containing protein 5 (FNDC5) (Boström et al. 2012). Irisin increases mitochondrial
7 biogenesis and energy expenditure by activating the conversion of white adipocytes to brown
8 adipocytes (Boström et al. 2012; Sanchis-Gomar et al. 2012). Circulating levels of irisin are
9 associated with obesity, type II diabetes mellitus, insulin resistance, hypertension, and
10 atherosclerosis. Recently, one of the agents predicted to be used in the prevention, follow-up,
11 and treatment of these metabolic diseases and/or pathologies is the irisin molecule (Korta et
12 al. 2019; Ma et al. 2021; Polyzos et al. 2018).

13 Vasoreactivity is of fundamental importance in various cardiovascular diseases such as
14 hypertension, headache, and stroke (Brunner et al. 2005). Although some studies have
15 demonstrated the peripheral vasodilator effect of irisin, little is known about the potential
16 vascular activity of this molecule, and the mechanisms underlying its effects on vascular
17 smooth muscle contraction-relaxation responses have not been fully elucidated yet. Besides,
18 studies with this newly discovered adipomyokine have had several conflicting results.
19 Therefore, more studies are needed to demonstrate the functional effects of irisin on vascular
20 tissue and the mechanisms involved in these effects.

3

Abbreviations: $[Ca^{2+}]_i$, intracellular free calcium concentration; ACh, acetylcholine; Akt, protein kinase B; AMPK, 5' adenosine monophosphate-activated protein kinase; BK_{Ca} , large-conductance calcium-activated potassium; cAMP, cyclic adenosine monophosphate; cGMP, guanosine 3', 5'-cyclic phosphate; DMSO, dimethyl sulfoxide; eNOS, endothelial nitric oxide synthase; FNDC5, fibronectin type III domain-containing protein 5; IP_3R , inositol-trisphosphate receptor; K_V , voltage-gated potassium; MEK1/2, mitogen-activated protein kinase kinase; NO, nitric oxide; PGI_2 , prostaglandin I_2 ; PHE, phenylephrine PVAT, perivascular adipose tissue; RyR, ryanodine receptor; SD, standard deviation; SK_{Ca} , small-conductance calcium-activated potassium; TEA, tetraethylammonium; TRPV4, transient receptor potential vanilloid subtype 4; UCP-1, uncoupling protein-1

In the present study, it was aimed to investigate the effects of irisin on vascular smooth muscle contractility on rat thoracic aorta, a conducting artery, and the mechanisms involved, the possible roles of mitogen-activated protein kinase kinase (MEK1/2), voltage-gated potassium (K_V) channels, small-conductance calcium-activated potassium (SK_{Ca}) channels, and large-conductance calcium-activated potassium (BK_{Ca}) channels.

2. Materials and methods

2.1. Ethics

This study was approved by the decision of Bursa Uludag University Animal Experiments Local Ethics Committee dated 03/04/2019 and numbered 2019-04/10.

2.2. Test animals

In this study, male Wistar Albino rats (10-12 weeks of age), obtained from Bursa Uludag University Experimental Animals Breeding Application and Research Center, were used. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Chemicals and reagents used in experiments

Irisin, phenylephrine hydrochloride (PHE), acetylcholine chloride (ACh), U0126, XE-991, apamin, tetraethylammonium (TEA), dimethyl sulfoxide (DMSO), and chemicals required to prepare Krebs solution used in the experiments were purchased from Sigma Aldrich (St Louis, MO, USA). Irisin, PHE, ACh, apamin, and TEA were dissolved in distilled water; U0126 and XE-991 were dissolved in DMSO.

2.4. Isolated organ bath experiments

2.4.1. Preparation of aortic rings and mounting in organ baths

The animals used in the experiments were decapitated without anesthesia, taking care that they were not exposed to any stress factors. The thoracic cavity of the animals was opened rapidly and then the thoracic aorta was gently removed and placed in a Petri dish containing cold (0-4 °C) Krebs solution. The solution contained (in mM): NaCl 119.0, KCl 4.7, MgSO₄ 1.5, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, and glucose 11.0. The dissected vessels were cleaned of adhering fat and connective tissue and sectioned into 2-3 mm rings afterward. To measure the isometric contractile responses, thoracic aorta rings were connected to the isometric force transducer (SS12LA force transducer, BIOPAC Systems, Inc. Aero Camino, USA) of the four-chamber organ bath (Commat Ltd., Ankara, Turkey) through steel wires as rapidly as possible. The temperature of the organ bath was kept constant at 37 °C (±0.5 °C) through a thermocirculator containing distilled water. The solution was continuously oxygenated with 95% O₂ - 5% CO₂ mixed gas during the course of the experiment to maintain a pH of 7.4. Briefly, the isolated organ bath model was used as previously described (Jespersen et al. 2015).

