



Research Article

Irisin Attenuates Oxidative Stress, Mitochondrial Dysfunction, and Apoptosis in the H9C2 Cellular Model of Septic Cardiomyopathy through Augmenting Fundc1-Dependent Mitophagy

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In the present study, we used lipopolysaccharide- (LPS-) stimulated H9C2 cardiomyocytes to investigate whether irisin treatment attenuates septic cardiomyopathy via Fundc1-related mitophagy. Fundc1 levels and mitophagy were significantly reduced in LPS-stimulated H9C2 cardiomyocytes but were significantly increased by irisin treatment. Irisin significantly increased ATP production and the activities of mitochondrial complexes I and III in the LPS-stimulated cardiomyocytes. Irisin also improved glucose metabolism and significantly reduced LPS-induced levels of reactive oxygen species by increasing the activities of antioxidant enzymes, glutathione peroxidase (GPX), and superoxide dismutase (SOD), as well as levels of reduced glutathione (GSH). TUNEL assays showed that irisin significantly reduced LPS-stimulated cardiomyocyte apoptosis by suppressing the activation of caspase-3 and caspase-9. However, the beneficial effects of irisin on oxidative stress, mitochondrial metabolism, and viability of LPS-stimulated H9C2 cardiomyocytes were abolished by silencing Fundc1. These results demonstrate that irisin abrogates mitochondrial dysfunction, oxidative stress, and apoptosis through Fundc1-related mitophagy in LPS-stimulated H9C2 cardiomyocytes. This suggests irisin is a potentially useful treatment for septic cardiomyopathy, though further investigations are necessary to confirm our findings.

1. Introduction

Sepsis refers to excessive activation of the immune system in response to microbial infections that result in inflammation-related injury [1, 2]. Sepsis-induced myocardial injury is termed as septic cardiomyopathy, which is associated with increased morbidity and mortality. Hemodynamic imbalances in response to sepsis render the heart unable to maintain adequate preload [3]. Besides, sepsis-related cytokines promote cardiomyocyte apoptosis and endothelial cell dysfunction, both of which diminish cardiac output and vascular tone [4]. The pathogenesis of septic cardiomyopathy includes oxidative stress, calcium imbalance, mitochondrial dysfunction, endoplasmic reticulum stress, autophagy inhi-

bition, activation of programmed cell death activation, and immune disorder [5, 6]. In our previous study, we demonstrated that cardiac dysfunction during LPS-induced sepsis involved mitochondrial fission-associated mitochondrial damage. Excessive mitochondrial fission promotes mitochondrial damage, reduced synthesis of mitochondrial ATP, and cardiomyocyte death via mitochondria-dependent apoptotic pathway, which results in reduced cardiac performance [7, 8]. Therefore, mitochondrial dysfunction is a potential molecular mechanism underlying septic cardiomyopathy [9, 10].

Fundc1-dependent mitophagy is a protective mechanism that involves degradation of injured mitochondria through the lysosomes [11–14]. Mitophagy protects against cardiovascular disorders through antioxidative, antiapoptotic,

and anti-inflammatory effects [15–18]. For example, hypoxia-induced mitophagy attenuates myocardial ischemia-reperfusion injury by reducing the death of cardiomyocytes [15, 19]. Mitophagy activation attenuates heart failure by increasing ATP production in the cardiomyocytes and correcting the redox balance [20]. Besides, mitophagy activation is also associated with cardiac progenitor cell differentiation and mitochondrial network remodeling [21]. Fundc1-mediated mitophagy is closely associated with inflammation through the STING pathway [22], thereby suggesting its potential role in septic cardiomyopathy.

Although protective effects of mitophagy have been widely observed, mitophagy is significantly suppressed under stressful conditions [23–25]. Therefore, several approaches have been designed to enhance mitophagy. For example, melatonin protects cardiac microvasculature against ischemia/reperfusion injury by activating mitophagy [26]. Empagliflozin is an antidiabetic drug that activates mitophagy in type-2 diabetes [27]. In our previous study, we suppressed septic cardiomyopathy by inhibiting DRP1-related mitochondrial fission using irisin [28]. In this study, we investigated if irisin treatment protects against sepsis-induced cardiomyopathy via Fundc1-related mitophagy using LPS-stimulated H9C2 cardiomyocytes as a model.

2. Materials and Methods

2.1. Cell Culture. H9C2 cardiomyocytes were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in DMEM medium containing 10% fetal bovine serum at 37°C and 5% CO₂ in a humidified incubator. When the cell density reached 70%–80%, the cells were subcultured in a 1:3 ratio after digestion with 0.25% trypsin [29]. We induced septic cardiomyopathy *in vitro* by culturing H9C2 cells in 1 µg/ml LPS for 24 h. Besides, cardiomyocytes were treated with irisin (20 nmol/L) 24 h before LPS stress.

2.2. MTT Assay. We incubated 1×10^4 /ml diluted H9C2 cells in 96-well plates at 37°C and 5% CO₂ in a humidified incubator. The culture solution was changed every day. After incubation for 24 h, 48 h, and 72 h, respectively, the culture solution was discarded, and the cells were washed with PBS [30]. Then, 20 µl of MTT solution was added to each well and incubated at 37°C for 4 h in the dark. After discarding the culture solution with MTT, we added 150 µl DMSO into each well [31]. The plate was constantly shaken for 10 min to fully dissolve the purple crystals. Then, the OD values were measured in an enzyme-linked immunosorbent detector at 490 nm wavelength [32, 33].

2.3. Western Blotting. The transfected cells were lysed in RIPA buffer (Thermo Scientific, Belmont, MA, USA) for 30 minutes at 4°C. Equal amounts of protein samples were separated on a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane (Roche) by electroblotting [34, 35]. The membrane was blocked by incubation with skimmed milk at 4°C overnight. Then, the membrane was incubated with the primary antibody over-

night at 4°C. GAPDH was used as internal reference and probed with anti-GAPDH antibody [36]. The membranes were incubated with HRP-conjugated secondary antibody at room temperature for 2 h. Finally, the membranes were developed using the ECL substrate reagent kit (GE Healthcare) and analyzed using the Gel Doc XR imaging system (Bio-Rad, USA). Each sample was analyzed in triplicate [37].

