

Leginsulin alleviates pathological cardiac hypertrophy potentially by regulating the oxidative stress-mediated MAPK signaling pathway

Chenguang Yao

Hubei University of Technology

Mengting Wu

Hubei University of Technology

Tianluo Shen

Hubei University of Technology

Zi Xiong

Hubei University of Technology

Xuejie Qi

Hubei University of Technology

Essam Hady

Al-Azhar University

Mei Liu

Hubei University of Technology

Xiaoke Shang

sxs_sxk@163.com

Hubei University of Technology

Research Article

Keywords: Leginsulin, Cardiac hypertrophy, Myocardial fibrosis, Oxidative stress, Mitochondrial function, Mitogen-activated protein kinase signaling

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11035353/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Pathological cardiac hypertrophy (PCH) is a critical driver of heart failure and is associated with oxidative stress, mitochondrial dysfunction, and abnormal stress-responsive signaling. This study aimed to investigate the protective effects of leginsulin, a naturally occurring soybean-derived peptide, against PCH and to elucidate its underlying mechanisms. Isoproterenol-induced cardiac hypertrophy was established in mice, and angiotensin II (AngII)-stimulated H9c2 and AC16 cardiomyocytes were used as complementary in vitro models. Cardiac remodeling, fibrosis, oxidative stress, mitochondrial function, and mitogen-activated protein kinase signaling were evaluated following leginsulin treatment. Leginsulin markedly attenuated cardiac hypertrophy and fibrosis in mice, as indicated by reduced cardiac enlargement, cardiomyocyte hypertrophy, and collagen accumulation. In AngII-stimulated H9c2 and AC16 cardiomyocytes, leginsulin suppressed the expression of hypertrophic and fibrotic markers. Furthermore, Leginsulin reduced oxidative stress by decreasing reactive oxygen species and malondialdehyde levels while enhancing superoxide dismutase activity. In parallel, leginsulin preserved mitochondrial membrane potential, increased adenosine triphosphate production, and improved mitochondrial network integrity. Mechanistically, leginsulin selectively suppressed AngII-induced activation of c-Jun N-terminal kinase and extracellular signal-regulated kinase signaling whereas p38 signaling was not significantly affected. Leginsulin protects against PCH and fibrosis by maintaining redox balance, preserving mitochondrial function, and regulating stress-responsive signaling pathways. These findings reveal a physiological role of leginsulin in cardiac stress adaptation and highlight its potential as a natural regulator of PCH.

1. Introduction

Pathological cardiac hypertrophy (PCH) is a major driver of cardiovascular disease progression, including hypertension, myocardial infarction, valvular heart disease, and cardiomyopathy, represents an important risk factor for heart failure and sudden cardiac death[1,2]. Although hypertrophy initially arises serving as an adaptive response to increased workload, sustained mechanical stress and neurohumoral activation drive fetal gene reactivation and excessive extracellular matrix deposition, leading to myocardial fibrosis, adverse cardiac remodeling, and functional deterioration[1,3,4]. Current therapies including renin-angiotensin system inhibitors, β -adrenergic receptor blockers, and sodium-glucose cotransporter 2 inhibitors, have improved clinical outcomes but cannot fully prevent PCH progression[2, 5]. Identifying additional regulatory mechanisms and effective interventions therefore remains necessary.

Oxidative stress is a central mechanism driving pathological cardiac remodeling[1,6]. Diverse pathological stimuli, including angiotensin II (Ang II), catecholamines, and mechanical stress, promote excessive reactive oxygen species (ROS) generation in cardiomyocytes[7,8]. When ROS production overwhelms endogenous antioxidant capacity, the resulting redox imbalance induces lipid peroxidation, protein oxidation, DNA damage, and activation of stress-responsive signaling pathways, thereby promoting cardiac remodeling[9, 10].

Mitochondria play essential roles in cardiomyocyte energy metabolism and redox regulation, but are highly vulnerable to oxidative stress-induced injury[11,12]. Excessive ROS accumulation impairs mitochondrial electron transport, decreases mitochondrial membrane potential and adenosine triphosphate (ATP) production, and disrupts mitochondrial dynamics, further enhancing ROS generation and establishing a vicious cycle between oxidative stress and mitochondrial dysfunction[13,14]. Consequently, mitochondrial impairment promotes metabolic abnormalities and activates downstream stress pathways involved in pathological cardiac remodeling[15,16]. Maintaining mitochondrial homeostasis therefore represents a key strategy for limiting oxidative stress-mediated cardiac injury.

The mitogen-activated protein kinase (MAPK) signaling pathway is a major downstream effector of oxidative stress and an important regulator of PCH progression[17,18]. ROS-induced activation of MAPK cascades, including c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 MAPK, regulates cardiomyocyte stress responses and pathological gene expression[19,20]. Activation of JNK and ERK promotes fetal gene re-expression, including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and β -myosin heavy chain (β -MHC), while enhancing transforming growth factor- β (TGF- β)-dependent profibrotic responses and extracellular matrix (ECM) accumulation[21,22]. Moreover, MAPK signaling interacts with inflammatory responses, apoptosis, autophagy, and mitochondrial dysfunction to regulate cardiac remodeling[23]. However, the functional contribution of individual MAPK isoforms varies among disease models; JNK and ERK are generally considered major mediators of hypertrophic signaling, whereas p38 MAPK exhibits stimulus- and context-dependent effects[24,25]. Collectively, these findings suggest that precise modulation of oxidative stress-related MAPK signaling may offer new therapeutic insights for PCH.

Leginsulin is a cysteine-rich bioactive peptide of plant origin belonging to the plant albumin 1 (PA1) family and exhibiting substantial sequence and structural similarity to PA1b. The originally identified Leginsulin was isolated from the radicles of germinated soybean seeds and consists of 37 amino acids, including six cysteine residues that form three disulfide bonds [26]. Leginsulin shares approximately 60% sequence identity and more than 80% sequence similarity with PA1b, indicating a close structural relationship between these peptides[27]. Previous studies have demonstrated that PA1b-like bioactive peptides can be isolated from pea seeds and possess diverse biological activities[28]. Building on this evidence, our laboratory isolated and prepared Leginsulin from pea seeds, and its biological activities were subsequently characterized in our previous study[29]. Accumulating evidence indicates that Leginsulin exhibits insulin-like activity by promoting glucose uptake and enhancing Akt activation and GLUT4 translocation to the plasma membrane in differentiated L6 and C2C12 myotubes[30]. Beyond its role in glucose metabolism, recent evidence suggests that Leginsulin also exerts cytoprotective effects by alleviating glucose toxicity-induced mitochondrial dysfunction and maintaining cellular ROS and ATP levels as well as mitochondrial membrane potential through interaction with voltage-dependent anion channel 1[29]. These findings suggest that the biological functions of Leginsulin extend beyond glucose metabolic regulation and that its ability to preserve mitochondrial homeostasis may contribute importantly to its cytoprotective effects. Despite these findings, the cardiovascular effects of Leginsulin remain largely unexplored, particularly its potential role in PCH and the underlying mechanisms. Given

that mitochondrial dysfunction is an important contributor to PCH and that Leginsulin has demonstrated mitochondrial-protective potential, we hypothesized that Leginsulin may attenuate PCH by preserving mitochondrial homeostasis in cardiomyocytes. Therefore, in the present study, we investigated the cardioprotective effects of Leginsulin in PCH and further explored the underlying molecular mechanisms. Our findings may provide new insights into the biological functions of plant-derived bioactive peptides in cardiovascular disease and contribute to the development of novel peptide-based strategies for preventing pathological cardiac remodeling.

In this study, we investigated the protective effects of leginsulin against PCH using an Isoproterenol (ISO) induced cardiac hypertrophy model in mice and an Ang II-stimulated cardiomyocyte model. We further examined whether these cardioprotective, leginsulin, effects are associated with the regulation of oxidative stress, mitochondrial function, and MAPK signaling.