2.4.2. Vessel viability testing

The aortic rings were given a resting tension of 2 g and then equilibrated for 60 min before the experiment. Krebs solution containing the tissues was replaced with fresh Krebs solution every 15 min during the equilibration period. After equilibration, the viability of the vessel and integrity of the endothelium was assessed by its stable and reproducible responses to the addition of 10⁻⁵ M PHE and 10⁻⁵ M ACh. Vessel segments with >60% relaxation to ACh were considered endothelium intact. To obtain endothelium-denuded aortas, the inside of the vessel was rubbed with a stainless-steel wire to remove the endothelium. The absence of ACh-induced relaxation was considered as evidence of successful peeling of the vessel segments from endothelium. Then, active and blocker/inhibitor substance applications were precisely performed into Krebs solution using an adjustable automatic pipette.

2.4.3. Preliminary experiments

First, in preliminary experiments, PHE was added to isolated organ bath chambers in cumulative concentrations (10^{-10} - 10^{-4} M) after the equilibration period to determine the submaximal contraction dose. In preliminary experiments, it was also investigated that whether irisin and blocker/inhibitor agents at the concentrations used in the study have significant effects on basal tone on rat thoracic aorta rings.

2.4.4. Measurements of vascular reactivity in isolated thoracic aortas

Cumulative concentrations (10^{-9} - 10^{-6} M) of irisin were used in all experimental groups. To determine the vasodilative effect of irisin on the endothelium-intact thoracic aorta, 10^{-5} M PHE was employed to induce a steady contraction in the aortic rings, and then the concentration-dependent responses of irisin were examined. The concentration-response curve obtained with the PHE pre-contraction protocol was used as a control record for the groups in which blocker/vehicle agents were used. Besides, the concentration-dependent vasorelaxant effects of irisin on the PHE pre-contracted rat thoracic aorta rings were studied with or without endothelium. The time-matched vehicle control (double-distilled water) group was also constructed.

In the next stage of the study, the effects of irisin on PHE-induced contraction responses were examined. For this purpose, after obtaining the concentration-response curves with PHE (10^{-9} - 10^{-5} M), the aortic rings were incubated separately with 10 nM, 100 nM, and 1 μ M irisin for 30 minutes, and then concentration-response curves were reconstructed with PHE. Next, the effects of irisin on endothelium-dependent relaxations were investigated. The concentration-response curves for ACh (10^{-9} - 10^{-5} M), which produces endothelium-dependent relaxation in thoracic aortic rings pre-contracted with PHE at submaximal concentration, were generated before and after 30 min incubation with 10 nM, 100 nM, and 1 μ M irisin.

To evaluate the role of vasoactive factors, endothelium-intact thoracic aorta rings were incubated with each of the following blockers/inhibitors: 1.5 μ M U0126 (MEK1/2 inhibitor) for 20 min, 30 μ M XE-991 (K_V channel blocker) for 20 min, 100 nM apamin (SK_{Ca} channel blocker) for 30 min, and 2 mM TEA (BK_{Ca} channel blocker) for 30 min before the addition of 10^{-5} M PHE and irisin.

Following the incubation of blockers/inhibitors used for mechanism researches, the responses developed depending on cumulative concentrations of irisin were compared with the control group, and then the differences between the relaxation amplitudes were calculated. Also, a "vehicle" control group was formed for DMSO, which was used as a solvent.

8 aortic preparations were used for each of the experimental groups formed according to the active, blocker/inhibitor, or vehicle agents used in the study.

Contraction-relaxation responses were measured isometrically by force transducer on the MP36 recording system (BIOPAC Systems, Inc. Aero Camino, USA). The records obtained were evaluated in a computer environment with Biopac Student Lab 3.7.7 program (BIOPAC Systems, Inc. Aero Camino, USA).

2.5. Statistical analysis

SPSS v.23.0 program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were expressed as mean $\pm$ SD (standard deviation). The statistical significance between the two independent groups was analyzed using the independent sample t-test. A one-way analysis of variance (one-way ANOVA) test was used for multiple comparisons. Dunnett's T3 post hoc test was used to determine the differences between groups. P values <0.05 were considered statistically significant.

3. Results

In preliminary experiments, submaximal contraction response was taken against 10^{-5} M PHE. In all subsequent experiments, pre-contractions were induced with the addition of 10^{-5} M PHE. Stable and reproducible contraction responses occurred in rat thoracic aorta rings with the submaximal concentration of PHE. In preliminary experiments, it was also determined that irisin and blocker/inhibitor agents at the concentrations used in the study had no significant effects on basal tone in rat thoracic aorta rings.