2.4. Quantitative Real-Time PCR (qRT-PCR). Total cellular RNA was isolated using total RNA extraction reagent (R401-01, Vazyme, Nanjing) according to manufacturer's instructions [38]. Then, 1 µg RNA was reversed transcribed to cDNA using Hiscript Q RT SuperMix for qPCR (R122-01, Vazyme, Nanjing) as described by manufacturer's instructions. Quantitative PCR was performed using ChamQ SYBR Master Mix (Q311-02, Vazyme, Nanjing) in a LightCycler 96 (Roche, Risch-Rotkreuz, Switzerland) with the following conditions: 5 min at 95°C, followed by 40 cycles at 95°C for 30 s, 60°C for 40 s, and 72°C for 60 s [39].

2.5. Immunofluorescence. The cells were fixed in formalin, dehydrated with alcohol, and blocked in PBST with 5% BSA for 1 h at RT. The cells were then incubated with primary antibodies at 4°C overnight followed by incubation with secondary antibodies for 1 h at RT [40]. Then, the samples were stained with DAPI, mounted on slides, and photographed using the Olympus microscope at 200x magnification [41, 42]. The average fluorescence intensity was calculated using Image J software. Five random fields were analyzed for every sample [43].

2.6. Detection of Reactive Oxygen Species (ROS). The cells were cultured in a 24-well plate. Then, after discarding the supernatant, the cells were incubated in the dark at 37°C for 20 min with 500 µl medium per well containing 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, Cayman Chemical, Michigan, USA, Catalog: 85155) to detect the levels of intracellular ROS [44]. After removing the medium with the dye, cells were twice rinsed with PBS and photographed under a fluorescence microscope [45].

2.7. Measurement of Antioxidant Enzyme Activities. The cells were trypsinized and centrifuged at 1,000 rpm for 10 min. After removing the supernatant [46, 47], the cell pellet was resuspended in 500 µl of PBS solution and homogenized by sonication for two seconds [48]. This was repeated ten times to obtain total cellular homogenate. Then, the activities of glutathione peroxidase (GSH-Px, Catalog: A005), GSH (CAT, Catalog: A007-1-1), and superoxide dismutase (SOD, Catalog: A001-3-2) were determined in the cell homogenates according to manufacturer's instructions [49].

2.8. Statistical Analysis. All *in vitro* experiments were performed in triplicate. All experiments were repeated three times. The experiments were performed using five rats per group. Data are presented as means ± SD and were analyzed using SPSS 23.0 software [49]. Student's *t*-test was used to compare data from two experimental groups. One-way ANOVA followed by Tukey's multiple comparisons test

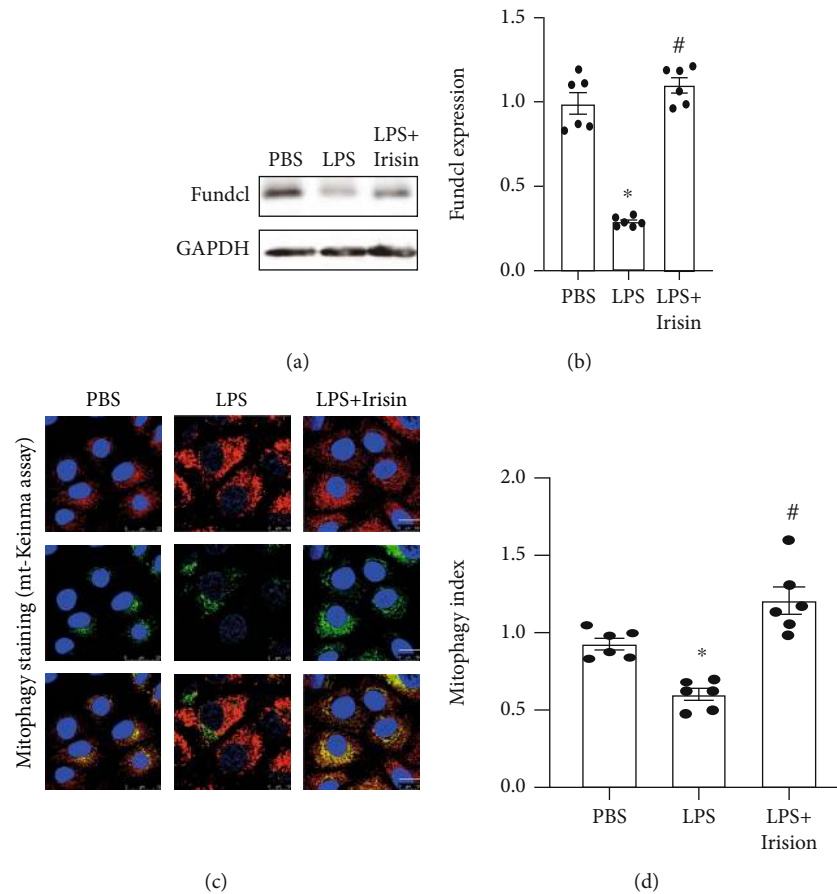


FIGURE 1: Irisin activates Fundc1-related mitophagy in LPS-stimulated cardiomyocytes. (a, b) Western blot analysis shows Fundc1 levels in control, LPS-stimulated, and irisin plus LPS-stimulated cardiomyocytes. (c, d) Immunofluorescence assay results demonstrate mitophagy index in control, LPS-stimulated, and irisin plus LPS-stimulated cardiomyocytes. Note: * denotes $p < 0.05$ vs. PBS group; # denotes $p < 0.05$ vs. LPS group; bar: 45 μm .

was used to evaluate differences between multiple groups. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Irisin Activates Fundc1-Related Mitophagy in LPS-Stimulated Cardiomyocytes. We first analyzed the effects of irisin on Fundc1-related mitophagy in LPS-stimulated cardiomyocytes. Western blot analysis showed that Fundc1 protein levels were rapidly downregulated in LPS-stimulated cardiomyocytes compared to the controls (Figures 1(a) and 1(b)). However, Fundc1 protein levels were significantly higher in irisin plus LPS-stimulated cardiomyocytes compared to the LPS-stimulated cardiomyocytes alone (Figures 1(a) and 1(b)). This suggested that irisin treatment is protected against defective Fundc1-related mitophagy in LPS-stimulated cardiomyocytes. We then analyzed the status of mitophagy using immunofluorescence. LPS treatment significantly reduced mitophagy compared to the control group, but these effects were reversed by irisin (Figures 1(c) and 1(d)). Taken together, these results demonstrated that Fundc1-related mitophagy was activated by irisin in LPS-stimulated cardiomyocytes.