2. Materials and Methods

2.1 Reagents

DMEM (4.5 g/L glucose, G4511), DMSO (D8370), and MitoTracker Red CMXRos (M9940) were obtained from Servicebio (Wuhan, China) or Solarbio (Beijing, China). ROS detection kit (S0033S), JC-1 mitochondrial membrane potential assay kit (C2006), ATP assay kit (S0027), and hematoxylin and eosin (H&E) staining kit (C0105S) were purchased from Beyotime (Shanghai, China). total superoxide dismutase (T-SOD) and malondialdehyde (MDA) assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Fluorescent Phalloidin (SB-YP0060) and Picosirius Red (PSR) staining solution (DC0041) were purchased from Sheng'er Biology (Shanghai, China) and Leigen Bio (Beijing, China), respectively. TRIzol reagent (15596018CN), reverse transcription kit (RT333-01), and SYBR Green Mix (Q411-02) were obtained from Thermo Fisher Scientific (USA) and Vazyme (Wuhan, China), respectively. Ang II (05-23-0101) and ISO (HY-B0468) were purchased from Sigma-Aldrich (USA) and MedChemExpress (USA), respectively. Leginsulin was prepared as previously described[29], dissolved in PBS, and sterilized through a 0.22 µm filter.

Primary antibodies against factor-beta 1 (TGF-β1), type I alpha 1 chain (COL1A1), ERK1/2, p-ERK1/2, JNK, p-JNK, p38 MAPK, p-p38, GAPDH, and β-actin were used for Western blotting. ERK1/2 antibody was obtained from Abmart (Shanghai, China), and other antibodies were purchased from Proteintech (Wuhan, China).

2.2 Animal Experiments

Mice used in the experiment are male C57BL/6 J mice (6 weeks old, 22 ± 3 g, Specific Pathogen Free grade) were supplied by Rat-Lover Biotechnology Co., Ltd. (Wuhan, China; animal production license no. SYXX(E)2021-0115). Mice were housed in the SPF animal facility at Hubei University of Technology under controlled conditions (22 ± 2°C, 50%-60% humidity, 12 h light/dark cycle) with free access to food and water.

After a 7-day acclimatization period, mice were randomly divided into four groups (n = 5/group): Control, ISO, ISO + Leg-20, and ISO + Leg-50. Control and ISO groups received saline gavage, whereas leginsulin-treated groups received leginsulin (20 or 50 mg/kg) daily for 30 days. From day 7 onward, ISO groups were injected intraperitoneally with ISO (7 mg/kg) once daily for 7 consecutive days to induce cardiac hypertrophy, while control mice received equal volumes of saline. Body weight and general health status were monitored during the experiment. At the endpoint, mice were euthanized, and cardiac tissues were collected for subsequent analyses.

2.3 Histological Analysis

Heart tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μ m thickness. H&E staining was performed for morphological evaluation, while PSR staining was conducted to assess myocardial fibrosis. Images were captured with a light microscope, and cardiomyocyte cross-sectional area (CSA) and collagen-positive area were quantified using ImageJ.

2.4 Cell Culture and Treatment

Rat cardiomyoblast H9c2 cells and human cardiomyocyte AC16 cells were obtained from Servicebio Biotechnology Co., Ltd. (Wuhan, China). Cells were cultured in high-glucose DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C with 5% CO₂. Cardiomyocyte hypertrophy was induced by Ang II (1 μ mol/L) stimulation, with or without leginsulin (20 or 50 μ g/mL), for 48 h.

2.5 Phalloidin and MitoTracker Red Staining

Cells were treated as described above. For Phalloidin staining, cells were fixed, permeabilized, and stained with fluorescent Phalloidin, followed by 4',6-diamidino-2-phenylindole staining. Cell morphology was observed using fluorescence microscopy, and cell area was quantified using ImageJ. For mitochondrial visualization, cells were incubated with MitoTracker Red CMXRos (200 nM) and analyzed using confocal microscopy. Mitochondrial fluorescence intensity was quantified using ImageJ.

2.6 ROS and Oxidative Stress Detection

Intracellular ROS production was detected using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining. After treatment, cells were incubated with DCFH-DA (10 μ mol/L), and fluorescence intensity was quantified using ImageJ. Cells were collected and lysed after treatment. Total protein concentrations were determined using a BCA assay kit. T-SOD activity and MDA levels were measured using commercial kits according to the manufacturer's instructions and normalized to total protein content.

2.7 Mitochondrial Function Analysis

Mitochondrial membrane potential ($\Delta\Psi$ m) was evaluated using a JC-1 assay kit. Cells were incubated with JC-1 working solution, and fluorescence images were captured by fluorescence microscopy. The

JC-1 red/green fluorescence intensity ratio was calculated using ImageJ. Intracellular ATP levels were measured using an ATP assay kit (Beyotime, Shanghai, China) and normalized to total protein concentrations.

2.8 RT-qPCR Analysis

Total RNA was extracted using TRIzol reagent and reverse-transcribed into cDNA. RT-qPCR was performed using SYBR Green Master Mix on a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the internal control. Primer sequences are listed in Table 1

Table 1. Primer sequences used for qPCR amplification

Primer name	Sequence5'→3'
ANP-F(human)	GAGCAAATCCCGTATACAGTGC
ANP-R(human)	ATCTTCTACCGGCATCTCCTCC
BNP-F(human)	GCTGCTGGAGCTGATAAGAGAA
BNP-R(human)	GTTCTTTTGTAGGGCCTTGGTC
β-MHC-F(human)	AGTTGCCTCTTGAGGTCCTC
β-MHC-R(human)	GCAGATCATCAAGGCCAAGG
GAPDH-F(human)	AGAAGGCTGGGGCTCATTTG
GAPDH-R(human)	AGGGGCCATCCACAGTCTTC
Anp-F(mouse)	ACAGATCTGATGGATTTCAAGAACCTG
Anp-R(mouse)	AGTGCGGCCCTGCTTCCTCA
Bnp-F(mouse)	GGATCGGATCCGTCAGTCGTT
Bnp-R(mouse)	AGACCCAGGCAGAGTCAGAAA
β-mhc-F(mouse)	CGGACCTTGGAAGACCAGAT
β-mhc-R(mouse)	GACAGCTCCCCATTCTCTGT
Gapdh-F(mouse)	CGACTTCAACAGCAACTCCCACTCTTCC
Gapdh -R(mouse)	TGGGTGGTCCAGGGTTTCTTACTCCTT

2.9 Western Blot Analysis

Total proteins were extracted using RIPA buffer containing protease and phosphatase inhibitors. For hypertrophy- and fibrosis-related proteins, cells were treated with Ang II and leginsulin for 48 h. For MAPK pathway analysis, cells were serum-starved, pretreated with leginsulin for 24 h, and stimulated

with Ang II for 30 min before protein extraction. Protein samples were separated by SDS-PAGE, transferred onto PVDF membranes, and incubated with specific primary antibodies followed by HRP-conjugated secondary antibodies. Protein signals were detected using an enhanced chemiluminescence system. Protein expression was quantified using ImageJ. Phosphorylated MAPK proteins were normalized to their corresponding total proteins, whereas TGF- β 1 and COL1A1 levels were normalized to GAPDH.

2.10 Statistical Analysis

Data are presented as mean $\pm$ SD. Each mouse was considered an independent experimental unit, and cell experiments were independently repeated at least three times. Statistical analyses were performed using GraphPad Prism 9. Data distribution assessed by normality testing. Multiple comparisons were performed using one-way ANOVA followed by Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Leginsulin Attenuates ISO-Induced Cardiac Pathological Remodeling in Mice

To evaluate the protective effects of leginsulin against PCH in vivo, a mouse model of ISO-induced cardiac hypertrophy was established. Male C57BL/6J mice were randomly divided into four groups: Control, ISO, ISO + Leg-20, and ISO + Leg-50. Mice received daily oral administration of leginsulin (20 or 50 mg/kg) for 30 days, and cardiac hypertrophy was induced by intraperitoneal injection of ISO (7 mg/kg) for 7 consecutive days beginning on day 7 (Fig. 1A). During the experimental period, no significant differences in body weight or food intake were observed among the groups (Fig. 1B,C), suggesting that neither ISO-induced cardiac stress nor leginsulin treatment substantially affected overall growth.

Gross morphological examination showed that ISO administration resulted in enlarged hearts with a rounded ventricular appearance compared with Control mice, whereas leginsulin treatment markedly improved these hypertrophic changes (Fig. 1D). Consistently, ISO significantly increased heart weight (HW) and heart weight/body weight ratio (HW/BW) by approximately 18.7% and 20.6%, respectively (Fig. 1E,F). Leginsulin administration significantly reduced both HW and HW/BW relative to ISO-treated mice, with the high-dose group exhibiting a stronger protective effect.