3.1. Irisin induced vasodilation in both endothelium-intact and –denuded rat thoracic aortas

Cumulatively applied irisin induced concentration-dependent relaxation responses on both endothelium-intact and -denuded rat thoracic aorta rings pre-contracted with PHE. The relaxing effect of irisin on endothelium-intact aortic rings was found to be significantly higher at concentrations of 10^{-8} , 10^{-7} , and 10^{-6} M compared to the control group (double distilled water-treated rat thoracic aorta rings) (p: 0.840 for 10^{-9} M; p: 0.000 for 10^{-8} M; p: 0.000 for 10^{-7} M; p: 0.000 for 10^{-6} M) (Figure 1A). Besides, when the endothelium is removed, irisin-induced relaxation responses decreased partially, however, the relaxing effect of irisin on endothelium-denuded aortic rings was found to be significantly higher at concentrations of 10^{-8} , 10^{-7} , and 10^{-6} M compared to the control group (double distilled water-treated rat thoracic aorta rings) (p: 0.269 for 10^{-9} M; p: 0.014 for 10^{-8} M; p: 0.000 for 10^{-7} M; p: 0.000 for 10^{-6} M) (Figure 1B).

Figure 1A, 1B

3.2. Irisin incubation did not affect PHE-mediated contraction in rat thoracic aortas but increased ACh-mediated relaxation

Incubation of the thoracic aortic rings with 10 nM, 100 nM, and 1 μ M irisin separately did not cause statistically significant changes in any concentration when the PHE contraction

protocol was applied (p: 1.000 for 10 nM; p: 0.245 for 100 nM; p: 0.156 for 1 μ M) (Figure 2A). In contrast, when the ACh relaxation protocol was applied, incubations of 100 nM and 1 μ M irisin significantly increased ACh-mediated relaxation and led to vasorelaxation (p: 1.000 for 10 nM; p: 0.039 for 100 nM; p: 0.016 for 1 μ M) (Figure 2B).

Figure 2A, 2B

3.3. MEK1/2 signaling pathway was involved in the irisin-induced relaxation of rat thoracic aortas

Incubation of tissues with MEK1/2 inhibitor U0126 (1.5 μ M) for 20 min caused a significant change in the relaxation responses to irisin. U0126 incubation resulted in a statistically significant decrease in the relaxation responses of irisin applied at cumulative concentrations of 10^{-7} and 10^{-6} M while it did not cause a significant change in the relaxation responses with a concentration of 10^{-8} M (p: 0.875 for 10^{-8} M; p: 0.010 for 10^{-7} M; p: 0.002 for 10^{-6} M). U0126 significantly inhibited relaxation responses to irisin (Figure 3).

Figure 3

3.4. Irisin induced vasodilation via K_V channels

Incubation of tissues with K_V channel blocker XE-991 (30 μ M) for 20 min caused a significant change in the relaxation responses to irisin. XE-991 incubation resulted in a statistically significant decrease in the relaxation responses of irisin applied at cumulative concentrations of 10^{-8} , 10^{-7} , and 10^{-6} M (p: 0.000 for 10^{-8} M; p: 0.000 for 10^{-7} M; p: 0.000 for 10^{-6} M). XE-991 almost completely inhibited relaxation responses to irisin (Figure 4A).

3.5. Irisin induced vasodilation via SK_{Ca} channels

Incubation of tissues with SK_{Ca} channel blocker apamin (100 nM) for 30 min caused a significant change in the relaxation responses to irisin. Apamin incubation resulted in a statistically significant decrease in the relaxation responses of irisin applied at cumulative concentrations of 10⁻⁷ and 10⁻⁶ M while it did not cause a significant change in the relaxation responses with a concentration of 10⁻⁸ M (p: 0.459 for 10⁻⁸ M; p: 0.014 for 10⁻⁷ M; p: 0.010 for 10⁻⁶ M). Apamin significantly inhibited relaxation responses to irisin (Figure 4B).

3.6. *Irisin induced vasodilation via BK_{Ca} channels*

Incubation of tissues with BK_{Ca} channel blocker TEA (2 mM) for 30 min caused a significant change in the relaxation responses to irisin. TEA incubation resulted in a statistically significant decrease in the relaxation responses of irisin applied at cumulative concentrations of 10⁻⁸, 10⁻⁷, and 10⁻⁶ M (p: 0.001 for 10⁻⁸ M; p: 0.000 for 10⁻⁷ M; p: 0.000 for 10⁻⁶ M). TEA significantly inhibited relaxation responses to irisin (Figure 4C).

Figure 4A, 4B, 4C

Incubation of tissues with DMSO, U0126 and XE-991 solvent, for 10 min did not cause a significant change in the relaxation responses to irisin. DMSO incubation at solvent concentration did not cause a remarkable change on any of the relaxation responses of irisin applied at cumulative concentrations of 10⁻⁸, 10⁻⁷, and 10⁻⁶ M (p: 1.000 for 10⁻⁸ M; p: 1.000 for 10⁻⁷ M; p: 1.000 for 10⁻⁶ M). DMSO did not affect the relaxation responses to irisin (Figure 5).

