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Formononetin improves heart failure with preserved ejection fraction in mice by activating the PPARα/PGC-1 pathway

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Abstract

Background: Formononetin (FMN) has beneficial effects in cardiovascular diseases but its functions and mechanisms in heart failure with preserved ejection fraction (HFpEF) remain unclear. This study aimed at determining whether FMN ameliorated HFpEF-induced cardiac dysfunction and exploring its underlying mechanisms. **Methods:** The mouse model of HFpEF was established through uninephrectomy surgery and d-aldosterone infusion in C57BL/6 mice. Cardiac remodeling and potential mechanisms of FMN in HFpEF were assessed by histological analysis, immunofluorescence, echocardiography, real-time PCR and western blotting sequentially. **Results:** FMN prevented myocardial dysfunction, fibrosis and cardiomyocyte apoptosis. The mRNA levels of left ventricular hypertrophy markers were increased in HFpEF mice but they remained unchanged in FMN-treated mice. In addition, the expression levels of PPARα and PGC-1 were increased in HFpEF mice for FMN treatment. The PPARα-PGC-1 complex affected the expression of fatty acid content and encoded enzymes in glucose metabolism. Both the hypertrophy and metabolic impairment due to FMN in HFpEF mice were alleviated after the addition of PPARα antagonist GW6471. **Conclusion:** In conclusion, FMN could prevent the cardiac hypertrophy in HFpEF mice by activating the PPARα/PGC-1 pathway and regulating energy metabolism, which provides a new therapeutic strategy for HFpEF patients.

Keywords: formononetin; heart failure with preserved ejection fraction; PPARα; PPARα/PGC-1 pathway; energy metabolism

Author contributions

Xu H and Zhang XQ contributed to the conceptualization, data curation, investigation, visualization, and writing of the original draft. Li F, Tian YY, Huang LM and Guo C contributed to data collection, statistical analysis, and reviewing of the original draft. Ma J, Ding Y, and Liu C contributed to conceptualization, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; Fen, fenofibrate; FMN, formononetin; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; LVH, left ventricular hypertrophy; HW/BW, heart weight to body weight; HW/TL, heart weight to tibia length; SSA, superstreptavidin; PPARα, Peroxisome proliferator-activated receptorα; qRT-PCR, quantitative real-time PCR; SD, standard deviation; KD, equilibrium dissociation constant.

Citation

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Highlights

FMN exhibited a preventive effect on myocardial dysfunction, fibrosis, and cardiomyocyte apoptosis. Additionally, FMN administration resulted in an upregulation of mRNA levels of Pdha1, Lcad, Cpt1a, and Cpt1b, while downregulating the mRNA level of Pfk1. These findings suggest that FMN may mitigate cardiac hypertrophy through the activation of the PPARα/PGC-1 pathway.

Medical history of objective

Formononetin is an isoflavone compound extracted from plants. This compound belongs to the phytoestrogens group. Formononetin was first reported in the literature in 1855 C.E. (Hlasiwetz H, Ueber die Wurzel der Ononis spinosa). It has been proved that the pharmacological benefits of FMN included anti-inflammation, anti-oxidation, and obesity prevention, which made it a potential therapeutic agent against hypertension, arteriosclerosis, and myocardial ischemia (Formononetin: a review of its anticancer potentials and mechanisms. *Frontiers in pharmacology* 2019).

Background

Heart failure with preserved ejection fraction (HFpEF) is a disease affecting over half of the patients with heart failure (HF) [1]. Diastolic dysfunction is a hallmark of HFpEF with a reduced filling function. Current interventions include such pharmacological therapies as beta-blockers and such device therapies as cardiac resynchronization therapy [2]. Although these treatments and technologies have reduced morbidity and mortality in some patients with HFpEF, the results are not satisfactory [3]. Therefore, it is of great importance to study the targets and the potential mechanisms of HFpEF for its effective prevention and treatment.

The normal adult mammalian heart obtains its required energy mainly through the fatty acid β-oxidation pathway [4]. In HF, an increase in glycolytic enzymes, lactic acid accumulation, and a marked decrease in ATP production is observed [5]. Therefore, it is important to maintain a balance between fatty acid and glucose metabolic flexibility and cardiac substrate utilization to increase energy ATP production capacity and improve cardiac function. Peroxisome proliferator-activated receptorα (PPARα) is the most important subtype of the PPARs family belonging to the type II nuclear receptor superfamily [5]. PPARα has been reported to promote the expression of fatty acid β-oxidation-related genes and accelerate fatty acid β-oxidation [6]. PPARα is a key regulatory protein in regulating lipid metabolism [7]. The expression of PPARα was reduced in hypertrophy hearts, and PPARα downstream genes was reversed by PPARα agonists [8]. PPARα-deficient mice exhibited a phenotype of heart failure and impaired fatty acid metabolism, suggesting PPARα may have a critical role in fatty acid/glucose metabolism [9]. PGC-1 is a PPARα cofactor-1, which is widely distributed in such tissues as the heart with high oxidative capacity. Expression of ATP synthesis, oxidative phosphorylation, and fatty acid oxidation can be regulated by PGC-1 [10]. PGC-1 exerts its biological effects by directly binding to the nuclear receptor PPARα and enhancing its transcriptional activity [11].