Histological analysis using (H&E) staining further demonstrated that ISO induced cardiomyocyte enlargement, myocardial fiber disorganization, and increased intercellular spaces, which were substantially alleviated following leginsulin treatment (Fig. 1G). Quantitative analysis showed that ISO nearly doubled cardiomyocyte CSA, whereas high-dose leginsulin reduced CSA by approximately 59% compared with the ISO group.

Because myocardial fibrosis is a major feature of pathological cardiac remodeling, PSR staining was performed to assess collagen deposition. ISO markedly increased collagen accumulation, with the collagen-positive area elevated approximately 2.3-fold compared with the Control group (Fig. 1H). Leginsulin treatment significantly reduced collagen deposition, with the high-dose group showing an approximately 43% decrease compared with ISO-treated mice. Together, these results demonstrate that leginsulin attenuates ISO-induced cardiac hypertrophic remodeling and myocardial fibrosis in mice.

3.2 Leginsulin Suppresses Ang II-Induced Cardiomyocyte Hypertrophy and Fibrotic Responses

To investigate the direct effects of leginsulin on cardiomyocyte remodeling, Ang II-induced hypertrophy models were established in H9c2 and AC16 cells. Cells were treated with Ang II (1 $\mu\text{mol/L}$) alone or in combination with leginsulin (20 or 50 $\mu\text{g/mL}$) for 48 h. Ang II stimulation induced characteristic hypertrophic alterations in both H9c2 and AC16 cells, as visualized by Phalloidin staining, which showed increased F-actin organization and enlarged cell size (Fig. 2A). Quantitative analysis showed that Ang II increased cardiomyocyte area by approximately 15.8% in H9c2 cells and 39.5% in AC16 cells compared with the control group (Fig. 2B). Leginsulin treatment significantly reversed these morphological changes, with the 50 $\mu\text{g/mL}$ dose producing a greater reduction in cell hypertrophy.

At the molecular level, Ang II activated the hypertrophic fetal gene program, resulting in marked upregulation of ANP, BNP, and β -MHC mRNA expression in both cell models (Fig. 2C,D). Leginsulin treatment substantially suppressed induction of these hypertrophic markers, indicating inhibition of Ang II-driven transcriptional remodeling. Because pathological hypertrophy is closely associated with extracellular matrix remodeling, the expression of fibrosis-related proteins was further examined. Western blot analysis revealed that Ang II markedly increased transforming growth TGF- β 1 and collagen COL1A1 protein abundance in H9c2 and AC16 cells, whereas leginsulin treatment significantly attenuated these profibrotic responses (Fig. 2E-G). Collectively, leginsulin counteracts Ang II-induced cardiomyocyte remodeling by simultaneously limiting cellular hypertrophy, suppressing fetal gene reactivation, and reducing profibrotic signaling.

3.3 Leginsulin Attenuates Ang II-Induced Oxidative Stress in Cardiomyocytes

Because oxidative stress is a key contributor to pathological cardiac remodeling, intracellular ROS generation, antioxidant capacity, and lipid peroxidation were evaluated in Ang II-treated H9c2 and AC16 cells (Fig. 3A-D). DCFH-DA staining showed that Ang II markedly increased intracellular ROS accumulation in both cell types, with ROS levels elevated approximately 2.1-fold and 2.5-fold in H9c2 and AC16 cells, respectively (Fig. 3A,B). Leginsulin treatment significantly reduced ROS accumulation, particularly at the high dose.

Consistent with ROS elevation, Ang II stimulation decreased T-SOD activity and increased MDA levels in both H9c2 and AC16 cells (Fig. 3C,D). Leginsulin treatment restored T-SOD activity and reduced MDA

accumulation, with high-dose leginsulin decreasing MDA levels by approximately 55.7% and 45.5% in H9c2 and AC16 cells, respectively, compared with the Ang II group. These results demonstrate that leginsulin alleviates Ang II-induced oxidative stress by reducing ROS accumulation, restoring antioxidant defense, and limiting lipid peroxidation in cardiomyocytes.

3.4 Leginsulin Ameliorates Ang II-Induced Mitochondrial Dysfunction in Cardiomyocytes

Because oxidative stress is closely associated with mitochondrial impairment, the effects of leginsulin on Ang II-induced mitochondrial dysfunction were evaluated by assessing membrane potential, ATP production, and mitochondrial morphology (Fig. 4A-D). JC-1 staining showed that Ang II markedly decreased the red/green fluorescence ratio in both H9c

2 and AC16 cells, indicating mitochondrial membrane depolarization (Fig. 4A). Leginsulin treatment significantly restored the JC-1 fluorescence ratio, with the high-dose group exhibiting a stronger effect. Ang II stimulation also markedly reduced intracellular ATP levels, whereas leginsulin treatment significantly increased ATP level. High-dose leginsulin restored ATP levels to approximately 95% and 92% of Control levels in H9c2 and AC16 cells, respectively (Fig. 4B).

MitoTracker Red staining further revealed that Ang II induced mitochondrial fragmentation and loss of network integrity, whereas leginsulin treatment preserved mitochondrial morphology and limited network disruption (Fig. 4C,D). Together, these findings demonstrate that leginsulin protects cardiomyocytes against Ang II-induced mitochondrial dysfunction by maintaining membrane potential, restoring energy production, and preserving mitochondrial structural integrity.

3.5 Leginsulin Suppresses Ang II-Induced Activation of the MAPK Signaling Pathway in Cardiomyocytes

To explore the molecular mechanism underlying the protective effects of leginsulin, MAPK pathway activation was assessed by Western blot analysis in Ang II-treated H9c2 and AC16 cells (Fig. 5A-D). Ang II stimulation markedly increased the phosphorylation levels of JNK, p38, and ERK without significantly affecting their total protein expression levels, indicating that MAPK signaling was activated primarily through phosphorylation. Leginsulin treatment significantly inhibited Ang II-induced JNK and ERK phosphorylation, with stronger effects observed at 50 µg/mL. In H9c2 cells, high-dose leginsulin reduced the Ang II-induced increases in p-JNK/JNK and p-ERK/ERK ratios from 1.28- and 1.51-fold to 0.74- and 1.18-fold, respectively. Similar inhibitory effects were observed in AC16 cells, where leginsulin treatment decreased these ratios from 1.76- and 1.29-fold to 1.04- and 1.02-fold. Although leginsulin showed a decreasing trend in p-p38/p38 levels, no significant differences were observed compared with the Ang II group. These results indicate that leginsulin selectively suppresses Ang II-induced activation of the JNK and ERK pathways, while exerting limited effects on p38 signaling, suggesting that JNK/ERK inhibition may contribute to its protective effects against pathological cardiac remodeling.

4. Discussion

This study systematically evaluated the protective effects of leginsulin against PCH and myocardial fibrosis. The results demonstrated that leginsulin significantly attenuated ISO-induced cardiac hypertrophy and myocardial fibrosis in mice, and effectively alleviated Ang II-induced cardiomyocyte hypertrophy, reactivation of the fetal gene program, and abnormal expression of profibrotic molecules. Moreover, leginsulin reduced oxidative stress, alleviated mitochondrial dysfunction, and selectively suppressed the aberrant activation of the JNK and ERK signaling pathways, indicating its multifaceted cardioprotective effects (Fig. 6).

PCH is a major pathological driver of cardiovascular disease progression toward heart failure and is characterized by cardiomyocyte hypertrophy, fetal gene reactivation, and myocardial interstitial remodeling[31, 32]. Persistent neurohumoral activation and mechanical stress induce abnormal expression of fetal genes, including ANP, BNP, and β -MHC, while promoting TGF- β 1-mediated fibroblast activation and ECM deposition, such as COL1A1 accumulation, ultimately resulting in myocardial fibrosis and cardiac dysfunction[33, 34]. Previous studies have shown that inhibition of Ang II- or pressure overload-induced cardiac hypertrophy reduces ANP, BNP, and β -MHC expression and alleviates myocardial fibrosis[35, 36]. Consistently, our study demonstrated that leginsulin suppressed hypertrophic remodeling and fetal gene reactivation. In vivo, leginsulin reduced HW and HW/BW ratios, attenuated cardiomyocyte hypertrophy, and decreased collagen deposition. In vitro, leginsulin inhibited Ang II-induced cardiomyocyte hypertrophy, downregulated ANP, BNP, and β -MHC expression, and reduced TGF- β 1 and COL1A1 protein levels. These findings indicate that leginsulin alleviates pathological cardiac remodeling by simultaneously improving hypertrophic responses and profibrotic alterations.