Figure 5

4. Discussion

The purpose of the present study was to investigate the vasorelaxant effects of irisin on rat thoracic aorta and its underlying mechanisms. We demonstrated for the first time that irisin can significantly induce vasorelaxation in both endothelium-intact and -denuded rat thoracic aortas, and the relaxing effects of irisin were related to the activity of the MEK1/2 signaling pathway, K_V channels, and SK_{Ca} and BK_{Ca} channels. The results suggest that the vasodilative mechanisms of irisin may be due to activation of nucleus-associated intracellular relaxant factors or inactivation of contraction factors formation through the MEK1/2 pathway and inhibition of extracellular Ca^{2+} entry via blocking both voltage-gated and calcium-activated K^+ channels.

Despite great advances in medicine, proper treatment of arterial hypertension, diabetes mellitus, and chronic kidney disease remains a major challenge. Undiagnosed and untreated arterial hypertension or diabetes mellitus may lead to the development of chronic kidney disease and, consequently, to cardiovascular disorders (Maciorkowska et al. 2019).

The frequency of insulin resistance, obesity, and type II diabetes mellitus, and cardiovascular diseases such as hypertension and atherosclerosis due to these diseases has increased.

Atherosclerosis, the main cause of coronary artery disease and stroke, remains the primary cause of global mortality (Byun and Lee 2020; Voutyrtsa et al. 2021).

Skeletal muscles have been defined as the endocrine organ in recent years (Pedersen et al. 2007). Cytokines known as myokines, released from skeletal muscles during and/or immediately after exercise, mediate the beneficial effects of exercise on metabolism and the cardiovascular system (Pedersen and Febbraio 2012; Strasser 2013).

Irisin, a newly described myokine, is increased with exercise and has the potential to play a preventive and/or therapeutic role in the management of the aforementioned chronic diseases (Boström et al. 2012; Demirel et al. 2021; Fu et al. 2016; Han et al. 2015; Hou et al. 2016;

Jiang et al. 2015; Ye et al. 2017; Zhang et al. 2015). In this context, it is considered that irisin may be an important therapeutic target in the prevention of the development of vascular pathologies and the treatment of atherosclerotic cardiovascular diseases. The precise functions of irisin related to the vascular tissue have not yet been clarified. However, few current studies have focused on the effects of irisin on vascular activity and the mechanisms involved.

Zhang et al. (2015) have found that central administration of irisin increases blood pressure and cardiac contractility. In contrast, peripheral administration of irisin reduces blood pressure in both control and spontaneously hypertensive rats. Irisin dilates mesenteric artery rings through ATP-sensitive potassium channels. Han et al. (2015) have provided the first evidence that irisin improves endothelial function in aortas of high-fat-diet-induced obese mice. The mechanism for this protective effect is related to the activation of the 5' adenosine monophosphate-activated protein kinase (AMPK)-endothelial nitric oxide synthase (eNOS) signaling pathway. Jiang et al. (2015) have investigated the effects of irisin on vascular activity and the mechanisms involved in mouse mesenteric arteries. The results have suggested that the endothelium-dependent relaxation of irisin is mediated by the nitric oxide (NO)-guanosine 3', 5'-cyclic phosphate (cGMP)-dependent pathway but not the prostaglandin I₂ (PGI₂)-cyclic adenosine monophosphate (cAMP)-dependent mechanism. Endothelium-independent relaxation may be depend on inhibiting Ca²⁺ influx through blocking voltage-dependent Ca²⁺ channels and intracellular Ca²⁺ release through both inositol-trisphosphate receptor (IP₃R) and ryanodine receptor (RyR) channels. Fu et al. (2016) have concluded that acute administration of irisin increases endothelium-dependent vasodilation and lowers the blood pressure of spontaneously hypertensive rats by amelioration of endothelial dysfunction of the mesenteric artery through the AMPK-protein kinase B (Akt)-eNOS-NO signaling pathway. Hou et al. (2016) have shown that irisin improves the anti-contractile properties of

perivascular adipose tissue (PVAT) from the thoracic aorta in diet-induced obese mice. The mechanism for protective effects of irisin appears to be related to upregulation of the heme oxygenase-1/adiponectin axis in PVAT and browning of PVAT. Ye et al. (2017) have stated that irisin-induced endothelium-dependent vasodilation is related to the stimulation of extracellular Ca^{2+} influx via transient receptor potential vanilloid subtype 4 (TRPV4) channels in rat mesenteric arteries.