Traditional Chinese medicines are highly preferred in the prevention and treatment of cardiovascular diseases because of their low toxicity and multi-target effects [12–14]. Formononetin (FMN) is a natural compound derived from Astragalus membranaceus and belongs to the class of isoflavones [15, 16]. It has been proved that the pharmacological benefits of FMN included anti-inflammation, anti-oxidation, and obesity prevention, which made it a potential therapeutic agent against hypertension, arteriosclerosis, and myocardial ischemia [17–19]. However, the functions of FMN in HFpEF and whether FMN could upregulate PPARα/PGC-1 expression in HFpEF are still unknown. As PPARα/PGC-1 pathway can regulate myocardial lipid regulation and glucose metabolism, we hypothesized that PPARα/PGC-1 pathway could be a treatment target for HFpEF. The purpose of this study is determining the effects and mechanisms of FMN against the HFpEF-induced cardiac dysfunction.

Materials & methods

Reagents

FMN, GW6471, Fenofibrate (Fen), and D-aldosterone were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Servicebio®RT First Strand cDNA Synthesis kit, and 2 × SYBR Green qPCR Master Mix (None ROX) were purchased from Wuhan Servicebio Technology Co., Ltd. (Wuhan, China).

Animals

Male C57BL/6 mice (8–12 weeks) were purchased from the Experimental Animal Center of the Fourth Military Medical University. The animal experiment was performed with the formal approval of the Medical Ethics Committee of the First Affiliated Hospital of the Fourth Military Medical University (KY20215015) at the data of 15–05–2021. The animal experiment was performed in line with the Guidelines for the Management and Use of Laboratory Animals by the Chinese National Institutes of Health.

All mice were housed at 23.0 ± 2 °C with a 12 h light-dark cycle. During the experiments, food and water were provided ad libitum. Mice were anesthetized with 1.5% isoflurane (Shenzhen Rayward Life Technology Co., Ltd., Shenzhen, China), intubated, and ventilated using a rodent ventilator (Taimeng Co., Ltd., Chengdu, China). Criteria established for euthanizing mice were performed according to ARRIVE 2.0 guidelines rigorously. Finally, mice from control and model groups were mercifully killed by excess CO_2.

FMN Treatment of HFpEF in mice

The 75 mice were divided into five groups randomly: sham, model, model + FMN (5 mg/kg), model + FMN (15 mg/kg), and model + FMN (50 mg/kg), with 5 mice in each group in different experiments. Non-nephrectomy was administered for mice in model group and model + FMN group with continuous infusion of d-aldosterone (0.15 mg/h) for 4 weeks successively [20]. After 24 h postoperatively, each mouse was orally administered FMN for 2 weeks. Following the completion of the treatment period, the mice were euthanized, and their heart tissues were extracted for further analysis. The cardiac index was determined by calculating the ratio of heart weight to body weight (HW/BW) and heart weight to tibia length (HW/TL) using the respective formulas. No mice met the criteria for exclusion with an ejection fraction (EF) lower than 45%.

To further clarify the therapeutic effects of FMN on HFpEF, 25 mice were divided into five groups: sham, model, model + FMN (25 mg/kg), model + Fen (PPAR-α agonists), and model + FMN + GW6471, with 5 mice in each group. The mice in model + Fen group were treated with oral gavage with Fen (80 mg/kg) each day for 2 weeks. The mice in the model + FMN + GW6471 group were subjected to intraperitoneal injection of GW6471 (3.5 mg/kg) each day for 2 weeks. Then the hearts of the mice were harvested to calculate HW/BW and HW/TL.

Echocardiography

Cardiac function of mice was measured using echocardiography (VisualSonics Vevo 2100 imaging system, Ontario, Canada), its continuous ECG detection system was used to assess cardiac rhythm in each state of the mouse, and tissue Doppler E'/A' ratios were used to assess cardiac diastolic function [21].

HE staining and Masson staining

The hearts of mice were taken out and perfused with 10% potassium chloride immediately to arrest diastole function. Then hearts were soaked in 10% formalin and embedded in paraffin, with the slides

dyed with hematoxylin and eosin. To assess the degree of myocardial fibrosis in heart failure, the paraffin-embedded hearts were sliced into 5 μm sections and stained with Masson's trichrome. Then the image was observed and captured by microscope.