Increasing evidence indicates that oxidative stress and mitochondrial dysfunction are central mechanisms driving PCH progression[37, 38]. Ang II stimulation activates NADPH oxidase and disrupts mitochondrial electron transport, leading to excessive ROS accumulation. Elevated ROS induces lipid peroxidation, protein oxidation, and DNA damage, while promoting mitochondrial membrane depolarization, ATP reduction, and fragmentation, thereby forming a vicious cycle that aggravates oxidative injury and cardiac remodeling[39, 40]. Consistent with previous findings, our study showed that leginsulin reduced ROS production, increased T-SOD activity, and decreased MDA accumulation. Moreover, leginsulin restored mitochondrial membrane potential, enhanced ATP production, and improved mitochondrial network integrity. These results suggest that leginsulin maintains redox balance and mitochondrial function, thereby protecting cardiomyocytes against Ang II-induced oxidative stress injury.

Oxidative stress also acts as an upstream regulator of stress-responsive signaling pathways. Among these, the MAPK signaling pathway is a critical mediator of ROS-associated cardiac remodeling[41, 42]. Previous studies have demonstrated that excessive ROS activates MAPK cascades and enhances phosphorylation of JNK, ERK, and p38, thereby regulating cardiomyocyte stress responses and

hypertrophic remodeling[17, 43]. JNK and ERK are considered major regulators of cardiomyocyte growth, fetal gene reactivation, and hypertrophic signaling, whereas p38 MAPK is more closely associated with inflammation, stress adaptation, and cell fate regulation, though its role remains context-dependent and controversial in cardiac remodeling[44, 45]. In our study, Ang II increased phosphorylation of JNK, ERK, and p38, whereas leginsulin selectively inhibited JNK and ERK phosphorylation without significantly affecting p38 activation. These findings indicate that leginsulin does not broadly suppress MAPK signaling but preferentially regulates specific MAPK branches, with JNK and ERK potentially serving as key downstream mediators of its cardioprotective effects.

Based on these findings, we propose that leginsulin exerts cardioprotective effects through regulation of the “oxidative stress-mitochondrial function-JNK/ERK signaling axis.” Leginsulin reduces ROS accumulation and enhances antioxidant capacity, while preserving mitochondrial membrane potential, ATP production, and mitochondrial integrity, thereby disrupting the vicious cycle between oxidative stress and mitochondrial dysfunction. Consequently, ROS-mediated activation of JNK and ERK is inhibited, resulting in reduced expression of hypertrophic and fibrotic markers, including ANP, BNP, β -MHC, TGF- β 1, and COL1A1, and ultimately attenuating pathological cardiac remodeling. This mechanism integrates the histological, cellular, and molecular findings of this study and provides a comprehensive explanation for the protective effects of leginsulin.

whereas previous studies mainly focused on the metabolic regulatory effects and insulin-like activities of leginsulin, this study provides the first systematic evaluation of its protective effects against PCH and reveals its multi-level cardioprotective actions involving physiological, cellular, and molecular regulation[29, 46]. These findings expand the biological functions of leginsulin and suggest that natural bioactive proteins may protect cardiovascular health through coordinated regulation of oxidative stress, mitochondrial function, and MAPK signaling, providing experimental evidence for their potential application in cardiovascular disease intervention.

Several limitations should be acknowledged. First, although ISO-induced mouse cardiac hypertrophy and Ang II-induced cardiomyocyte models reproduce several pathological characteristics of PCH, they cannot fully reflect clinical disease complexity. Second, the proposed mechanisms were mainly based on correlative evidence. The lack of genetic or pharmacological validation, including MAPK-specific inhibitors, gene knockout, or overexpression approaches, limits definitive confirmation of the causal role of JNK/ERK signaling in leginsulin-mediated cardioprotection. Third, the direct molecular targets and upstream receptors of leginsulin remain unknown. Future studies integrating RNA sequencing, proteomics, and receptor screening approaches are needed to identify its direct targets and further clarify its signaling network.

Ang II induces oxidative stress and mitochondrial dysfunction, activating ERK/JNK signaling and promoting hypertrophic markers (ANP, BNP, and β -MHC) and fibrotic markers (COL1A1 and TGF- β), thereby contributing to cardiomyocyte hypertrophy and fibrosis. Leginsulin attenuates these effects by reducing oxidative stress, preserving mitochondrial function, and inhibiting ERK/JNK activation

Declarations

Acknowledgements

We thank all the participants for participating in our study.

Funding

This study is supported by the National Key R&D Program of China (Grant No. 2023YFC2706203) to X.Shang, and the Collaborative Grant-in-Aid of the HBUT National “111” Center for Cellular Regulation to C. Yao.

Author Contributions

Chenguang Yao: Conceptualization, Supervision, Writing - review & editing, Writing - original draft, Project administration, Funding acquisition, Formal analysis. Mengting Wu: Investigation (animal and cellular experiments), Writing - original draft, Visualization, Formal analysis, Data curation. Tianluo Shen: Investigation (animal and cellular experiments), visualization, data curation. Zi Xiong and Xuejie Qi: Investigation (cellular experiments). Essam Hady: Writing-review & editing. Mei Liu and Xiaoke Shang: Writing-review & editing, project administration, funding acquisition, formal analysis.

Competing interest

The authors report no conflicts of interest.

Ethics Approval Statement

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Hubei University of Technology and conducted in the animal facility of the School of Life Engineering and Health Engineering.

Data Availability Statement

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

References

1. Ritterhoff J, Tian R (2023) Metabolic mechanisms in physiological and pathological cardiac hypertrophy: new paradigms and challenges. *Nat Rev Cardiol* 20:812–829.
<https://doi.org/10.1038/s41569-023-00887-x>
2. Martin TG, Juarros MA, Leinwand LA (2023) Regression of cardiac hypertrophy in health and disease: mechanisms and therapeutic potential. *Nat Rev Cardiol* 20:347–363.
<https://doi.org/10.1038/s41569-022-00806-6>

3. Wen J, Liu G, Liu M, Wang H, Wan Y, Yao Z, Gao N, Sun Y, Zhu L (2023) Transforming growth factor- β and bone morphogenetic protein signaling pathways in pathological cardiac hypertrophy. *Cell Cycle* 22:2467–2484. <https://doi.org/10.1080/15384101.2023.2293595>
4. Harvey PA, Leinwand LA (2011) The cell biology of disease: cellular mechanisms of cardiomyopathy. *J Cell Biol* 194:355–365. <https://doi.org/10.1083/jcb.201101100>
5. Bozkurt B (2024) Contemporary pharmacological treatment and management of heart failure. *Nat Rev Cardiol* 21:545–555. <https://doi.org/10.1038/s41569-024-00997-0>
6. Ramachandra CJA, Cong S, Chan X, Yap EP, Yu F, Hausenloy DJ (2021) Oxidative stress in cardiac hypertrophy: From molecular mechanisms to novel therapeutic targets. *Free Radic Biol Med* 166:297–312. <https://doi.org/10.1016/j.freeradbiomed.2021.02.040>
7. Zablocki D, Sadoshima J (2013) Angiotensin II and oxidative stress in the failing heart. *Antioxid Redox Signal* 19:1095–1109. <https://doi.org/10.1089/ars.2012.4588>
8. Tappia PS, Hata T, Hozaima L, Sandhu MS, Panagia V, Dhalla NS (2001) Role of oxidative stress in catecholamine-induced changes in cardiac sarcolemmal Ca²⁺ transport. *Arch Biochem Biophys* 387:85–92. <https://doi.org/10.1006/abbi.2000.2234>
9. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M (2023) Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch Toxicol* 97:2499–2574. <https://doi.org/10.1007/s00204-023-03562-9>
10. Heusch G, Andreadou I, Bell R, Bertero E, Botker H-E, Davidson SM, Downey J, Eaton P, Ferdinandy P, Gersh BJ, Giacca M, Hausenloy DJ, Ibanez B, Krieg T, Maack C, Schulz R, Sellke F, Shah AM, Thiele H, Yellon DM, Di Lisa F (2023) Health position paper and redox perspectives on reactive oxygen species as signals and targets of cardioprotection. *Redox Biol* 67:102894. <https://doi.org/10.1016/j.redox.2023.102894>
11. Li Y, Lin R, Peng X, Wang X, Liu X, Li L, Bai R, Wen S, Ruan Y, Chang X, Tang R, Liu N (2022) The Role of Mitochondrial Quality Control in Anthracycline-Induced Cardiotoxicity: From Bench to Bedside. *Oxid Med Cell Longev* 2022:3659278. <https://doi.org/10.1155/2022/3659278>
12. Xia D, Liu Y, Wu P, Wei D (2023) Current Advances of Mitochondrial Dysfunction and Cardiovascular Disease and Promising Therapeutic Strategies. *Am J Pathol* 193:1485–1500. <https://doi.org/10.1016/j.ajpath.2023.06.013>
13. Ali MA, Gioscia-Ryan R, Yang D, Sutton NR, Tyrrell DJ (2024) Cardiovascular aging: spotlight on mitochondria. *Am J Physiol Heart Circ Physiol* 326:H317–H333. <https://doi.org/10.1152/ajpheart.00632.2023>
14. Wang Y, Ding Y, Sun P, Zhang W, Xin Q, Wang N, Niu Y, Chen Y, Luo J, Lu J, Zhou J, Xu N, Zhang Y, Xie W (2022) Empagliflozin-Enhanced Antioxidant Defense Attenuates Lipotoxicity and Protects Hepatocytes by Promoting FoxO3a- and Nrf2-Mediated Nuclear Translocation via the CAMKK2/AMPK Pathway. *Antioxidants (Basel)* 11:799. <https://doi.org/10.3390/antiox11050799>
15. Oh CM, Ryu D, Cho S, Jang Y (2020) Mitochondrial Quality Control in the Heart: New Drug Targets for Cardiovascular Disease. *Korean Circ J* 50:395–405. <https://doi.org/10.4070/kcj.2019.0416>