3.2. Irisin Enhances ATP Production and Mitochondrial Metabolism through Fundc1-Related Mitophagy in LPS-Stimulated Cardiomyocytes. Next, we analyzed the effects of irisin on ATP levels in LPS-stimulated cardiomyocytes, and its relationship with Fundc1-related mitophagy. ATP levels were significantly reduced in LPS-stimulated cardiomyocytes compared to the control group, but these effects were reversed by irisin (Figure 2(a)). Next, we analyzed the relationship between Fundc1-related mitophagy and irisin-mediated upregulation of ATP levels in LPS-stimulated cardiomyocytes by transfecting H9C2 cells with siRNA against Fundc1. Then, we analyzed ATP levels in the presence and absence of Fundc1. As shown in Figure 2(a), the ATP levels were significantly reduced in irisin-treated Fundc1-silenced cardiomyocytes compared to the irisin-treated cardiomyocytes alone. These results demonstrated that irisin enhanced ATP production in LPS-stimulated cardiomyocytes via Fundc1-related mitophagy.

We then analyzed the activities of mitochondrial respiration complexes that regulate mitochondrial ATP production. LPS treatment significantly reduced the activity of mitochondrial respiration complexes I and III compared to the control group, but these effects were reversed by irisin

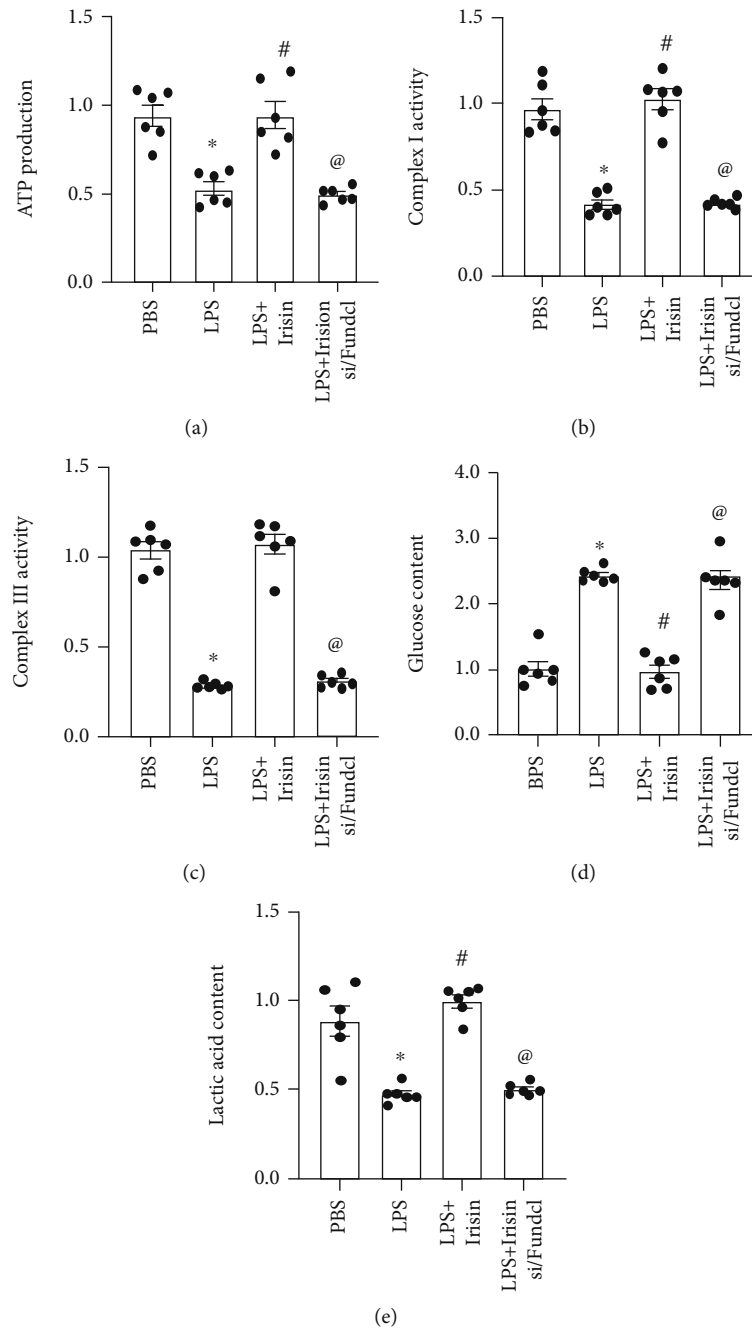


FIGURE 2: Irisin enhances mitochondrial metabolism and ATP production in LPS-stimulated cardiomyocytes through Fundc1-related mitophagy. (a) ELISA analysis shows ATP levels in control, LPS-stimulated, irisin plus LPS-stimulated cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. (b, c) ELISA results show activities of mitochondrial complexes I and III in control, LPS-stimulated, irisin plus LPS-stimulated cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. (d, e) ELISA results show the levels of (d) glucose and (e) lactic acid in the medium from control, LPS-stimulated, irisin plus LPS-stimulated H9C2 cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. * denotes $p < 0.05$ vs. PBS group; # denotes $p < 0.05$ vs. LPS group; @ denotes $p < 0.05$ vs. LPS + irisin.

(Figures 2(b) and 2(c)). However, the beneficial effects of irisin on the activities of complex I/III were abolished by Fundc1 silencing (Figures 2(b) and 2(c)). Decreased mitochondrial ATP production is closely associated with increased production of lactic acid, a by-product of glucose metabolism [50, 51]. Therefore, we analyzed changes in glu-

cose and lactic acid levels in the medium of different experimental groups of cardiomyocytes. The levels of glucose were significantly higher, and lactic acid levels were significantly reduced in the growth medium of LPS-stimulated cardiomyocytes compared to the control group (Figures 2(d) and 2(e)). This suggested an arrest in mitochondrial oxidative