Molecular docking

Auto Dock 4.2.6 software was used for molecular docking of FMN to PPARα proteins. The PubChem database was applied to obtain 3D structure of FMN. Before docking, a grid box (60 × 60 × 60 Å3) was built to cover the entire bonding pocket. Then the docking file was converted to PDBQT format by the ADFR 1.0 program. For docking, the rigid setting was PPARα protein and the flexible setting was FMN ligand, and the optimal binding pose for FMN was obtained using the Lamarckian genetic algorithm. After the docking was repeated three times, the lowest scoring pose was selected from the docking results. Then it was visualized and analyzed with PyMol-open-source 2.6 and the academic version of Maestro.

Bio-layer interferometry (BLI)

The binding affinity of FMN to PPARα was determined through the Fortebio Octet system (ForteBio). Prior to the assay, the superstreptavidin (SSA) biosensor was immersed in Tween 20 (Thermo Fisher Scientific Inc., Waltham, MA, USA) (0.1 mol/L phosphate buffer, ph 7.4, 4 ˚C) for 10 min. PPARα protein was incubated with the SSA sensor for 15 min to detect the adsorption and desorption process of FMN and vector. In the subsequent 6 min, the SSA sensor was transferred to FMN solution and serially diluted 5-fold to a concentration of 1,000 μM. Data were analyzed using software 11.0 (ForteBio), and Graph Pad Prism 8 software was used to create binding curve plots.

Immunofluorescence

Sections with 10 μm thickness were incubated with anti-PPARα. Then they were incubated with FITC-conjugated anti-rabbit and Texas Res-conjugated anti-mouse whole IgG. Finally, the stained images were observed and photographed with a microscope.

Western blotting

Cold lysis buffer solution was used to homogenize heart tissues and then the tissues were separated by SDS-PAGE and polyvinylidene difluoride (PVDF) membranes with 5% (w/v) milk blocking. Membranes were incubated overnight with the primary antibody at 4 ˚C and incubated with secondary antibody after washed. Immunoreactive bands were analyzed with a chemiluminescence system (Amersham Bioscience Co., Ltd., Buckinghamshire, UK) and the results were quantified with ImageJ software.

Quantitative real-time PCR (qRT-PCR)

After total RNA was extracted from the frozen tissues, it was reversely transcribed into cDNA. Specific RNA extraction was conducted using RNAiso Plus reagent (Takara Bio Inc., Beijing, China) and cDNA reverse transcription was performed using PrimeScript RT kit with gDNA Eraser (Takara Bio Inc., Beijing, China). Real-time PCR was performed using the ABI Prism 7500 system (Applied Biosystems, Foster City, CA, USA). The real-time qRT-PCR primers for this study are shown in Table 1.

Statistical analysis

All quantitative data gathered in this study were meticulously analyzed using SPSS 22.0 software. The results were subsequently reported as mean values accompanied by their corresponding standard deviation (SD). To assess the statistical significance, a one-way ANOVA analysis was conducted. In accordance with standard practices, a significance level of $P < 0.05$ was employed as the threshold to ascertain statistical significance.

Results

FMN improved the diastolic function in HFpEF mice

HE staining of left ventricular hypertrophy (LVH) showed that the cardiomyocyte of mice in model group was remarkably bigger than that in sham group. The ratio of HW/BW and HW/TL in model group was significantly increased and FMN treatment notably decreased HW/BW and HW/TL in a dose-response manner (Figure 1A). Compared with the mice in sham model group, mRNA expression of the mice in model group showed a remarkable increase of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) mRNA, which were markers of left ventricular hypertrophy LVH. The mRNAs in the mice treated with FMN were significantly reduced compared with model group (Figure 1B). Compared to the mice in the sham group, the model group mice showed increased myocardial fibrosis, as indicated by Masson trichrome staining, which was consistent with HE staining. FMN treatment alleviated the myocardial interstitial fibrosis of the mice with HFpEF (Figure 1C).

FMN upregulated PPARα and PGC-1 in HFpEF mice

The fluorescence intensity of PPARα was decreased in model group but it was increased significantly after FMN treatment (Figure 2A). The expressions of PPARα and PGC-1 at both mRNA and protein levels were affected by FMN. Real-time PCR and western blot analysis showed a remarkable decrease of protein levels of PPARα in model group (Figure 2B). Moreover, FMN treatment significantly increased the protein level of PPARα in heart tissues. Compared with model group, PPARα and PGC-1 gene expression levels were significantly upregulated after FMN treatment (Figure 2C, 2D).

FMN regulated the expression of metabolic genes in HFpEF mice

Compared to sham model group, it was observed that the Cpt1a, Cpt1b, Lcad, and Pdha1 mRNA levels declined while Pfk1 increased in model group. However, there is a significant increase of Cpt1a, Cpt1b, Lcad, and Pdha1 mRNA levels after FMN treatment while the mRNA level of Pfk1 was significantly decreased (Figure 2E).

