ORIGINAL ARTICLE

# Selective sodium-glucose cotransporter two inhibitor empagliflozin ameliorates diabetic cardiomyopathy by activating the AMPK/TFEB signaling pathway

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## Abstract

**The highly selective SGLT2 inhibitor (SGLT2i) is reported to have beneficial effects on diabetic cardiac hypertrophy; however, the molecular mechanisms underlying the cardioprotection of SGLT2i are not fully understood. In this study, we investigated the impact of the SGLT2 Inhibitor empagliflozin (EMPA) on diabetic hearts and its regulatory mechanisms in high-fat-diet (HFD)- and streptozotocin (STZ)-treated rats. Male rats orally administered HFD/STZ treatment for eight weeks, with or without EMPA (10 mg/kg), were used as our *in vivo* model. Hematoxylin and eosin (H&E) staining was used for histological examination. Western blot analysis and immunohistochemistry were used to analyze the expression of proteins. Daily EMPA administration prevented the HFD/STZ treatment-induced cardiac hypertrophy by activating the AMP-activated protein kinase (AMPK)/transcription factor EB (TFEB)-mediated upregulation of autophagy- and antioxidant-related proteins. Moreover, EMPA treatment decreased oxidative stress by increasing the antioxidant capacity and protein expression of antioxidant proteins while downregulating the levels of 4-hydroxy-2E-nonenal in the hearts of diabetic rats. Furthermore, EMPA treatment decreased cardiomyocyte apoptosis and increased heart mitochondrial function. The AMPK/TFEB signaling-mediated increase in autophagy, antioxidant capacity, mitochondrial function, and attenuated cardiomyocyte apoptosis may be crucial in the anti-hypertrophic effect conferred by SGLT2i. Our clinical implications suggest a novel pharmacological approach for treating diabetic cardiomyopathy by modulating autophagy and redox homeostasis.**

*Keywords:* AMP-activated protein kinase, Diabetic cardiomyopathy, Empagliflozin, Sodium-glucose cotransporter two inhibitors, Transcription factor EB

## 1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to insufficient insulin production (type 1) or insulin resistance (type 2 diabetes, T2DM) [1,2]. Prolonged hyperglycemia leads to serious complications, including cardiovascular diseases, nephropathy, neuropathy, and retinopathy [3–5]. Among these, cardiac hypertrophy represents one of the most significant cardiovascular complications [5,6], characterized by abnormal thickening and enlargement of the heart muscle that substantially increases the risk of heart failure in diabetic patients [5–8]. The pathogenesis of diabetic cardiac hypertrophy involves complex mechanisms [7,8] driven primarily by metabolic disturbances from hyperglycemia and insulin resistance [9–11]. Chronic hyperglycemia promotes the formation of advanced glycation end-products (AGEs),

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contributing to oxidative stress, inflammation, and myocardial remodeling [6,7,12]. Additionally, insulin resistance alters lipid metabolism, leading to myocardial lipid accumulation (lipotoxicity), which induces cardiomyocyte death and hypertrophy while impairing cardiac function [13,14]. Understanding the underlying mechanisms and implications of diabetic cardiac hypertrophy is crucial for developing effective preventive and therapeutic strategies.

Autophagy is a highly conserved catabolic process that degrades unnecessary or dysfunctional cellular components through lysosomes [15], maintaining cellular homeostasis by recycling cytoplasmic contents, including damaged proteins and organelles [16]. In cardiovascular cells, autophagy plays a crucial protective role for cardiomyocytes under stress conditions, such as hyperglycemia and hyperlipidemia [17,18]. However, autophagy dysregulation occurs in diabetic cardiomyocytes [6,16,19]. Studies have shown that hyperglycemia [16] and dyslipidemia [20] inhibit autophagy, while insulin resistance can lead to autophagy hyperactivation, which hinders cardiomyocyte survival [6,16,17,21]. This dual effect suggests that regulating autophagy represents a potential therapeutic strategy for improving diabetic cardiomyopathy and heart failure [19].