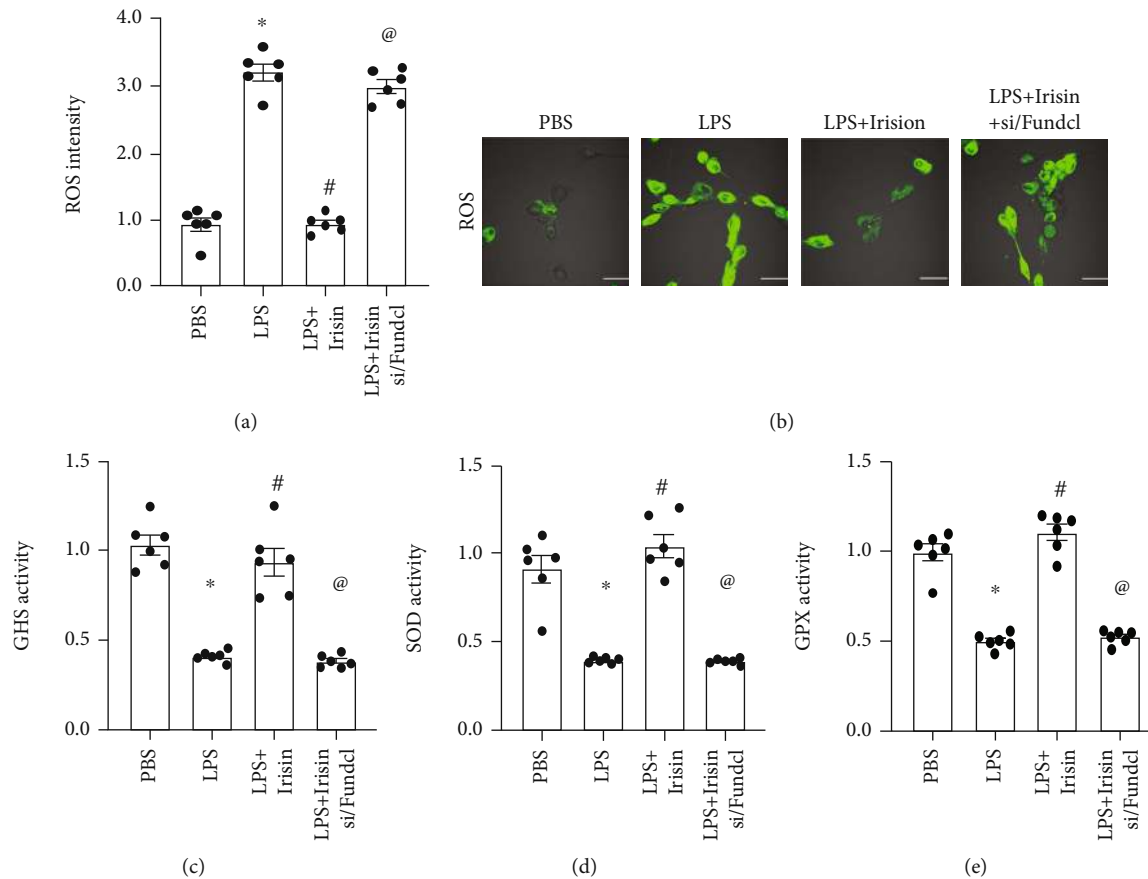


FIGURE 3: Irisin attenuates oxidative stress in LPS-stimulated cardiomyocytes through Fundc1-related mitophagy. (a, b) Immunofluorescence assay results show ROS levels in control, LPS-stimulated, irisin plus LPS-stimulated cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. (c–e) ELISA assay results show activities of antioxidant enzymes, GSH, SOD, and GPX in the control, LPS-stimulated, irisin plus LPS-stimulated cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. * denotes $p < 0.05$ vs. PBS group; # denotes $p < 0.05$ vs. LPS group; @ denotes $p < 0.05$ vs. LPS + irisin; bar: 85 μm .

phosphorylation. However, irisin treatment increased the levels of lactic acid and decreased glucose levels in the medium of LPS-stimulated cardiomyocytes (Figures 2(d) and 2(e)). This demonstrated that irisin promoted glucose metabolism in the LPS-stimulated cardiomyocytes. However, the beneficial effects of irisin on glucose metabolism were abolished by Fundc1 knockdown. Overall, these results demonstrated that irisin enhanced mitochondrial metabolism and ATP production in LPS-stimulated cardiomyocytes through Fundc1-related mitophagy.

3.3. Oxidative Stress Is Attenuated by Irisin through Fundc1-Related Mitophagy. Impaired mitochondrial metabolism is closely associated with mitochondrial oxidative stress [52–54]. Therefore, we analyzed the effects of irisin on oxidative stress in LPS-stimulated cardiomyocytes and the role of Fundc1-related mitophagy. The levels of ROS were significantly increased in the LPS-stimulated cardiomyocytes compared to the control group (Figures 3(a) and 3(b)). Irisin treatment suppressed the levels of ROS in LPS-stimulated cardiomyocytes, but these effects were abrogated by Fundc1 knockdown (Figures 3(a) and 3(b)). In addition to ROS production, we also measured the levels of antioxidative

enzymes, such as GSH, SOD, and GPX in the cardiomyocytes. The activities of GSH, SOD, and GPX were significantly reduced in the LPS-stimulated cardiomyocytes compared to the control group (Figures 3(c)–3(e)), thereby suggesting decreased antioxidative capacity. Irisin treatment increased the activities of GSH, SOD, and GPX in LPS-stimulated cardiomyocytes, but these effects were abolished by Fundc1 silencing (Figures 3(c)–3(e)). These data suggested that the antioxidative capacity of LPS-stimulated cardiomyocytes was enhanced by irisin through a mechanism related to Fundc1-related mitophagy. Taken together, our results demonstrated that irisin suppressed oxidative stress in LPS-stimulated cardiomyocytes via Fundc1-related mitophagy.

3.4. Mitochondria-Mediated Cardiomyocyte Death Is Inhibited by Irisin through Fundc1-Related Mitophagy. Mitochondrial ATP reduction and oxidative stress promote cardiomyocyte apoptosis by activating the mitochondria-dependent programmed cell death pathway [55–57]. Therefore, we analyzed if irisin attenuated cardiomyocyte apoptosis by activating Fundc1-related mitophagy. We used TUNEL staining assay to analyze the number of apoptotic