Vascular smooth muscle contraction is triggered by an increase in intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$), promoting actin-myosin cross-bridge formation. Growing evidence indicates that vascular function is also regulated by calcium-independent mechanisms involving mitogen-activated protein kinase signaling. Vascular smooth muscle cell $[\text{Ca}^{2+}]_i$ not only determines the contractile state but also influences the activity of many calcium-dependent transcription factors and proteins thereby impacting the relaxing function. Changes in vascular smooth muscle cell signaling and altered function influence vascular reactivity and tone, important determinants of vascular resistance and blood pressure (Cuiñas et al. 2013; Korkmaz et al. 2011; Touyz et al. 2018). In this study, we found that the MEK1/2 pathway is involved in irisin-induced vasodilation in rat thoracic aorta.

The activity of K^+ channels, including a number of voltage-gated K^+ channels, is a major regulator of vascular smooth muscle membrane potential. K_V channels, through their regulation of membrane potential, have a major influence on intracellular Ca^{2+} by modulating the open probability of L-type Ca^{2+} channels (and perhaps T-type channels). Physiological activation of K_V channels hyperpolarizes and relaxes vascular smooth muscle by decreasing the activity of voltage-gated Ca^{2+} channels and therefore Ca^{2+} influx, whereas their inhibition promotes contraction (Nieves-Cintrón et al. 2018). So we first investigated the role of K_V channels on the concentration-dependent relaxation responses of irisin. In the present

study, the vasorelaxant effect of irisin was significantly attenuated by pretreatment with K_v channel blocker XE-991, suggesting that K_v channels may participate in irisin-induced vasodilation.

Calcium-activated K^+ channels, especially SK_{Ca} and BK_{Ca} channels, have been demonstrated to have an important role in the regulation of vascular tone. In the plasma membranes of vascular smooth muscle cells, activation of SK_{Ca} and BK_{Ca} channels leads to the efflux of K^+ results in hyperpolarization that inhibits the opening of voltage-gated Ca^{2+} channels. Under these conditions, cytosolic Ca^{2+} concentrations are relatively low and Ca^{2+} -dependent contraction is inhibited, followed by vasodilatation (Bondarenko et al. 2018; Dong et al. 2016; Guéguinou et al. 2014). In this study, we found that SK_{Ca} and BK_{Ca} channels are involved in irisin-induced vasodilation.

Differences could be observed in the vasodilator activity of irisin on different vascular preparations obtained from various species. It is thought that this may be due to methodological differences such as the species from which the tissues were removed, the vascular tissues in which the experiments were performed (locations and diameters of vascular tissues), and/or the agents used to induce pre-contraction. Vasoreactivities of the arteries may be different from each other. It is known that the density and types of ion transport mechanisms and/or physiological/pharmacological receptors, as well as differences in substances released from the endothelium, could have an important role in changes in the responses of vascular smooth muscles to various agents (Clark and Fuchs 1997; Mulvany and Aalkjaer 1990). For instance, the effects of hyperpolarizing factors increase inversely with vessel diameter (de Wit and Wölfle 2007). Therefore, in the present study, the relaxing effects of irisin were investigated for the first time in thoracic aorta preparations isolated from

Wistar Albino rats. Furthermore, the possible action mechanisms of irisin performed in this study have not been tested on any species and/or vascular tissues before.

In this context, our results showed that 10^{-8} , 10^{-7} , and 10^{-6} M concentrations of irisin applied cumulatively have a vasodilative effect on rat thoracic aorta, a conducting artery. Although removal of the endothelium caused a slight decrease in the vasorelaxant effect of irisin, the level of vasodilator effect was largely preserved, suggesting that the vasorelaxation was mainly endothelium-dependent. We next determined whether 10 nM, 100 nM, and 1 μ M irisin incubations have any vasorelaxant effect in thoracic aortas. Preincubation of irisin, by itself, had no direct significant vasorelaxant effect on PHE-mediated vasoconstriction. However, it augmented ACh-mediated vasorelaxation in aortic rings with incubations of 100 nM and 1 μ M, but not with 10 nM. Taken together, our results suggested that irisin may have a direct effect on vascular smooth muscle cells and exerts its relaxing effect by activating endothelium-dependent mechanisms. Thus, we reported that irisin has relaxing effects on vascular smooth muscle contractility both at mean plasma concentration (10^{-7} M) (Fu et al. 2016) and a wide range of concentrations, including the upper and lower levels of mean concentration (10^{-8} - 10^{-6} M). Pretreatment with DMSO, a solvent of U0126 and XE-991 did not modulate irisin-induced relaxation. It was further demonstrated that the irisin-induced vasorelaxation effects on endothelium-intact rat thoracic aorta were reduced by pretreatment with MEK1/2 inhibitor U0126, K_V channel blocker XE-991, SK_{Ca} channel blocker apamin, and BK_{Ca} channel blocker TEA. This study provides the first evidence that irisin-induced vasodilation is related to these mechanisms.