Transcription factor EB (TFEB) is a member of the microphthalmia family of transcription factors [22] that plays a crucial role in cellular processes [23]. TFEB overexpression increases lysosome numbers and lysosomal enzyme activity, elevating lysosomal catabolic function [24]. It can bind to autophagy gene promoter regions to induce autophagosome biogenesis and autophagosome-lysosome fusion [25], thereby stimulating both lysosome generation and autophagy activation. Research indicates that glucolipotoxicity inhibits autophagy and damages cardiomyocytes, potentially through reduced lysosome and intracellular TFEB content [26]. Increasing TFEB expression may promote lipophagy to combat obesity and metabolic syndrome [27], suggesting the essential role of TFEB in cardiomyocyte autophagy.

Glucose reabsorption occurs through sodium-glucose co-transporters (SGLTs) in kidney tubules [28]. SGLT2 is responsible for 80–90% of glucose reabsorption, while SGLT1 handles 10–20% [29–33]. SGLT2 inhibitors (SGLT2i) reduce glucose reabsorption, lowering blood sugar independently of insulin secretion [34–36]. FDA-approved SGLT2i include canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin [34]. Cardiovascular safety outcome trials demonstrate that SGLT2i reduce hospitalization for heart failure by 30–35% in diabetic patients [37–40]. Proposed mechanisms include empagliflozin's ability to shift ATP production from fatty acid oxidation toward glucose oxidation [41] and dapagliflozin's reduction of interstitial fluid without affecting arterial blood filling [42]. Despite these clinical benefits, the precise mechanisms underlying the cardioprotective effects of SGLT2 inhibitors (SGLT2i) remain unclear. Given the vital role of autophagy in cardiomyocytes and its potential relationship with TFEB, investigating the impact of SGLT2 inhibitors (SGLT2i) on autophagy and TFEB expression in cardiac hypertrophy represents an important research direction for understanding these therapeutic benefits and developing targeted interventions for diabetic cardiac complications. In this project, we investigated the effects of SGLT2 inhibitors (SGLT2i) on autophagy and TFEB expression in cardiac hypertrophy.

**Abbreviations**

| | |
|---|---|
| α-SMA | α-smooth muscle actin |
| β-MHC | β-myosin heavy chain |
| 4HNE | 4-hydroxy-2E-nonenal |
| ANP | atrial natriuretic peptide |
| AMPK | AMP-activated protein kinase |
| Bcl-2 | B-cell lymphoma 2 |
| BNP | B-type natriuretic peptide |
| CVD | cardiovascular disease |
| EMPA | Empagliflozin |
| H&E | hematoxylin and eosin |
| HbA1c | Glycated hemoglobin |
| HF | heart failure |
| HFD | High-fat diet |
| LVW | left ventricular weights |
| SGLT2i | Sodium-glucose co-transporter two inhibitor |
| STZ | Streptozotocin |
| T2DM | Type 2 diabetes mellitus |
| TFEB | Transcription factor EB |

## 2. Materials and methods

### 2.1. Reagents

EMPA was purchased from Boehringer Ingelheim (Rhein, Germany). Rabbit antibodies for AMPK (A12718), TFEB (A7311), and mouse antibody against β-actin (AC004) were purchased from ABclonal (Woburn, MA, USA). Rabbit antibody for p-TFEB (Ser211) (AF3708) from Affinity Biosciences (OH, USA). Streptozotocin (STZ) was obtained from Sigma–Aldrich (Saint Louis, MO, USA). Rabbit antibody against protein phosphatase 2B (PP2B, sc-9070) was obtained from Santa Cruz Biotechnology

(Santa Cruz, CA, USA). Mouse antibody against 4-hydroxy-2E-nonenal (4-HNE, MAB3249) and goat antibody against glutathione peroxidase (GPx, AF3798) were obtained from R&D Systems (Minneapolis, MN, USA). Rabbit antibody against heme oxygenase-1 (HO-1, ADI-SPA-895) was obtained from Assay Designs (Plymouth Meeting, PA, USA). Rabbit antibodies against the mammalian target of rapamycin (mTOR, #2983), microtubule-associated protein 1A/1B-light chain (LC3, #4108), sequestosome 1 (p62, #5114), and p-AMPK were purchased from Cell Signaling Technology (Danvers, MA, USA). Rabbit antibodies against superoxide dismutase 1 (SOD1, ab13498), SOD2 (ab13533), B-cell lymphoma 2 (Bcl-2, ab59348), caspase-3 (ab32351) and mouse antibody for cytochrome c oxidase subunit 1 (MTCO1, ab14705), ATP assay kit (ab83355), and total antioxidant capacity fluorometric assay (ab65329) kits were obtained from Abcam (Cambridge, MA, USA).