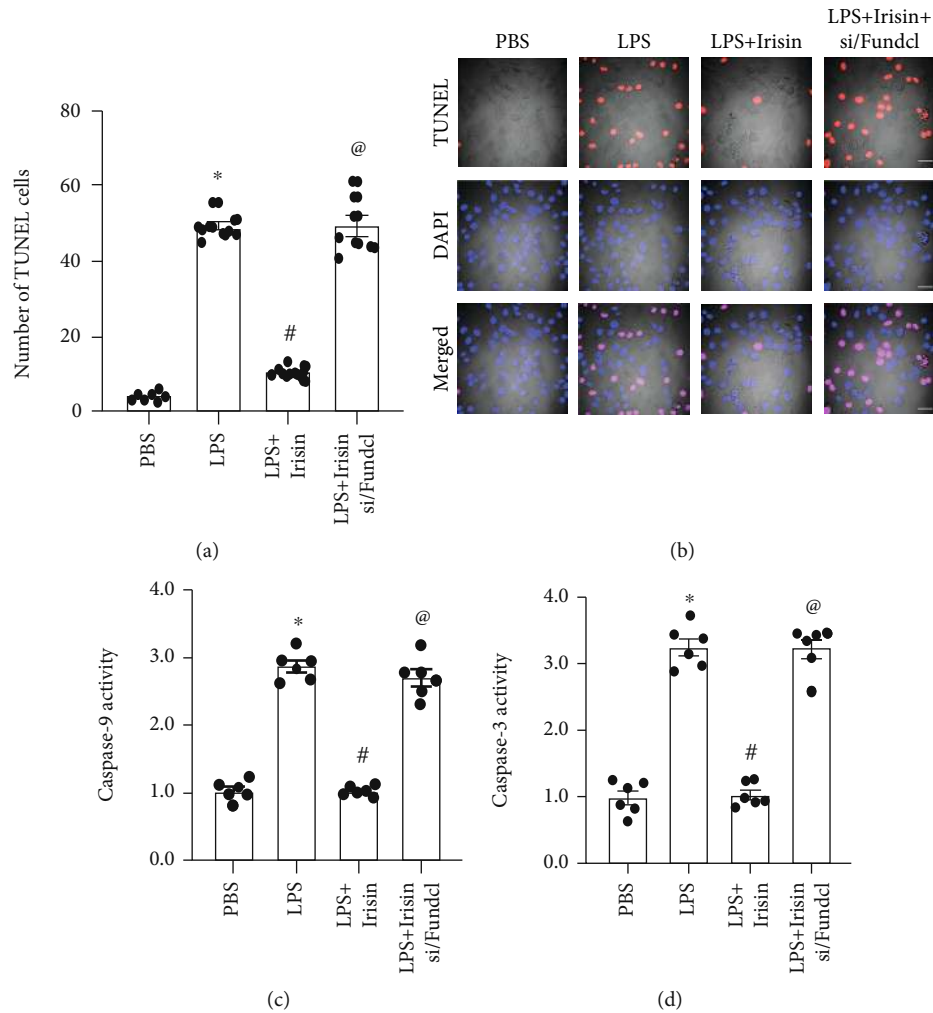


FIGURE 4: Mitochondria-mediated cardiomyocyte death is inhibited by irisin through Fundc1-related mitophagy. (a, b) TUNEL assay results show the percentages of apoptotic cells in control, LPS-stimulated, irisin plus LPS-stimulated cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. (c, d) ELISA assay results show caspase-9 and caspase-3 activities in control, LPS-stimulated, irisin plus LPS-stimulated cardiomyocytes, and irisin plus LPS-stimulated Fundc1-silenced cardiomyocytes. * denotes $p < 0.05$ vs. PBS group; # denotes $p < 0.05$ vs. LPS group; @ denotes $p < 0.05$ vs. LPS + irisin group; bar: $120 \mu\text{m}$.

cardiomyocytes in response to irisin treatment under LPS stress. The percentage of TUNEL-positive cardiomyocytes (apoptotic cells) was significantly higher in the LPS-stimulated group compared to the control group (40% vs. 10%; Figures 4(a) and 4(b)). Irisin significantly reduced the number of TUNEL-positive LPS-stimulated cardiomyocytes, but these effects were reversed by Fundc1 silencing (15% vs. 45%; Figures 4(a) and 4(b)). Taken together, these results suggested that LPS-stimulated cardiomyocyte apoptosis was inhibited by irisin through activation of Fundc1-related mitophagy. We then analyzed caspase-9 activity in LPS-stimulated cardiomyocytes. The activity of caspase-9 was significantly higher in LPS-stimulated H9C2 cells compared to the control group, but was significantly reduced by irisin treatment (Figure 4(c)). However, silencing of Fundc1 abolished the effects of irisin on caspase-9 (Figure 4(c)). Furthermore, caspase-3 activity was significantly elevated in LPS-stimulated cardiomyocytes but was reduced by irisin treatment in Fundc1-dependent manner

(Figure 4(d)). However, knockout of Fundc1 increased caspase-3 activation in irisin-treated LPS-stimulated H9C2 cardiomyocytes (Figure 4(d)). These data confirmed that mitochondria-dependent apoptosis was inhibited by irisin in LPS-stimulated cardiomyocytes via Fundc1-dependent mitophagy.

4. Discussion

Cardiac dysfunction is associated with increased morbidity and mortality in sepsis patients. Sepsis is a systemic inflammatory response that significantly reduces cardiac output and contributes to septic cardiomyopathy [58]. The risk factors associated with septic cardiomyopathy include upregulation of cytokines, hemodynamics disorder, sympathetic activation, decreased urine output, rapid rise in body temperature, and a sharp decrease in blood pressure [59]. However, therapeutic drugs are not available for the treatment of septic cardiomyopathy because molecular mechanisms underlying

sepsis-related myocardial dysfunction are not well known [60]. Our previous studies suggested that mitochondrial fission was a potential mechanism that triggered cardiomyocyte apoptosis during LPS-induced sepsis. Therefore, mitochondrial damage was probably a key feature of septic cardiomyopathy. In this study, we demonstrated that Fundc1-related mitophagy was suppressed in LPS-stimulated cardiomyocytes. We also demonstrated that irisin administration restored mitophagy and attenuated mitochondrial dysfunction by increasing mitochondrial ATP production, suppressing mitochondrial oxidative stress, and inhibiting mitochondria-dependent pathway of cardiomyocyte apoptosis. This suggested that sepsis-related myocardial dysfunction may be effectively treated by targeting mitochondrial homeostasis.

Mitophagy promotes degradation of damaged mitochondrial components and is critical for maintaining mitochondrial homeostasis [61–63]. Mitophagy activation prevents ischemia-reperfusion injury by modulating oxidative stress and inflammation response [63]. Mitophagy activation also reduces calcium deposition from the vascular smooth muscle cells via AMPK/Opa1 signaling pathway [64]. Besides, hypoxia-related hypertension is attenuated by mitophagy through endothelial uncoupling protein 2 [65]. High-fat-diet impairs cardiac contraction by inactivating mitophagy [66]. Mitophagy activation improves endothelial dysfunction and microvascular damage by activating antioxidative, anti-inflammatory, and antiapoptotic mechanisms [18, 67]. In the present study, mitophagy induction through irisin treatment increased ATP content and attenuated oxidative stress and apoptosis in the LPS-stimulated cardiomyocytes. This suggested that induction of mitophagy was a potential mechanism to improve cardiomyocyte function in patients with sepsis.