16. Zong Y, Li H, Liao P, Chen L, Pan Y, Zheng Y, Zhang C, Liu D, Zheng M, Gao J (2024) Mitochondrial dysfunction: mechanisms and advances in therapy. *Signal Transduct Target Ther* 9:124. <https://doi.org/10.1038/s41392-024-01839-8>
17. Muslin AJ (2008) MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. *Clin Sci (Lond)* 115:203–218. <https://doi.org/10.1042/CS20070430>
18. Molkenkin JD (2004) Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. *Cardiovasc Res* 63:467–475. <https://doi.org/10.1016/j.cardiores.2004.01.021>
19. McCubrey JA, Lahair MM, Franklin RA (2006) Reactive oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxid Redox Signal* 8:1775–1789. <https://doi.org/10.1089/ars.2006.8.1775>
20. Sag CM, Santos CXC, Shah AM (2014) Redox regulation of cardiac hypertrophy. *J Mol Cell Cardiol* 73:103–111. <https://doi.org/10.1016/j.yjmcc.2014.02.002>
21. Kerkelä R, Ulvila J, Magga J (2015) Natriuretic Peptides in the Regulation of Cardiovascular Physiology and Metabolic Events. *J Am Heart Assoc* 4:e002423. <https://doi.org/10.1161/JAHA.115.002423>
22. Takano APC, Senger N, Barreto-Chaves MLM (2020) The endocrinological component and signaling pathways associated to cardiac hypertrophy. *Mol Cell Endocrinol* 518:110972. <https://doi.org/10.1016/j.mce.2020.110972>
23. Wang X, Liu R, Liu D (2025) The Role of the MAPK Signaling Pathway in Cardiovascular Disease: Pathophysiological Mechanisms and Clinical Therapy. *Int J Mol Sci* 26:2667. <https://doi.org/10.3390/ijms26062667>
24. Zhang W, Elimban V, Nijjar MS, Gupta SK, Dhalla NS (2003) Role of mitogen-activated protein kinase in cardiac hypertrophy and heart failure. *Exp Clin Cardiol* 8:173–183
25. Romero-Becerra R, Santamans AM, Folgueira C, Sabio G (2020) p38 MAPK Pathway in the Heart: New Insights in Health and Disease. *Int J Mol Sci* 21:7412. <https://doi.org/10.3390/ijms21197412>
26. Watanabe Y, Barbashov SF, Komatsu S, Hemmings AM, Miyagi M, Tsunasawa S, Hirano H (1994) A peptide that stimulates phosphorylation of the plant insulin-binding protein. Isolation, primary structure and cDNA cloning. *Eur J Biochem* 224:167–172. <https://doi.org/10.1111/j.1432-1033.1994.tb20008.x>
27. Da Silva P, Rahioui I, Laugier C, Jouvencal L, Meudal H, Chouabe C, Delmas AF, Gressent F (2010) Molecular requirements for the insecticidal activity of the plant peptide pea albumin 1 subunit b (PA1b). *J Biol Chem* 285:32689–32694. <https://doi.org/10.1074/jbc.M110.147199>
28. Jiang H, Feng J, Du Z, Zhen H, Lin M, Jia S, Li T, Huang X, Ostenson C-G, Chen Z (2014) Oral administration of soybean peptide Vglycin normalizes fasting glucose and restores impaired pancreatic function in Type 2 diabetic Wistar rats. *J Nutr Biochem* 25:954–963. <https://doi.org/10.1016/j.jnutbio.2014.04.010>

29. Huang H, Zeng X, Zhang L, Cheng H, Hu K, Shang X, Yao C (2025) PA1b-like peptides alleviate mitochondrial dysfunction induced by glucose toxicity through interaction with VDAC1 in β -cells. *Food Funct* 16:4325–4342. <https://doi.org/10.1039/d5fo00054h>
30. Hashidume T, Sakano T, Mochizuki A, Ito K, Ito S, Kawarasaki Y, Miyoshi N (2018) Identification of soybean peptide leginsulin variants in different cultivars and their insulin-like activities. *Sci Rep* 8:16847. <https://doi.org/10.1038/s41598-018-35331-5>
31. Zhu F, Li P, Sheng Y (2022) Treatment of myocardial interstitial fibrosis in pathological myocardial hypertrophy. *Front Pharmacol* 13:1004181. <https://doi.org/10.3389/fphar.2022.1004181>
32. Caturano A, Vetrano E, Galiero R, Salvatore T, Docimo G, Epifani R, Alfano M, Sardu C, Marfella R, Rinaldi L, Sasso FC (2022) Cardiac Hypertrophy: From Pathophysiological Mechanisms to Heart Failure Development. *Rev Cardiovasc Med* 23:165. <https://doi.org/10.31083/j.rcm2305165>
33. Hong J-H, Zhang H-G (2022) Transcription Factors Involved in the Development and Prognosis of Cardiac Remodeling. *Front Pharmacol* 13:828549. <https://doi.org/10.3389/fphar.2022.828549>
34. Maruyama K, Imanaka-Yoshida K (2022) The Pathogenesis of Cardiac Fibrosis: A Review of Recent Progress. *Int J Mol Sci* 23:2617. <https://doi.org/10.3390/ijms23052617>
35. Zhen C, Wu X, Zhang J, Liu D, Li G, Yan Y, He X, Miao J, Song H, Yan Y, Zhang Y (2023) Ganoderma lucidum polysaccharides attenuates pressure-overload-induced pathological cardiac hypertrophy. *Front Pharmacol* 14:1127123. <https://doi.org/10.3389/fphar.2023.1127123>
36. Li R, Shan Y, Gao L, Wang X, Wang X, Wang F (2019) The Glp-1 Analog Liraglutide Protects Against Angiotensin II and Pressure Overload-Induced Cardiac Hypertrophy via PI3K/Akt1 and AMPKa Signaling. *Front Pharmacol* 10:537. <https://doi.org/10.3389/fphar.2019.00537>
37. Yang D, Liu H-Q, Liu F-Y, Guo Z, An P, Wang M-Y, Yang Z, Fan D, Tang Q-Z (2021) Mitochondria in Pathological Cardiac Hypertrophy Research and Therapy. *Front Cardiovasc Med* 8:822969. <https://doi.org/10.3389/fcvm.2021.822969>
38. Martins D, Garcia LR, Queiroz DAR, Lazzarin T, Tonon CR, Balin P da S, Polegato BF, de Paiva SAR, Azevedo PS, Minicucci MF, Zornoff L (2022) Oxidative Stress as a Therapeutic Target of Cardiac Remodeling. *Antioxidants (Basel)* 11:2371. <https://doi.org/10.3390/antiox11122371>
39. Bhullar SK, Dhalla NS (2022) Angiotensin II-Induced Signal Transduction Mechanisms for Cardiac Hypertrophy. *Cells* 11:3336. <https://doi.org/10.3390/cells11213336>
40. Yang J, Guo Q, Feng X, Liu Y, Zhou Y (2022) Mitochondrial Dysfunction in Cardiovascular Diseases: Potential Targets for Treatment. *Front Cell Dev Biol* 10:841523. <https://doi.org/10.3389/fcell.2022.841523>
41. Soga M, Matsuzawa A, Ichijo H (2012) Oxidative Stress-Induced Diseases via the ASK1 Signaling Pathway. *Int J Cell Biol* 2012:439587. <https://doi.org/10.1155/2012/439587>
42. Meijles DN, Cull JJ, Markou T, Cooper STE, Haines ZHR, Fuller SJ, O’Gara P, Sheppard MN, Harding SE, Sugden PH, Clerk A (2020) Redox Regulation of Cardiac ASK1 (Apoptosis Signal-Regulating Kinase 1) Controls p38-MAPK (Mitogen-Activated Protein Kinase) and Orchestrates Cardiac

Remodeling to Hypertension. Hypertension 76:1208–1218.