Table 1 Sequences of primers in qRT-PCR analysis

Gene	Forward Primer	Reverse Primer
ANP	CTTCTTCCTCGTCTTGGCCTTT	TCCAGGTGGTCTAGCAGGTTCT
BNP	CTTTATCTGTCACCGCTGGGAG	ACAACTTCAGTGCGTTACAGCC
PPARα	CACTACGGAGTTCACGCATGT	GTGACATCCCGACAGACAGGC
PCG-1α	CTGGGTGGATTGAAGTGGTGTA	AGTGGTCACGGCTCCATCTGT
PCG-1β	CCAGGTGCTGACGAGAAGTAAA	TGTATCTGGGCCAACGGAAGT
Cpt1α	GCACGAGGAAAAAATAAGCAATCT	TTGTCAAACCACCTGTCGAAAC
Cpt1β	GTACTTTCTGAACCCTGGAGCCT	TGAGTATTTGCTGGAGATGTGGAA
Lcad	TTGCTTGGCATCAACATCGC	ATACACTTGCCCGCCGTCAT
Pdha1	ACCAGAGAGGATGGGCTCAAGT	AGGTGGTCCGTAGGGTTTATGC
Pfk1	TGTTCGCTCTACCGTGAGGATT	AAACTGCTTCCTGCCTTCCATC

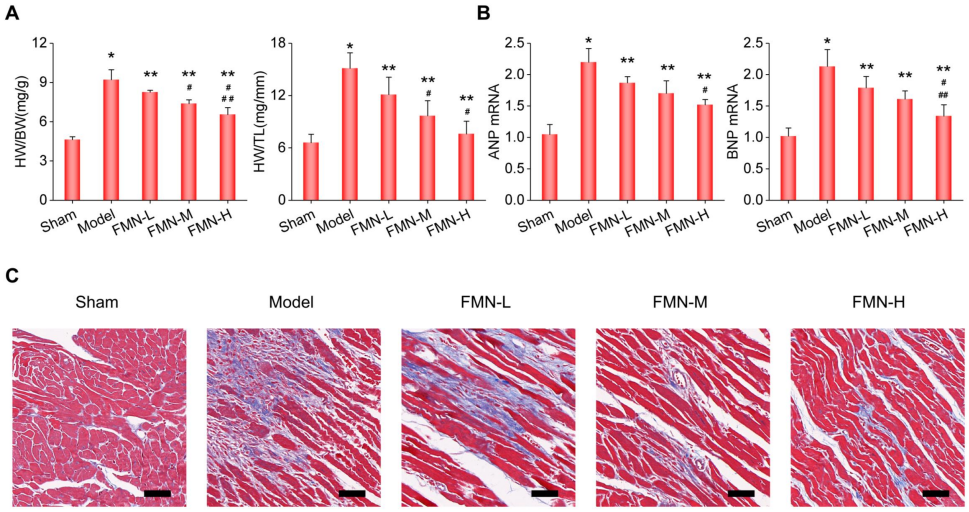

Figure 1 Effect of FMN on HFpEF phenotype. (A) Quantitation of the ratio of HW/BW and HW/TL. (B) qRT-PCR analysis of ANP and BNP expression. (C) Representative images of Masson's trichrome staining for myocardial interstitial fibrosis. Scale bars: 50 μm (C). Data are mean ± SD (A-B, n = 5). Statistical significance was assessed by one-way ANOVA. $^{*}P < 0.05$ vs. Sham group, $^{**}P < 0.05$ vs. Model group, $^{\#}P < 0.05$ vs. FMN-L group, $^{\#\#}P < 0.05$ vs. FMN-M group. FMN, formononetin.