In conclusion, in this study, the first physiological findings were obtained regarding the functional relaxing effects of irisin in rat thoracic aorta. The findings showed that irisin induces concentration-dependent relaxation responses in endothelium-intact and

endothelium-denuded aortic rings. Also, this study is the first to report that irisin-induced relaxation responses are related to the activity of the MEK1/2 pathway, K_v channels, SK_{Ca} channels, and BK_{Ca} channels. The results of this experimental study suggest that irisin might be a useful agent in the treatment of abnormal vasoconstriction-related diseases such as hypertension and atherosclerosis, in addition to its key role in regulating energy metabolism, resulting in thermogenesis and weight loss, by increasing uncoupling protein-1 (UCP-1) expression in adipose tissue. This study further supports the beneficial effect of exercise on vascular function, depending on the release of irisin, an exercise hormone.

However, the findings presented in our study are preliminary. Endothelium-derived factors regarding the ACh-mediated vasorelaxation effect of irisin should be investigated. Also, the precise roles of the MEK1/2 pathway, K_v channels, and SK_{Ca} and BK_{Ca} channels in irisin-mediated vasodilation need to be further elucidated in clinically relevant animal models. More detailed mechanism studies and *in vivo* studies are necessary to establish the efficacy of irisin in the management and treatment of cardiovascular diseases. In this regard, investigating the vasoactive effects of irisin in different pathological conditions including hypertensive models may provide a more comprehensive evaluation.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: This study was funded by the Scientific and Technological Research Council of Turkey [Project No. 219S306].

References

Bondarenko A.I., Panasiuk O., Drachuk K., Montecucco F., Brandt K.J., and Mach F. 2018. The quest for endothelial atypical cannabinoid receptor: BK_{Ca} channels act as cellular

- 1 sensors for cannabinoids in in vitro and in situ endothelial cells. *Vascul. Pharmacol.* 102: 44-
2 55. <https://doi.org/10.1016/j.vph.2018.01.004>.
- 3 Boström P., Wu J., Jedrychowski M.P., Korde A., Ye L., Lo J.C., et al. 2012. A PGC1- α -
4 dependent myokine that drives brown-fat-like development of white fat and thermogenesis.
5 *Nature* 481: 463-468. <https://doi.org/10.1038/nature10777>.
- 6 Brunner H., Cockcroft J.R., Deanfield J., Donald A., Ferrannini E., Halcox J., et al.; Working
7 Group on Endothelins and Endothelial Factors of the European Society of Hypertension.
8 2005. Endothelial function and dysfunction. Part II: association with cardiovascular risk
9 factors and diseases. A statement by the Working Group on Endothelins and Endothelial
10 Factors of the European Society of Hypertension. *J. Hypertens.* 23: 233-246.
11 <https://doi.org/10.1097/00004872-200502000-00001>.
- 12 Byun K. and Lee S. 2020. The potential role of irisin in vascular function and atherosclerosis:
13 a review. *Int. J. Mol. Sci.* 21: 7184. <https://doi.org/10.3390/ijms21197184>.
- 14 Clark S.G. and Fuchs L.C. 1997. Role of nitric oxide and Ca^{++} -dependent K^{+} channels in
15 mediating heterogeneous microvascular responses to acetylcholine in different vascular beds.
16 *J. Pharmacol. Exp. Ther.* 282: 1473-1479. PMID: 9316861.
- 17 Cuññas A., Elíes J., Orallo F., and Campos-Toimil M. 2013. Cyclic AMP relaxation of rat
18 aortic smooth muscle is mediated in part by decrease of depletion of intracellular $\text{Ca}(2+)$
19 stores and inhibition of capacitative calcium entry. *Vascul. Pharmacol.* 58: 98-104.
20 <https://doi.org/10.1016/j.vph.2012.08.007>.
- 21 de Wit C. and Wölfe S.E. 2007. EDHF and gap junctions: important regulators of vascular
22 tone within the microcirculation. *Curr. Pharm. Biotechnol.* 8: 11-25.
23 <https://doi.org/10.2174/138920107779941462>.