In the present study, we used irisin to improve cardiomyocyte function and survival by modulating mitophagy. The cardioprotective actions of irisin have also been previously reported. For example, serum irisin levels were reduced in diabetic patients and negatively correlated with cardiovascular events [68]. In type 2 diabetes model mice and hepatocytes, irisin inhibited hepatic gluconeogenesis and increased glycogen synthesis through the PI3K/Akt pathway [69]. Yin et al. reported that irisin acted as a mediator between obesity and vascular inflammation [70]. Furthermore, supplementation of irisin delayed progression of carotid atherosclerosis in dialysis patients [71]. Moreover, irisin improved endothelial proliferation through miRNA126-5p. In the present study, we demonstrated that irisin restored Fundc1-related mitophagy in LPS-stimulated cardiomyocytes as a model of sepsis cardiomyopathy.

There are several limitations in the present study. Our findings were not confirmed in animal models. Moreover, we did not investigate the association between mitophagy and inhibition of mitochondrial fission. Therefore, future investigations are required to confirm our findings and establish clinical relevance of irisin for treatment of septic cardiomyopathy.

In conclusion, our study demonstrates that irisin improved mitochondrial function and survival of LPS-stimulated cardiomyocytes via Fundc1-related mitophagy.

Data Availability

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

XQJ, SMC, and YHJ conceived and designed the study. FW, JH, and XXW acquired and interpreted the data. YT and YW drafted and revised the manuscript. All authors read and approved the final manuscript.

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References

- [1] M. W. Merx and C. Weber, "Sepsis and the heart," *Circulation*, vol. 116, pp. 793–802, 2007.
- [2] T. Gori, J. Lelieveld, and T. Münzel, "Perspective: cardiovascular disease and the Covid-19 pandemic," *Basic Research in Cardiology*, vol. 115, p. 32, 2020.
- [3] M. R. Celes, C. M. Prado, and M. A. Rossi, "Sepsis: going to the heart of the matter," *Pathobiology*, vol. 80, pp. 70–86, 2013.
- [4] A. Rudiger and M. Singer, "Mechanisms of sepsis-induced cardiac dysfunction," *Critical Care Medicine*, vol. 35, pp. 1599–1608, 2007.
- [5] Y. S. Hassan, M. Settergren, and L. Henareh, "Sepsis-induced myocardial depression and takotsubo syndrome," *Acute cardiac care*, vol. 16, pp. 102–109, 2014.
- [6] Y. Li, P. Liang, B. Jiang et al., "CARD9 promotes autophagy in cardiomyocytes in myocardial ischemia/reperfusion injury via interacting with Rubicon directly," *Basic Research in Cardiology*, vol. 115, p. 29, 2020.
- [7] F. L. Abel, "Myocardial function in sepsis and endotoxin shock," *The American Journal of Physiology*, vol. 257, pp. R1265–R1281, 1989.
- [8] F. Cao, M. L. Maguire, M. A. DJ et al., "Overexpression of mitochondrial creatine kinase preserves cardiac energetics without ameliorating murine chronic heart failure," *Basic Research in Cardiology*, vol. 115, p. 12, 2020.
- [9] R. J. Levy, "Mitochondrial dysfunction, bioenergetic impairment, and metabolic down-regulation in sepsis," *Shock*, vol. 28, pp. 24–28, 2007.
- [10] A. C. Fender, S. Kleeschulte, S. Stolte et al., "Thrombin receptor PAR4 drives canonical NLRP3 inflammasome signaling in the heart," *Basic Research in Cardiology*, vol. 115, p. 10, 2020.
- [11] J. Wang, P. Zhu, R. Li, J. Ren, and H. Zhou, "Fundc1-dependent mitophagy is obligatory to ischemic preconditioning-conferred renoprotection in ischemic AKI via suppression of Drp1-mediated mitochondrial fission," *Redox Biology*, vol. 30, p. 101415, 2020.