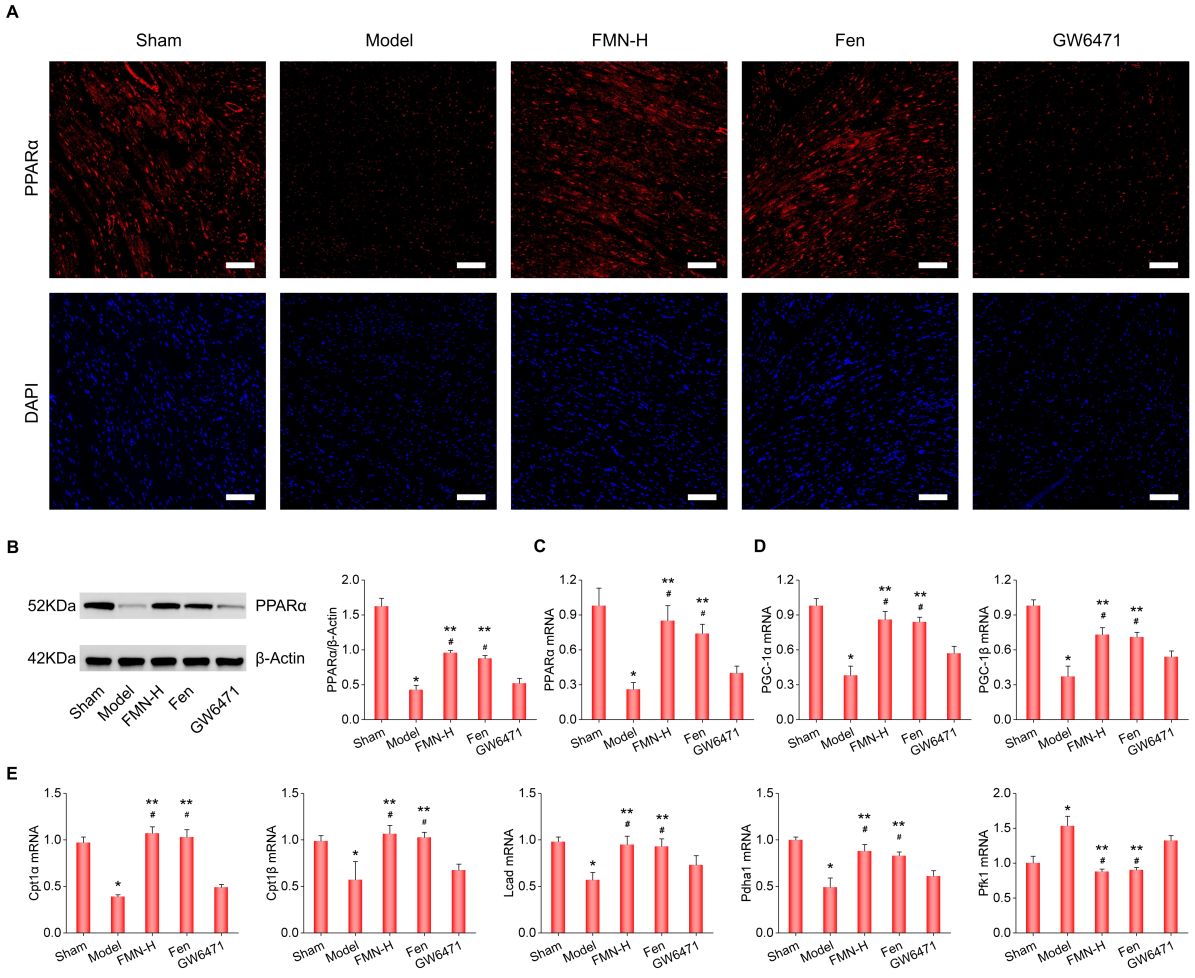

Figure 2 FMN modulated PPARα and PPARα-targeted genetic expression in HFpEF. (A) Representative PPAR immunofluorescence staining. (B) Western blotting analysis of PPARα expression. (C) qRT-PCR analysis of PPARα gene expression. (D) qRT-PCR analysis of PGC-1α and PGC-1β gene expression. (E) qRT-PCR analysis of the expression of PPARα-targeted genes (Cpt1a, Acacb, Cpt1b, Lcad, Pdha1, and Pfk1). Cpt1a, carnitine palmitoyltransferase 1A; Cpt1b, carnitine palmitoyltransferase 1B; Lcad, long-chain acyl-CoA dehydrogenase; Pdha1, pyruvate dehydrogenase E1 component subunit alpha; Pfk1, phosphofructokinase 1. Scale bars: 100 μm (A). Data are mean ± SD (B–E, n = 5). Statistical significance was assessed by one-way ANOVA. $^{*}P < 0.05$ vs. Sham group, $^{**}P < 0.05$ vs. Model group, $^{\#}P < 0.05$ vs. GW6471 group. FMN, formononetin; Fen, fenofibrate; PPARα, peroxisome proliferator-activated receptorα.

FMN inhibited the apoptotic protein expression in HFpEF mice

Bax, cleaved caspase-3, and cleaved PARP are pro-apoptosis-related proteins, while Bcl-2 is anti-apoptosis-related protein. To further verify the beneficial role of FMN on HFpEF-induced cardiomyocyte apoptosis, we treated mice with FMN. Western blotting analysis showed that FMN alleviated cardiomyocyte apoptosis. The expressions of Bax, cleaved caspase3, and cleaved PARP were increased while Bcl-2 was decreased in the hearts of HFpEF mice. However, FMN decreased the expressions of Bax, cleaved caspase3, and cleaved PARP but increased the expression of Bcl2 in the hearts (Figure 3A, 3B).