- 1 Demirel S., Şahintürk S., and Özyener F. 2021. Irisin and its effects on vascular contractility.
2 Med. J. SDU. 28: 163-170. <https://doi.org/10.17343/sdutfd.718412>.
- 3 Dong D.-L., Bai Y.-L., and Cai B.-Z. 2016. Calcium-activated potassium channels: potential
4 target for cardiovascular diseases. Adv. Protein Chem. Struct. Biol. 104: 233-261.
5 <https://doi.org/10.1016/bs.apcsb.2015.11.007>.
- 6 Fu J., Han Y., Wang J., Liu Y., Zheng S., Zhou L., et al. 2016. Irisin lowers blood pressure
7 by improvement of endothelial dysfunction via AMPK-Akt-eNOS-NO pathway in the
8 spontaneously hypertensive rat. J. Am. Heart. Assoc. 5: e003433.
9 <https://doi.org/10.1161/JAHA.116.003433>.
- 10 Guéguinou M., Chantôme A., Fromont G., Bougnoux P., Vandier C., and Potier-Cartreau
11 M. 2014. K_{Ca} and Ca²⁺ channels: the complex thought. Biochim. Biophys. Acta. 1843:
12 2322-2333. <https://doi.org/10.1016/j.bbamcr.2014.02.019>.
- 13 Han F., Zhang S., Hou N., Wang D., and Sun X. 2015. Irisin improves endothelial function in
14 obese mice through the AMPK-eNOS pathway. Am. J. Physiol. Heart Circ. Physiol. 309:
15 H1501-H1508. <https://doi.org/10.1152/ajpheart.00443.2015>.
- 16 Hou N., Liu Y., Han F., Wang D., Hou X., Hou S., and Sun X. 2016. Irisin improves
17 perivascular adipose tissue dysfunction via regulation of the heme oxygenase-1/adiponectin
18 axis in diet-induced obese mice. J. Mol. Cell. Cardiol. 99: 188-196.
19 <https://doi.org/10.1016/j.yjmcc.2016.09.005>.
- 20 Jespersen B., Tykocki N.R., Watts S.W., and Cobbett P.J. 2015. Measurement of smooth
21 muscle function in the isolated tissue bath-applications to pharmacology research. J. Vis.
22 Exp. (95): 52324. <https://doi.org/10.3791/52324>.

- 1 Jiang M., Wan F., Wang F., and Wu Q. 2015. Irisin relaxes mouse mesenteric arteries
2 through endothelium-dependent and endothelium-independent mechanisms. *Biochem.*
3 *Biophys. Res. Commun.* 468: 832-836. <https://doi.org/10.1016/j.bbrc.2015.11.040>.
- 4 Korkmaz B., Buharalioglu K., Sahan-Firat S., Cuez T., Tuncay Demiryurek A., and Tunctan
5 B. 2011. Activation of MEK1/ERK1/2/iNOS/sGC/PKG pathway associated with
6 peroxynitrite formation contributes to hypotension and vascular hyporeactivity in
7 endotoxemic rats. *Nitric Oxide*, 24: 160-172. <https://doi.org/10.1016/j.niox.2011.02.004>.
- 8 Korta P., Pocheć E., and Mazur-Biały A. 2019. Irisin as a multifunctional protein:
9 implications for health and certain diseases. *Medicina (Kaunas)*, 55: 485.
10 <https://doi.org/10.3390/medicina55080485>.
- 11 Ma C., Ding H., Deng Y., Liu H., Xiong X., and Yang Y. 2021. Irisin: a new code uncover
12 the relationship of skeletal muscle and cardiovascular health during exercise. *Front. Physiol.*
13 12: 620608. <https://doi.org/10.3389/fphys.2021.620608>.
- 14 Maciorkowska M., Musiałowska D., and Małyszko J. 2019. Adropin and irisin in arterial
15 hypertension, diabetes mellitus and chronic kidney disease. *Adv. Clin. Exp. Med.* 28: 1571-
16 1575. <https://doi.org/10.17219/acem/104551>.
- 17 Mulvany M.J. and Aalkjaer C. 1990. Structure and function of small arteries. *Physiol. Rev.*
18 70: 921-961. <https://doi.org/10.1152/physrev.1990.70.4.921>.
- 19 Nieves-Cintrón M., Syed A.U., Nystoriak M.A., and Navedo M.F. 2018. Regulation of
20 voltage-gated potassium channels in vascular smooth muscle during hypertension and
21 metabolic disorders. *Microcirculation*, 25: 10.1111/micc.12423.
22 <https://doi.org/10.1111/micc.12423>.