- [12] H. Zhou, P. Zhu, J. Wang, S. Toan, and J. Ren, "DNA-PKcs promotes alcohol-related liver disease by activating Drp1-related mitochondrial fission and repressing FUNDC1-required mitophagy," *Signal Transduction and Targeted Therapy*, vol. 4, p. 56, 2019.
- [13] A. Grogan, A. Coleman, H. Joca et al., "Deletion of obscurin immunoglobulin domains Ig58/59 leads to age-dependent cardiac remodeling and arrhythmia," *Basic Research in Cardiology*, vol. 115, p. 60, 2020.
- [14] A. Jusic and Y. Devaux, "Mitochondrial noncoding RNA-regulatory network in cardiovascular disease," *Basic Research in Cardiology*, vol. 115, p. 23, 2020.
- [15] H. Zhou, P. Zhu, J. Guo et al., "Ripk3 induces mitochondrial apoptosis via inhibition of FUNDC1 mitophagy in cardiac IR injury," *Redox Biology*, vol. 13, pp. 498–507, 2017.
- [16] H. Zhu, Y. Tan, W. Du et al., "Phosphoglycerate mutase 5 exacerbates cardiac ischemia-reperfusion injury through disrupting mitochondrial quality control," *Redox Biology*, vol. 38, p. 101777, 2021.
- [17] Y. Tan, D. Mui, S. Toan, P. Zhu, R. Li, and H. Zhou, "SERCA overexpression improves mitochondrial quality control and attenuates cardiac microvascular ischemia-reperfusion injury," *Molecular Therapy-Nucleic Acids*, vol. 22, pp. 696–707, 2020.
- [18] H. Zhou, J. Wang, P. Zhu et al., "NR4A1 aggravates the cardiac microvascular ischemia reperfusion injury through suppressing FUNDC1-mediated mitophagy and promoting Mff-required mitochondrial fission by CK2alpha," *Basic Research in Cardiology*, vol. 113, p. 23, 2018.
- [19] D. J. Hausenloy, M. Ntsekhe, and D. M. Yellon, "A future for remote ischaemic conditioning in high-risk patients," *Basic Research in Cardiology*, vol. 115, p. 35, 2020.
- [20] S. E. Shires and Å. B. Gustafsson, "Mitophagy and heart failure," *Journal of Molecular Medicine (Berlin, Germany)*, vol. 93, pp. 253–262, 2015.
- [21] M. A. Lampert, A. M. Orogo, R. H. Najor et al., "BNIP3L/NIX and FUNDC1-mediated mitophagy is required for mitochondrial network remodeling during cardiac progenitor cell differentiation," *Autophagy*, vol. 15, pp. 1182–1198, 2019.
- [22] D. A. Sliter, J. Martinez, L. Hao et al., "Parkin and PINK1 mitigate STING-induced inflammation," *Nature*, vol. 561, pp. 258–262, 2018.
- [23] J. Wang and H. Zhou, "Mitochondrial quality control mechanisms as molecular targets in cardiac ischemia-reperfusion injury," *Acta Pharmaceutica Sinica B*, vol. 10, pp. 1866–1879, 2020.
- [24] H. Zhu, S. Toan, D. Mui, and H. Zhou, "Mitochondrial quality surveillance as a therapeutic target in myocardial infarction," *Acta Physiologica (Oxford, England)*, vol. 231, article e13590, 2021.
- [25] H. Zhou, J. Ren, S. Toan, and D. Mui, "Role of mitochondrial quality surveillance in myocardial infarction: from bench to bedside," *Ageing Research Reviews*, vol. 66, p. 101250, 2021.
- [26] H. Zhou, Y. Zhang, S. Hu et al., "Melatonin protects cardiac microvasculature against ischemia/reperfusion injury via suppression of mitochondrial fission-VDAC1-HK2-mPTP-mitophagy axis," *Journal of pineal research*, vol. 63, 2017.
- [27] H. Zhou, S. Wang, P. Zhu, S. Hu, Y. Chen, and J. Ren, "Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission," *Redox Biology*, vol. 15, pp. 335–346, 2018.
- [28] Y. Tan, H. Ouyang, X. Xiao, J. Zhong, and M. Dong, "Irisin ameliorates septic cardiomyopathy via inhibiting DRP1-related mitochondrial fission and normalizing the JNK-LATS2 signaling pathway," *Cell Stress & Chaperones*, vol. 24, pp. 595–608, 2019.
- [29] D. Bausch, S. Fritz, L. Bolm et al., "Hedgehog signaling promotes angiogenesis directly and indirectly in pancreatic cancer," *Angiogenesis*, vol. 23, pp. 479–492, 2020.
- [30] A. L. Bayliss, A. Sundararaman, C. Granet, and H. Mellor, "Raftlin is recruited by neuropilin-1 to the activated VEGFR2 complex to control proangiogenic signaling," *Angiogenesis*, vol. 23, pp. 371–383, 2020.
- [31] R. K. Adapala, A. K. Kanugula, S. Paruchuri, W. M. Chilian, and C. K. Thodeti, "TRPV4 deletion protects heart from myocardial infarction-induced adverse remodeling via modulation of cardiac fibroblast differentiation," *Basic Research in Cardiology*, vol. 115, p. 14, 2020.
- [32] C. Schinner, S. Olivares-Florez, A. Schlipp et al., "The inotropic agent digitoxin strengthens desmosomal adhesion in cardiac myocytes in an ERK1/2-dependent manner," *Basic Research in Cardiology*, vol. 115, no. 4, p. 46, 2020.
- [33] X. Lu, Y. He, C. Tang et al., "Triad3A attenuates pathological cardiac hypertrophy involving the augmentation of ubiquitination-mediated degradation of TLR4 and TLR9," *Basic Research in Cardiology*, vol. 115, no. 2, p. 19, 2020.
- [34] O. Bakhta, A. Pascaud, X. Dieu et al., "Tryptophane-kynurenine pathway in the remote ischemic conditioning mechanism," *Basic Research in Cardiology*, vol. 115, p. 13, 2020.
- [35] K. Qiao, Y. Liu, Z. Xu et al., "RNA m6A methylation promotes the formation of vasculogenic mimicry in hepatocellular carcinoma via Hippo pathway," *Angiogenesis*, vol. 24, no. 1, pp. 83–96, 2021.
- [36] M. Lv, X. Zhuang, Q. Zhang et al., "Acetyl-11-keto- β -boswellic acid enhances the cisplatin sensitivity of non-small cell lung cancer cells through cell cycle arrest, apoptosis induction, and autophagy suppression via p21-dependent signaling pathway," *Cell Biology and Toxicology*, vol. 37, no. 2, pp. 209–228, 2021.
- [37] M. T. Islam, "Angiostatic effects of ascorbic acid: current status and future perspectives," *Angiogenesis*, vol. 23, pp. 275–277, 2020.
- [38] S. K. Lahiri, A. P. Quick, B. Samson-Couterie et al., "Nuclear localization of a novel calpain-2 mediated junctophilin-2 C-terminal cleavage peptide promotes cardiomyocyte remodeling," *Basic Research in Cardiology*, vol. 115, p. 49, 2020.
- [39] M. Lindner, H. Mehel, A. David et al., "Fibroblast growth factor 23 decreases PDE4 expression in heart increasing the risk of cardiac arrhythmia; Klotho opposes these effects," *Basic Research in Cardiology*, vol. 115, p. 51, 2020.
- [40] N. Ludwig, S. S. Yerneni, J. H. Azambuja, and D. G. Gillespie, "Tumor-derived exosomes promote angiogenesis via adenosine A2B receptor signaling," *Angiogenesis*, vol. 23, no. 4, pp. 599–610, 2020.
- [41] A. Umamathy, L. W. Chamley, and J. L. James, "Reconciling the distinct roles of angiogenic/anti-angiogenic factors in the placenta and maternal circulation of normal and pathological pregnancies," *Angiogenesis*, vol. 23, pp. 105–117, 2020.
- [42] A. Vasseur, L. Cabel, O. Tredan et al., "Prognostic value of CEC count in HER2-negative metastatic breast cancer patients treated with bevacizumab and chemotherapy: a prospective