<https://doi.org/10.1161/HYPERTENSIONAHA.119.14556>

43. Asrih M, Mach F, Nencioni A, Dallegri F, Quercioli A, Montecucco F (2013) Role of mitogen-activated protein kinase pathways in multifactorial adverse cardiac remodeling associated with metabolic syndrome. *Mediators Inflamm* 2013:367245. <https://doi.org/10.1155/2013/367245>
44. Liang Y, Sheikh F (2016) Scaffold Proteins Regulating Extracellular Regulated Kinase Function in Cardiac Hypertrophy and Disease. *Front Pharmacol* 7:37. <https://doi.org/10.3389/fphar.2016.00037>
45. Liang Q, Molkentin JD (2003) Redefining the roles of p38 and JNK signaling in cardiac hypertrophy: dichotomy between cultured myocytes and animal models. *J Mol Cell Cardiol* 35:1385–1394. <https://doi.org/10.1016/j.yjmcc.2003.10.001>
46. Hu K, Huang H, Li H, Wei Y, Yao C (2023) Legume-Derived Bioactive Peptides in Type 2 Diabetes: Opportunities and Challenges. *Nutrients* 15:1096. <https://doi.org/10.3390/nu15051096>

Figures

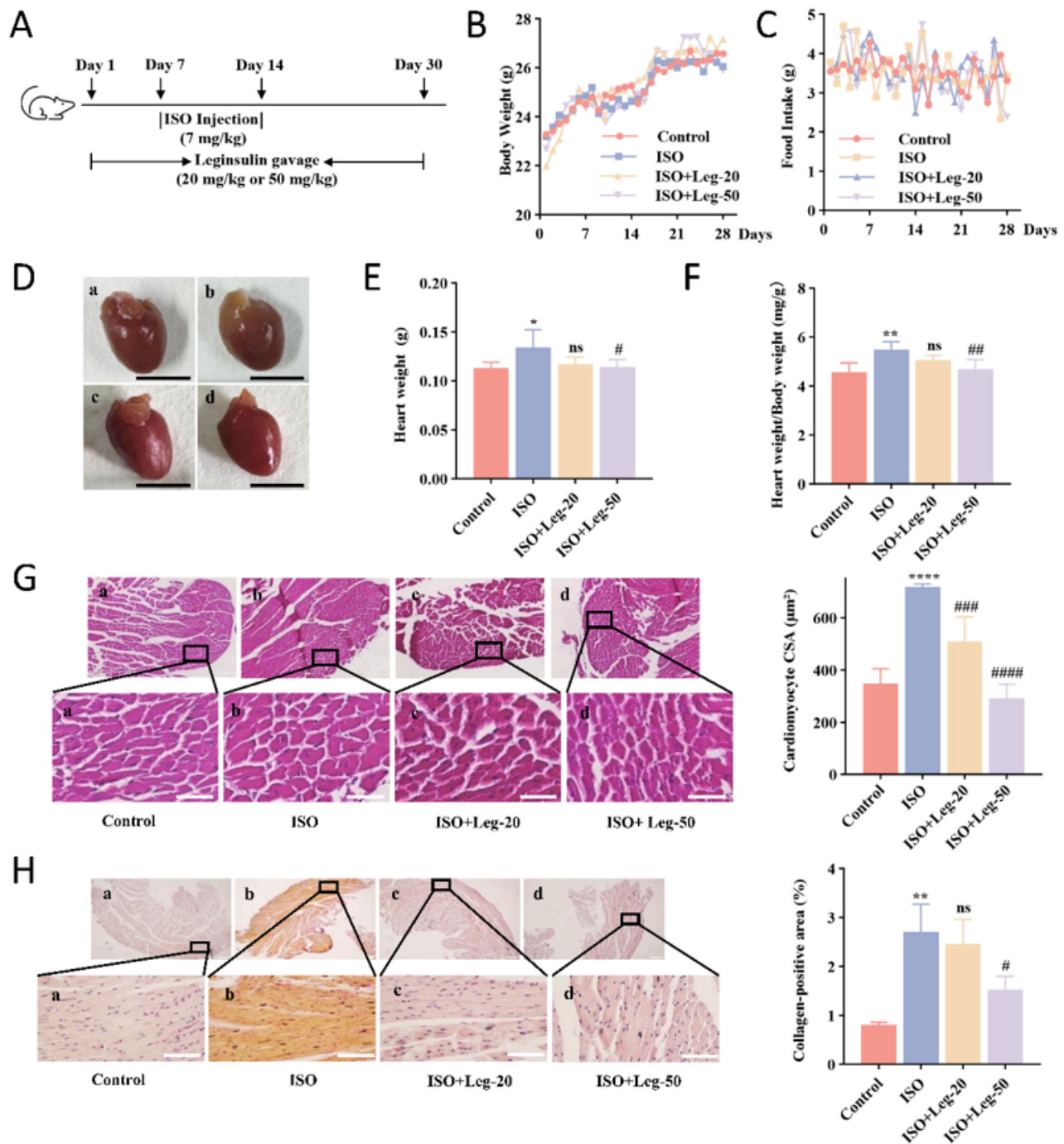


Figure 1

Leginsulin attenuates isoproterenol-induced pathological cardiac remodeling in mice

(A) Experimental design. (B) Body weight changes during the experimental period. (C) Food intake. (D) Representative gross morphology of hearts. Scale bar = 0.5 cm. (E) Heart weight (HW). (F) Heart weight-to-body weight ratio (HW/BW). (G) Representative hematoxylin and eosin (H&E) staining of left ventricular sections and quantitative analysis of cardiomyocyte cross-sectional area (CSA). Scale bar = 100 μ m. (H) Representative Picrosirius Red (PSR) staining of left ventricular sections and quantitative analysis of collagen-positive area. Scale bar = 100 μ m. Data are presented as mean $\pm$ SD (n = 5).

Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus Control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, and #### $P < 0.0001$ versus ISO; ns, not significant