- 1 Pedersen B.K. and Febbraio M.A. 2012. Muscles, exercise and obesity: skeletal muscle as a
2 secretory organ. *Nat. Rev. Endocrinol.* 8: 457-465. <https://doi.org/10.1038/nrendo.2012.49>.
- 3 Pedersen B.K., Akerström T.C., Nielsen A.R., and Fischer C.P. 2007. Role of myokines in
4 exercise and metabolism. *J. Appl. Physiol.* 103: 1093-1098.
5 <https://doi.org/10.1152/japplphysiol.00080.2007>.
- 6 Polyzos S.A., Anastasilakis A.D., Efstathiadou Z.A., Makras P., Perakakis N., Kountouras J.,
7 and Mantzoros C.S. 2018. Irisin in metabolic diseases. *Endocrine*, 59: 260-274.
8 <https://doi.org/10.1007/s12020-017-1476-1>.
- 9 Sanchis-Gomar F., Lippi G., Mayero S., Perez-Quilis C., and García-Giménez J.L. 2012.
10 Irisin: a new potential hormonal target for the treatment of obesity and type 2 diabetes. *J.*
11 *Diabetes*, 4: 196. <https://doi.org/10.1111/j.1753-0407.2012.00194.x>.
- 12 Strasser B. 2013. Physical activity in obesity and metabolic syndrome. *Ann. N. Y. Acad. Sci.*
13 1281: 141-159. <https://doi.org/10.1111/j.1749-6632.2012.06785.x>.
- 14 Touyz R.M., Alves-Lopes R., Rios F.J., Camargo L.L., Anagnostopoulou A., Arner A., and
15 Montezano A.C. 2018. Vascular smooth muscle contraction in hypertension. *Cardiovasc.*
16 *Res.* 114: 529-539. <https://doi.org/10.1093/cvr/cvy023>.
- 17 Voutyritsa E., Kyriakos G., Patsouras A., Damaskos C., Garmpi A., Diamantis E., et al. 2021.
18 Experimental agents for the treatment of atherosclerosis: new directions. *J. Exp. Pharmacol.*
19 13: 161-179. <https://doi.org/10.2147/JEP.S265642>.
- 20 Ye L., Xu M., Hu M., Zhang H., Tan X., Li Q., et al. 2017. TRPV4 is involved in irisin-
21 induced endothelium-dependent vasodilation. *Biochem. Biophys. Res. Commun.* 495: 41-45.
22 <https://doi.org/10.1016/j.bbrc.2017.10.160>.

1 Zhang W., Chang L., Zhang C., Zhang R., Li Z., Chai B., et al. 2015. Central and peripheral
2 irisin differentially regulate blood pressure. *Cardiovasc. Drugs. Ther.* 29: 121-127.
3 <https://doi.org/10.1007/s10557-015-6580-y>.

4 **Figure Captions**

5 **Figure 1.** Concentration-dependent relaxant effects of irisin on PHE pre-contracted **(A)**
6 endothelium-intact and **(B)** endothelium-denuded rat thoracic aorta rings. The control groups
7 were treated with double-distilled water. The relaxant effects of irisin on thoracic aorta rings
8 were calculated as a percentage of the contraction in response to 10^{-5} M PHE. The data were
9 expressed as the mean $\pm$ SD. (n = 8). *: $p < 0.05$ vs. control. **: $p < 0.001$ vs. control.

10 **Figure 2A.** Effects of irisin incubations at 10 nM, 100 nM, and 1 μ M concentrations on the
11 contraction responses induced by PHE (10^{-9} - 10^{-5} M) applied at cumulative concentrations in
12 endothelium-intact rat thoracic aortas. Results were given as a percentage of the maximum
13 contraction response with 45 mM KCl. The data were expressed as the mean $\pm$ SD. (n = 8 for
14 each group).

15 **Figure 2B.** Effects of irisin incubations at 10 nM, 100 nM, and 1 μ M concentrations on the
16 relaxation responses induced by ACh (10^{-9} - 10^{-5} M) applied at cumulative concentrations in
17 endothelium-intact rat thoracic aortas. Results were given as a percentage of contraction
18 response with 10^{-5} M PHE. The data were expressed as the mean $\pm$ SD. (n = 8 for each
19 group). *: $p < 0.05$ vs. control.

20 **Figure 3.** Effects of U0126 on irisin-induced relaxation in rat thoracic aorta. Relaxant effects
21 of irisin on endothelium-intact thoracic aorta rings were measured in the absence (control) or
22 presence of U0126. The relaxant effects were calculated as a percentage of the contraction in

response to 10^{-5} M PHE. The data were expressed as the mean $\pm$ SD. (n=8). *: $p<0.05$ vs. control.

Figure 4. Effects of different pharmacological K^+ channel blockers on irisin-induced relaxation in rat thoracic aorta. Relaxant effects of irisin on endothelium-intact thoracic aorta rings were measured in the absence (control) or presence of (A) XE-991, (B) apamin, or (C) TEA. The relaxant effects were calculated as a percentage of the contraction in response to 10^{-5} M PHE. The data were expressed as the mean $\pm$ SD. (n=8). *: $p<0.05$ vs. control. **: $p<0.001$ vs. control.

Figure 5. Effects of DMSO on irisin-induced relaxation in rat thoracic aorta. Relaxant effects of irisin on endothelium-intact thoracic aorta rings were measured in the absence (control) or presence of DMSO. The relaxant effects were calculated as a percentage of the contraction in response to 10^{-5} M PHE. The data were expressed as the mean $\pm$ SD. (n=8).

1 **Figures**

3 **Figure 1A**

5 **Figure 1B**

Figure 2A

Figure 2B

Figure 3

Figure 4A

Figure 4B

Figure 4C

Figure 5

Draft