FMN prevented cardiac dysfunction by activating PPARα/PGC-1 pathway in HFpEF mice

Echocardiographical detection showed that GW6471 treatment abolished the prevention of FMN in HFpEF mice (Figure 4A). GW6471 treatment aggravated the pathological hypertrophy phenotypes, such as those mediated by non-nephrectomy surgery and d-aldosterone infusion, including the increased cell size (Figure 4B, 4C) and the expression of hypertrophic genes (Figure 4D). Besides, Masson staining analysis showed that the anti-fibrotic effects of FMN on the hearts of HFpEF mice were blunted by PPARα inhibitor (Figure 4E). GW6471 treatment decreased the expressions of PPARα and PGC-1 (Figure 2B–2D). Similarly, the expression of downstream targets of PPARα also demonstrated the corresponding changes (Figure 2E). Fen, as a positive control agent, showed similar efficacy to FMN in reversing hypertrophy indexes and improving PPARα/PGC-1 pathway.

Detection of the structural interaction between FMN and PPARα in HFpEF mice

The optimal docking mode and configuration of FMN to PPARα is shown in Figure 5A. Molecular docking simulations found that FMN effectively interacted with PPARα and demonstrated the hydrophobic effects in the ligand-binding domain of PPARα (Figure 5B). In addition, the calculated results of in vitro binding affinity of FMN to PPARα showed that FMN could bind to PPARα directly and its affinity was proportional to FMN concentration (Figure 5C). The binding kinetics of the experiment were assessed, and several parameters were reported, including the equilibrium dissociation constant (KD), association rate constant (Kon), dissociation rate constant (Kdis), and R2 values. The KD value can be calculated by dividing the Kdis value by the Kon value. This calculation allows for the determination of the strength of the binding interaction between the molecules under investigation. The value of dissociation constant KD was 6.24×10^{-5} M.

Discussion

FMN significantly reduced infarct volume and improved cardiac function in rats with ischemic reperfusion injury [18]. FMN also demonstrated good effects in preventing and treating atherosclerosis for its low side effects and high efficacy [22].Our study found that FMN ameliorated non-nephrectomy and d-aldosterone infusion-induced cardiac dysfunction in HFpEF mice. However, the cardioprotective effect of FMN on HFpEF and its underlying mechanisms have not been investigated. Therefore, our study focused on the cardioprotective effects of FMN on the mice with HFpEF and its potential mechanisms.

PPAR, as a nuclear receptor, is able to regulate the body's systemic metabolism, such as controlling fatty acid uptake, β-oxidation, and fatty acid sensors and is activated by natural ligands, including saturated, monounsaturated and polyunsaturated fatty acids and their metabolites [23]. PPARs are specifically expressed in the heart and mainly regulate lipid and glucose metabolism [24]. Although the heart consumes mainly fatty acids under normal conditions, it still shows flexibility in fuel utilization and generates energy using different carbon substrates. PPARs are switches that regulate the enzymatic mechanisms of cardiomyocytes [25]. Previous reports have shown a remarkable decrease of mRNA levels of PPARα and PGC-1α in an ovariectomized rat model of stress-induced cardiac hypertrophy compared with the rats in sham-operated group [26, 27].

Similarly, our study found that there was a decreased mRNA expression of PPARα, PGC-1α and PGC-1β of the mice in model group compared with sham group. Some studies have shown that the increased fatty acid oxidation ameliorated cardiac dysfunction induced by stress overload in heart-specific acetyl-CoA carboxylase 2 (ACC2) -deficient mice [28]. Activating PPARα could induce the expression of genes related to fatty acid pathway in myocardium [29]. Our finding that the expression of genes involved in fatty acid β-oxidation (Cpt1a, Cpt1b, Lcad, and Pdha1) was remarkably decreased in the hearts of the mice in model groupindicates that PPARα/PGC-1 pathway is deficient in HFpEF. Mitochondria are the powerhouse of cells and the site of energy generation [30, 31]. It is reported that mitochondrial function was associated with cardiovascular diseases [32]. Several studies have found that FMN attenuated ox-LDL-mediated inflammatory responses in HUVECs through activation of PPAR-γ [33].

Table 2 Echocardiography parameters of each group

Parameter	Sham	Model	FMN-H	Fen	GW6471
LVEF (%)	73.7 ± 4.2	69.5 ± 5.4	72.1 ± 4.6	71.8 ± 5.1	70.7 ± 3.9
LVFS (%)	39.4 ± 3.3	37.4 ± 4.6	38.8 ± 4.1	38.1 ± 3.2	37.9 ± 3.7

Data are presented as mean ± SD. LVEF, Left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