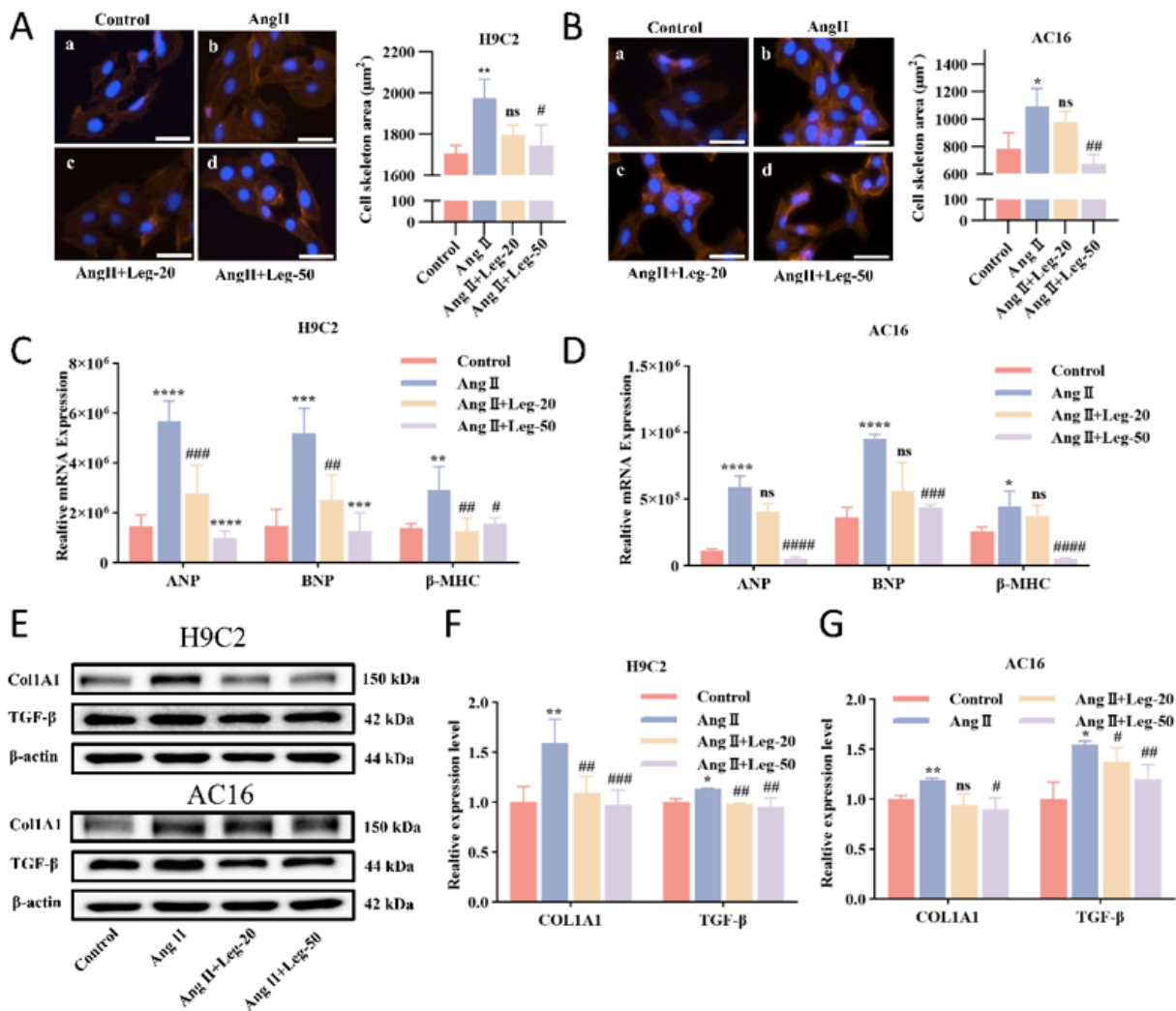


Figure 2

Leginsulin attenuates Ang II-induced cardiomyocyte hypertrophy and profibrotic responses

(A, B) Representative phalloidin staining images and quantitative analysis of cell area in H9c2 and AC16 cells. Scale bar = 100 μm. (C, D) Relative mRNA expression of ANP, BNP, and β-MHC. (E) Representative Western blots of COL1A1 and TGF-β1. (F, G) Quantitative analysis of COL1A1 and TGF-β1 protein expression. Data are presented as mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus Control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, and #### $P < 0.0001$ versus Ang II; ns, not significant

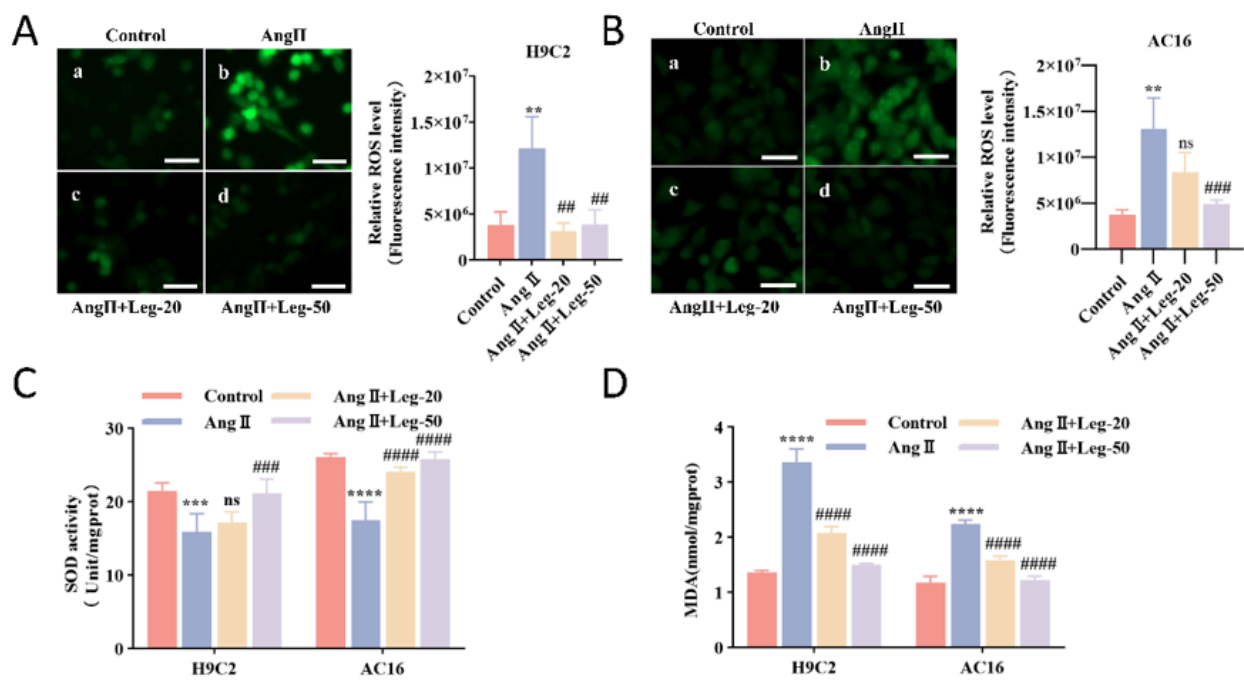


Figure 3

Leginsulin attenuates Ang II-induced oxidative stress in cardiomyocytes (A, B) Representative intracellular ROS staining and quantitative analysis of fluorescence intensity in H9c2 and AC16 cells. Scale bar = 100 μ m. (C) T-SOD activity. (D) MDA content. Data are presented as mean $\pm$ SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 versus Control; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 versus Ang II; ns, not significant

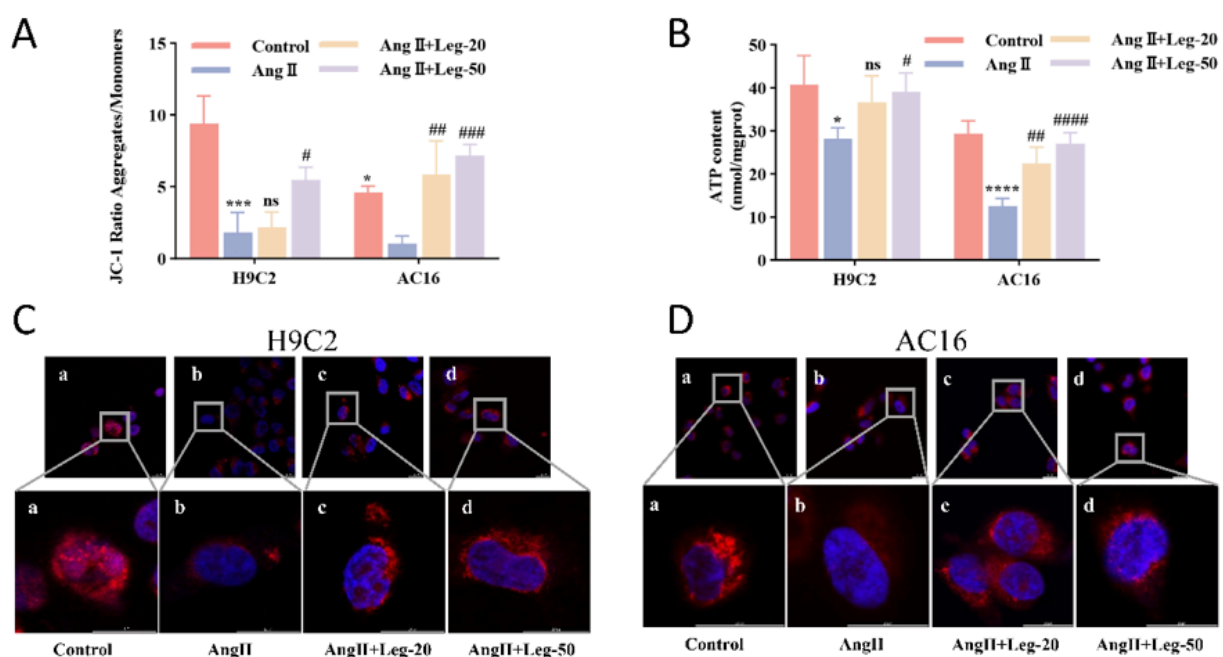


Figure 4

Leginsulin ameliorates Ang II-induced mitochondrial dysfunction in cardiomyocytes