- validation study (UCBG COMET),” *Angiogenesis*, vol. 23, no. 2, pp. 193–202, 2020.
- [43] E. H. Moon, Y. H. Kim, P. N. Vu et al., “TMEM100 is a key factor for specification of lymphatic endothelial progenitors,” *Angiogenesis*, vol. 23, pp. 339–355, 2020.
- [44] M. P. Winter, S. Sharma, J. Altmann et al., “Interruption of vascular endothelial growth factor receptor 2 signaling induces a proliferative pulmonary vasculopathy and pulmonary hypertension,” *Basic Research in Cardiology*, vol. 115, p. 58, 2020.
- [45] S. A. Watson, A. Dendorfer, T. Thum, and F. Perbellini, “A practical guide for investigating cardiac physiology using living myocardial slices,” *Basic Research in Cardiology*, vol. 115, p. 61, 2020.
- [46] Y. Zhang, S. Wang, and A. C. Dudley, “Models and molecular mechanisms of blood vessel co-option by cancer cells,” *Angiogenesis*, vol. 23, pp. 17–25, 2020.
- [47] P. Szaraz, P. Mander, N. Gasner, M. Librach, F. Iqbal, and C. Librach, “Glucose withdrawal induces endothelin 1 release with significant angiogenic effect from first trimester (FTM), but not term human umbilical cord perivascular cells (HUCPVC),” *Angiogenesis*, vol. 23, pp. 131–144, 2020.
- [48] C. Tacconi, Y. He, L. Ducoli, and M. Detmar, “Epigenetic regulation of the lineage specificity of primary human dermal lymphatic and blood vascular endothelial cells,” *Angiogenesis*, vol. 24, no. 1, pp. 67–82, 2021.
- [49] P. Wischmann, V. Kuhn, T. Suvorava et al., “Anaemia is associated with severe RBC dysfunction and a reduced circulating NO pool: vascular and cardiac eNOS are crucial for the adaptation to anaemia,” *Basic Research in Cardiology*, vol. 115, p. 43, 2020.
- [50] G. Heusch, “Coronary microvascular obstruction: the new frontier in cardioprotection,” *Basic Research in Cardiology*, vol. 114, p. 45, 2019.
- [51] W. E. Hughes, A. M. Beyer, and D. D. Gutterman, “Vascular autophagy in health and disease,” *Basic Research in Cardiology*, vol. 115, p. 41, 2020.
- [52] Y. Z. Li, X. D. Wu, X. H. Liu, and P. F. Li, “Mitophagy imbalance in cardiomyocyte ischaemia/reperfusion injury,” *Acta Physiologica (Oxford, England)*, vol. 225, article e13228, 2019.
- [53] M. Makrecka-Kuka, E. Liepinsh, A. J. Murray et al., “Altered mitochondrial metabolism in the insulin-resistant heart,” *Acta Physiologica (Oxford, England)*, vol. 228, article e13430, 2020.
- [54] P. García-Gómez and M. Valiente, “Vascular co-option in brain metastasis,” *Angiogenesis*, vol. 23, pp. 3–8, 2020.
- [55] J. Wang, S. Toan, and H. Zhou, “New insights into the role of mitochondria in cardiac microvascular ischemia/reperfusion injury,” *Angiogenesis*, vol. 23, pp. 299–314, 2020.
- [56] A. Del Campo, “Mitophagy as a new therapeutic target for sarcopenia,” *Acta Physiologica (Oxford, England)*, vol. 225, article e13219, 2019.
- [57] E. A. Kuczynski and A. R. Reynolds, “Vessel co-option and resistance to anti-angiogenic therapy,” *Angiogenesis*, vol. 23, pp. 55–74, 2020.
- [58] A. C. Sharma, S. J. Motew, S. Farias et al., “Sepsis alters myocardial and plasma concentrations of endothelin and nitric oxide in rats,” *Journal of Molecular and Cellular Cardiology*, vol. 29, pp. 1469–1477, 1997.
- [59] H. Zhang, D. Liu, X. Wang et al., “Melatonin improved rat cardiac mitochondria and survival rate in septic heart injury,” *Journal of Pineal Research*, vol. 55, pp. 1–6, 2013.
- [60] A. C. Sharma, “Sepsis-induced myocardial dysfunction,” *Shock*, vol. 28, pp. 265–269, 2007.
- [61] H. Zhou, C. Shi, S. Hu, H. Zhu, J. Ren, and Y. Chen, “BII1 is associated with microvascular protection in cardiac ischemia reperfusion injury via repressing Syk-Nox2-Drp1-mitochondrial fission pathways,” *Angiogenesis*, vol. 21, pp. 599–615, 2018.
- [62] H. Zhou, S. Toan, P. Zhu, J. Wang, J. Ren, and Y. Zhang, “DNA-PKcs promotes cardiac ischemia reperfusion injury through mitigating BI-1-governed mitochondrial homeostasis,” *Basic Research in Cardiology*, vol. 115, p. 11, 2020.
- [63] H. Zhou, P. Zhu, J. Wang, H. Zhu, J. Ren, and Y. Chen, “Pathogenesis of cardiac ischemia reperfusion injury is associated with CK2alpha-disturbed mitochondrial homeostasis via suppression of FUNDC1-related mitophagy,” *Cell Death and Differentiation*, vol. 25, pp. 1080–1093, 2018.
- [64] W. R. Chen, Y. J. Zhou, J. Q. Yang, F. Liu, X. P. Wu, and Y. Sha, “Melatonin attenuates calcium deposition from vascular smooth muscle cells by activating mitochondrial fusion and mitophagy via an AMPK/OPA1 signaling pathway,” *Oxidative Medicine and Cellular Longevity*, vol. 2020, Article ID 5298483, 23 pages, 2020.
- [65] M. Haslip, I. Dostanic, Y. Huang et al., “Endothelial uncoupling protein 2 regulates mitophagy and pulmonary hypertension during intermittent hypoxia,” *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 35, pp. 1166–1178, 2015.
- [66] T. D. Larsen, K. H. Sabey, A. J. Knutson et al., “Diabetic pregnancy and maternal high-fat diet impair mitochondrial dynamics in the developing fetal rat heart by sex-specific mechanisms,” *International journal of molecular sciences*, vol. 20, no. 12, p. 3090, 2019.
- [67] S. Hu, Y. Gao, H. Zhou et al., “New insight into mitochondrial changes in vascular endothelial cells irradiated by gamma ray,” *International Journal of Radiation Biology*, vol. 93, pp. 470–476, 2017.
- [68] Z. M. Khorasani, R. K. Bagheri, M. A. Yaghoubi et al., “The association between serum irisin levels and cardiovascular disease in diabetic patients,” *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, vol. 13, pp. 786–790, 2019.
- [69] T. Y. Liu, C. X. Shi, R. Gao et al., “Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes,” *Clinical Science (London, England)*, vol. 129, pp. 839–850, 2015.
- [70] C. Yin, W. Hu, M. Wang, W. Lv, T. Jia, and Y. Xiao, “Irisin as a mediator between obesity and vascular inflammation in Chinese children and adolescents,” *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 30, pp. 320–329, 2020.
- [71] M. J. Lee, S. A. Lee, B. Y. Nam et al., “Irisin, a novel myokine is an independent predictor for sarcopenia and carotid atherosclerosis in dialysis patients,” *Atherosclerosis*, vol. 242, pp. 476–482, 2015.