### 2.2. Animal preparation

Male Whistar-Kyoto rats (160–200 g) were allowed free access to rat chow and water and housed two per cage in a 12-h light/dark cycle animal room. Periodic checks of the cages and body weights ensured that the food was administered appropriately. The temperature of the animal breeding room was controlled at 20 ± 2 °C. The Animal Care and Use Committee of National Taiwan University approved the study protocol. The induction steps of T2DM were as follows: rats were fed a high-fat diet (HFD; D12331; 58% fat, 16% protein, and 26% carbohydrate) for four weeks. Then STZ (40 mg/kg) was injected twice intraperitoneally (i.p.). One week after the injection of STZ, fasting blood glucose (FBG) was detected. The fasting time was 12–16 h, but the rats had free access to water. FBG was detected by collecting a small drop of blood from the tail, applying it to blood glucose test paper (Ascensia ELITE Test Strips), and then using a blood glucose test machine (Ascensia ELITE XL Blood Glucose Meter, Bayer, Mishawaka, IN, USA). Rats with FBG value greater than 140 mg $dL^{-1}$ were confirmed to have successfully induced T2DM and would be randomly divided into different groups: (1) T2DM group (HFD/STZ); (2) T2DM treated with empagliflozin group (HFD/STZ + EMPA). EMPA was orally given 10 mg/kg/day for eight weeks [43–45]. During this step, rats were fed with standard chow (Fig. 1A). All rats were humanely euthanized at the same age to collect blood and heart for further experiments.

### 2.3. Histological examination

The hearts were fixed with 10% formalin and embedded in paraffin. Paraffin blocks were cut into 8 μm sections, dewaxed with Hemo-De, and rehydrated from alcohol to deionized water for further staining. For hematoxylin and eosin (H&E) staining, the sections were stained with hematoxylin at 25 °C for 30 s and then stained with eosin Y at 25 °C for 20 s. After staining, sections were dehydrated in ascending alcohol solutions and mounted with mounting gel. For immunohistochemistry, the deparaffinized sections were incubated in retrieval buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) for 10 min at 37 °C. The sections were blocked with 2% BSA for 60 min at 37 °C and incubated with primary antibody for 2 h at 37 °C, and then with the FITC-conjugated secondary antibody overnight at 4 °C. Images were observed under a Zeiss LSM 880 confocal microscope with Zen software (Carl Zeiss AG, Oberkochen, Germany).

### 2.4. Western blot analysis

The hearts were lysed in immunoprecipitation lysis buffer (50 mM Tris pH 7.5, 5 mM EDTA, 300 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL aprotinin, and phosphatase inhibitor cocktail II and III) and the total protein was extracted. Aliquots of protein (50 μg) mixed with 5 μL loading dye (250 mM Tris HCl, pH 6.8, 500 mM dithiothreitol, 10% SDS, 50% glycerol, and bromophenol blue) were separated on 8%, 12% SDS gels and then transblotted onto the PVDF membrane (Millipore, Bedford, MA, USA). After being blocked with 5% skim milk, the blotting membrane was incubated with the primary antibodies, followed by the corresponding horseradish peroxidase-conjugated secondary antibodies. The protein bands were visualized using an enzyme-linked chemiluminescence detection kit (Perkin, Waltham, MA, USA), and the band density was measured using the quantitative software (TotalLab, Newcastle upon Tyne, UK).