(A) Quantitative analysis of the JC-1 red/green fluorescence intensity ratio in H9c2 and AC16 cells. (B) Intracellular ATP levels. (C, D) Representative MitoTracker Red staining and quantitative analysis of mitochondrial morphology in H9c2 and AC16 cells. Scale bar = 20 μ m. Data are presented as mean $\pm$ SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 versus Control; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 versus Ang II; ns, not significant

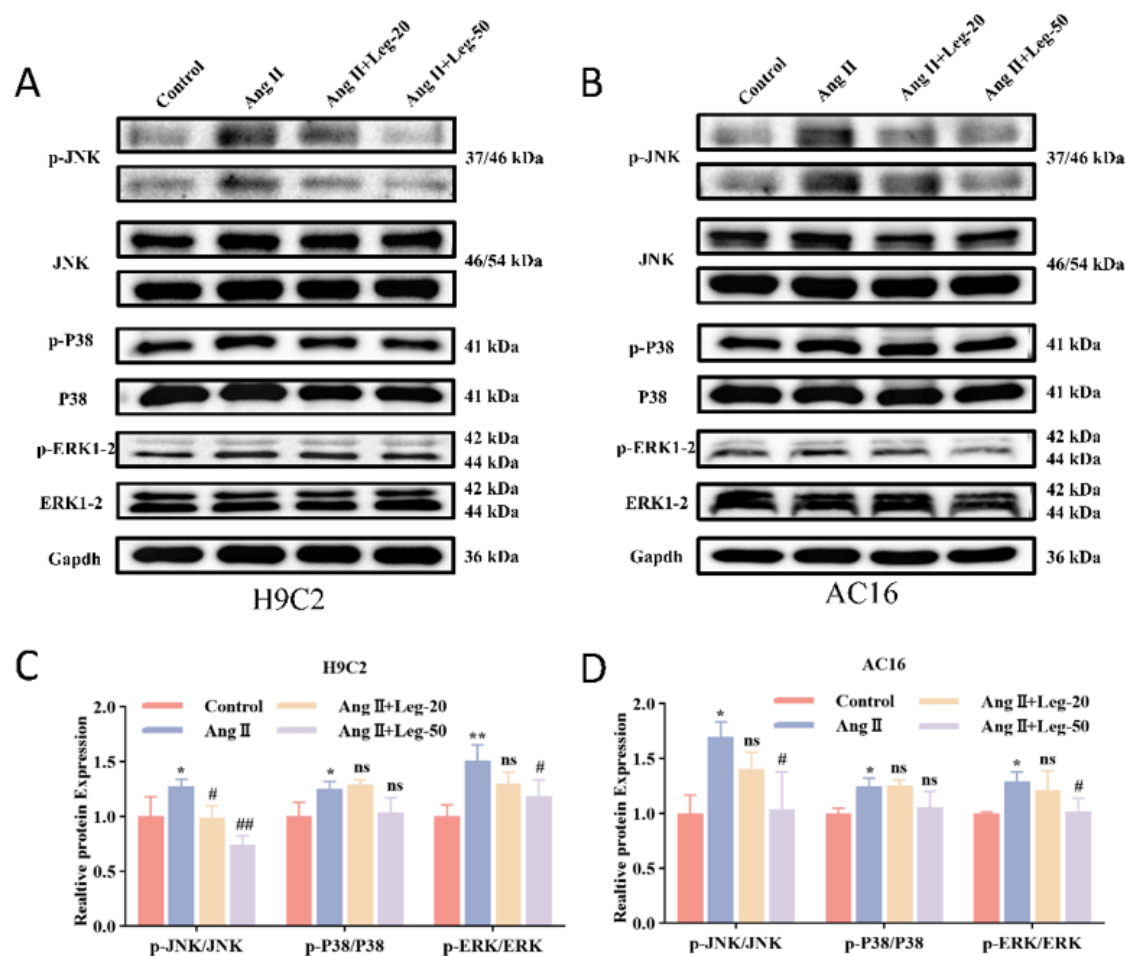


Figure 5

Leginsulin suppresses Ang II-induced MAPK pathway activation in cardiomyocytes

(A, B) Representative Western blots of total and phosphorylated JNK, p38, and ERK1/2 in H9c2 and AC16 cells. (C, D) Quantitative analysis of p-JNK/JNK, p-p38/p38, and p-ERK1/2/ERK1/2 ratios. Data are presented as mean $\pm$ SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by

Tukey's multiple comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 versus Control; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 versus Ang II; ns, not significant

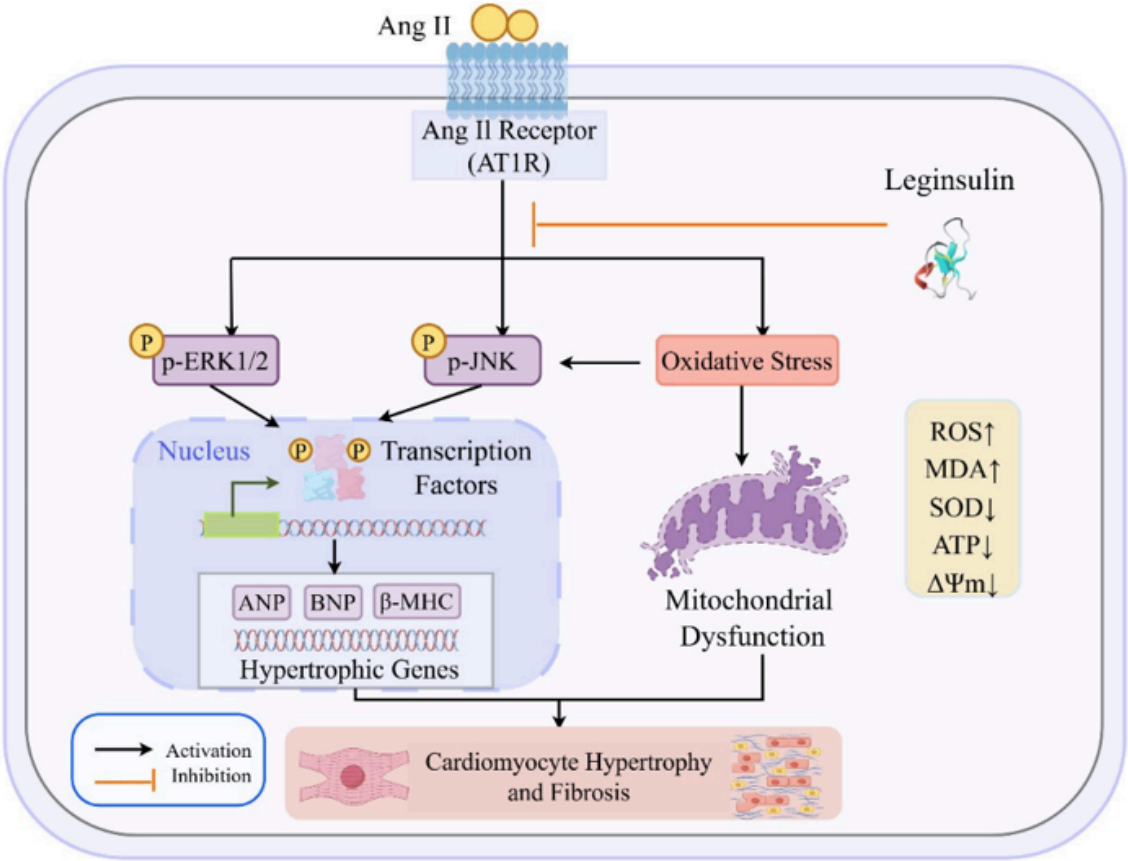


Figure 6

Proposed mechanism of Leginsulin against cardiac hypertrophy and fibrosis