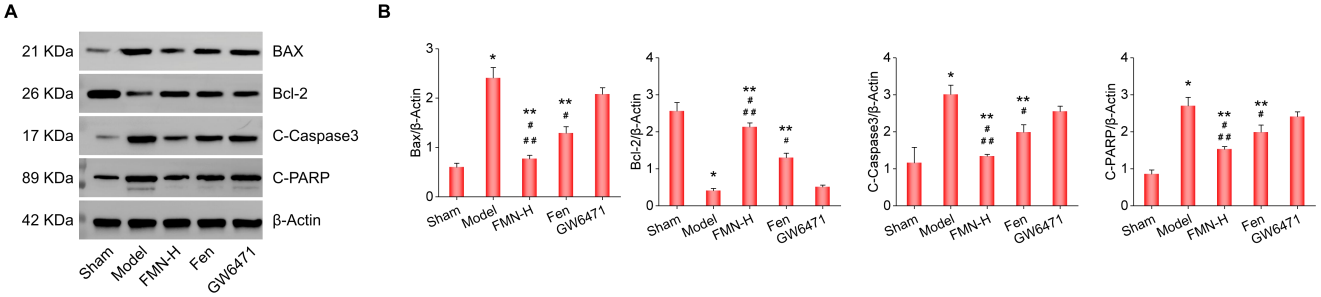

Figure 3 FMN inhibited the expression of apoptotic proteins in HFpEF mice. Heart tissues were lysed and the proteins were subjected to immunoblotting with antibodies against the apoptosis-related proteins (Bax, Bcl-2, cleaved caspase-3, and cleaved PARP). Data are mean ± SD (n = 5). Statistical significance was assessed by one-way ANOVA. $^{*}P < 0.05$ vs. Sham group, $^{**}P < 0.05$ vs. Model group, $^{\#}P < 0.05$ vs. GW6471 group. FMN, formononetin; Fen, fenofibrate.

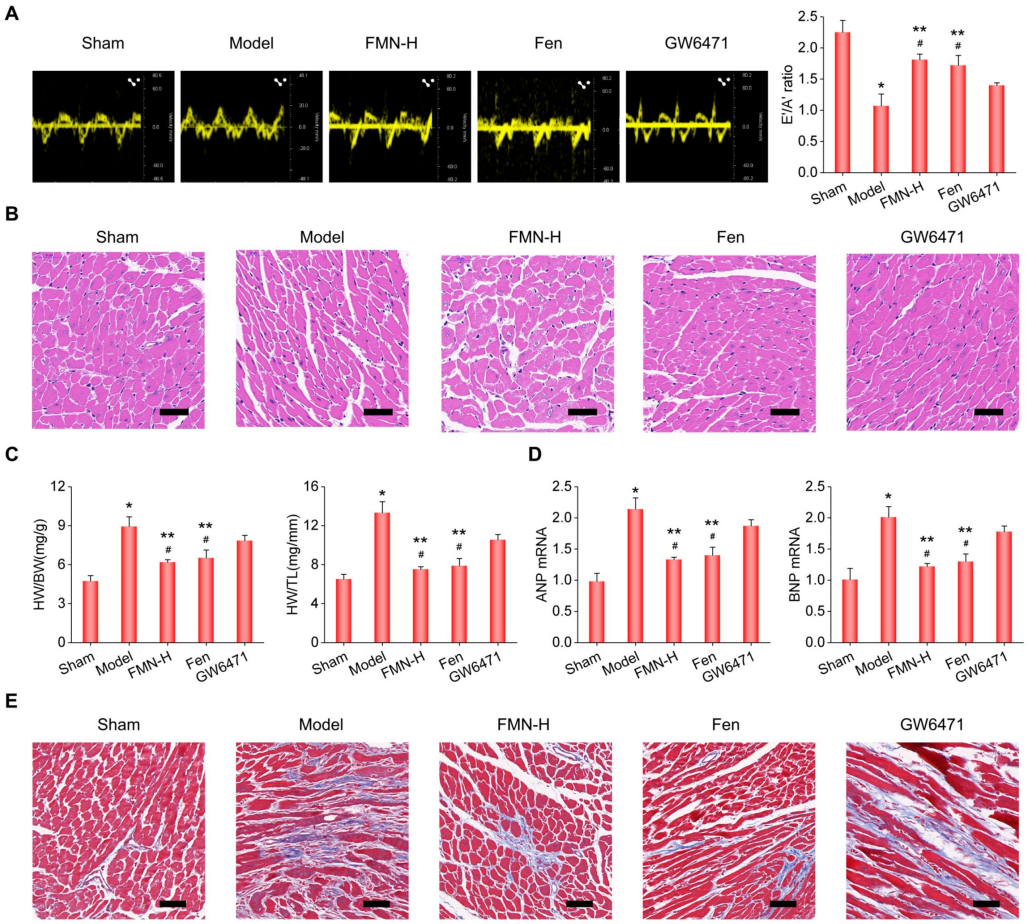

Figure 4 FMN prevented cardiac dysfunction by activating PPARα/PGC-1 pathway in HFpEF mice. (A) Representative images of tissue Doppler and tissue Doppler E'/A' ratio. (B) Representative images of hearts. (C) Quantitation of the ratio of HW/BW and HW/TL. (D) qRT-PCR analysis of ANP and BNP expression. (E) Representative images of Masson's trichrome staining for myocardial interstitial fibrosis. Scale bars: 50 μm (B, E). Data are mean ± SD (A, C, D, n = 5). Statistical significance was assessed by one-way ANOVA. $^{*}P < 0.05$ vs. Sham group, $^{**}P < 0.05$ vs. Model group, $^{\#}P < 0.05$ vs. GW6471 group. FMN, formononetin; Fen, fenofibrate; HW/BW, heart weight to body weight; HW/TL, heart weight to tibia length; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

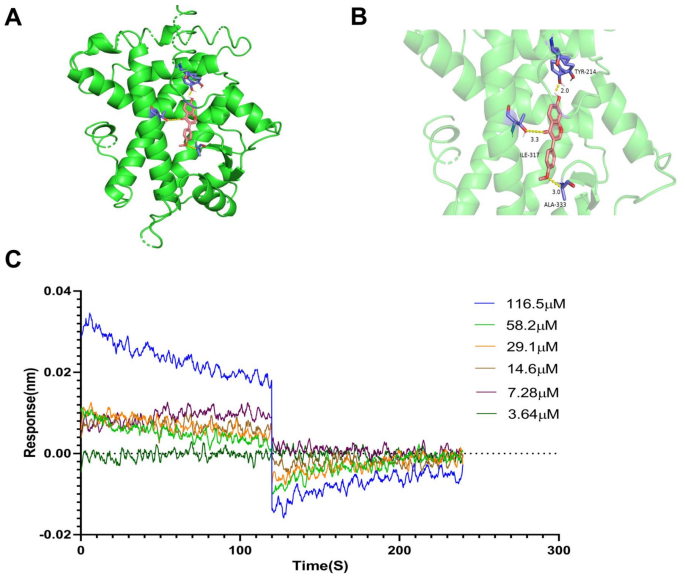

Figure 5 Docking configuration of FMN and PPARα. Docking simulation between FMN and PPARα was identified by Autodock 4.2.6 software. (A) Docking pattern of FMN with PPARα proteins. (B) Interaction of FMN with the ligand-binding domain of PPARα in TYR-214, ILE-317 and ALA-333 residues to form three hydrogen bonds, shown by dashed yellow line. (C) Binding affinity of FMN to PPARα. The left par represents the dissociation curves of FMN and PPARα; the right part shows the relationof curves with FMN at concentrations of 3.64, 7.28, 14.6, 29.1, 58.2 and 116.5 μM.

Therefore, the dependence on carbohydrate metabolism may represent a state of energy deficit, leading to dysfunction in heart failure. We have observed significant fibrosis in the mice of model group, indicating that cardiomyocyte apoptosis in HFpEF mice was aggravated. It is evident that FMN treatment could ameliorate the fibrosis and cardiomyocyte apoptosis of HFpEF mice. In this research endeavor, our findings revealed a significant enhancement in cardiac function among mice treated with FMN, which was attributed to the augmented fatty acid β-oxidation through the activation of PPARα agonists. We hypothesize that the cardioprotective impact of FMN could potentially be a consequence of the boosted fatty acid β-oxidation via the activation of PPARα. Additionally, our investigations unveiled that FMN triggered an upregulation in the expressions of PPARα, PCG-1, and the target genes of PPARα. Notably, this upregulation was impeded by GW6471, further suggesting that the enhanced fatty acid β-oxidation renders a protection on HFpEF.

This study has some limitations. The specific way in which FMN activated PPARα and the relationship between FMN and PGC-1α need further investigation. Although we have successfully constructed a mouse model of HFpEF induced by non-nephrectomy surgery and d-aldosterone infusion, there is a difference between this mouse model and human clinical model of HFpEF. In addition, we just determine the KD value of FMN group, but did not compare the KD between FMN group and the positive control.

Conclusions

Our investigation has yielded compelling evidence regarding the beneficial impact of FMN on energy metabolism and cardiac function in mice afflicted with HFpEF. Notably, FMN administration has demonstrated the capacity to enhance the expression of PPARα, PGC-1, and genes associated with fatty acid metabolism, while concomitantly suppressing the expression of genes related to glucose metabolism. These effects collectively contribute to an improved ability to utilize fatty acids and enhance mitochondrial function. Additionally, FMN exhibits the ability to mitigate cardiac fibrosis, thereby ameliorating HFpEF-induced LVH and diastolic dysfunction. These multifaceted benefits are facilitated by FMN's modulation of the PPARα/PGC-1 expression and the regulation of fatty acid β-oxidation. Consequently, FMN holds promise as a therapeutic intervention against the detrimental effects of HFpEF through its ability to enhance myocardial fatty acid β-oxidation.

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