### 2.5. Terminal deoxynucleotidyl transferase UTP nick end labeling (TUNEL) assay

The TUNEL assay was conducted using a Situ Cell Death Detection Kit obtained from Roche (Basel, Switzerland). In brief, formalin-fixed tissue sections were dewaxed and subsequently permeabilized by incubating sections in 0.1% Triton X-

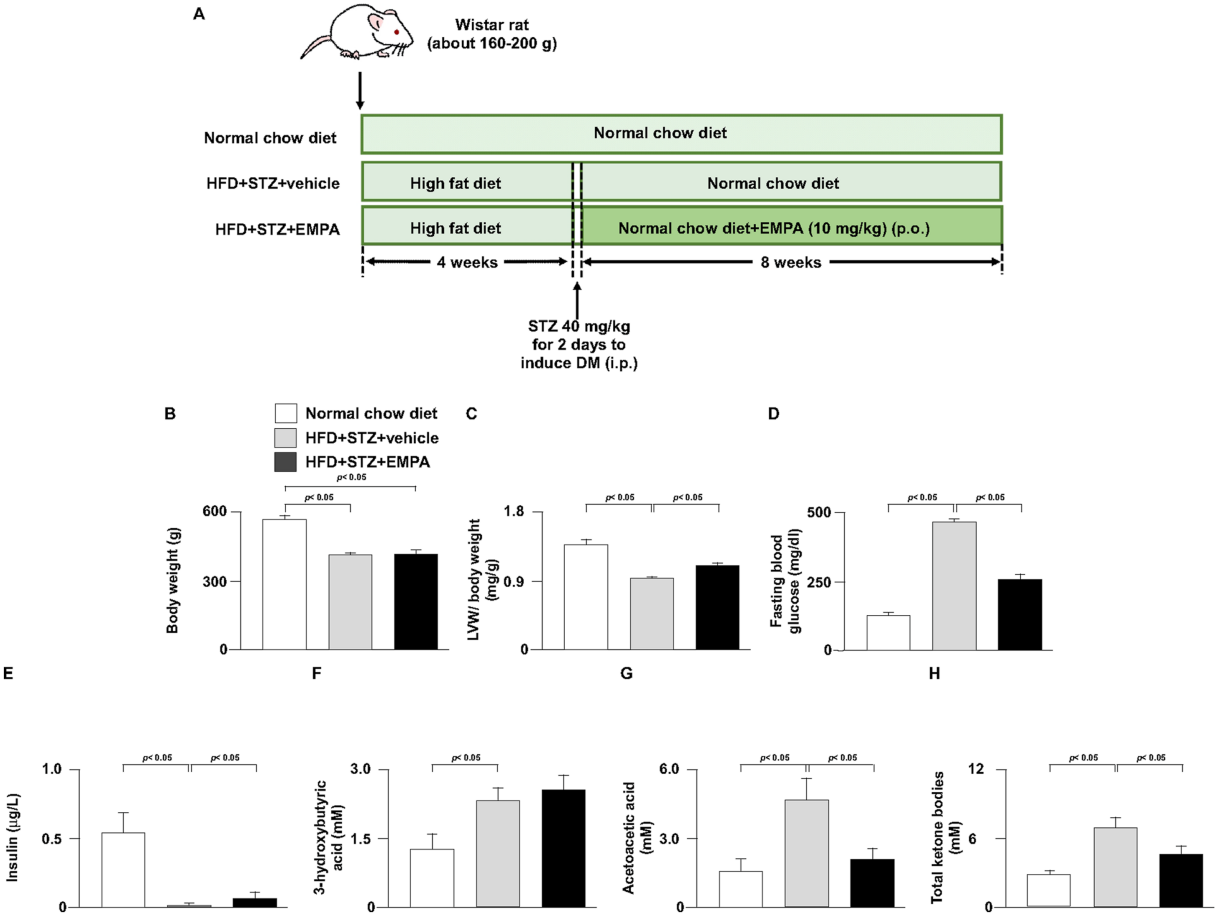


*Fig. 1. **Schematic diagram of the experimental procedure to establish a rat model of diabetes mellitus (DM).** (A) The induction steps were as follows: rats were fed a high-fat diet (HFD; D12331; 58% fat, 16% protein, and 26% carbohydrate) for four weeks. Then, streptozotocin (STZ; 40 mg/kg) was administered intraperitoneally (i.p.) twice. One week after the STZ injection, fasting blood glucose (FBG) was measured after 12–16 h of fasting with free access to water. Rats with FBG values greater than 140 mg/dL were considered successfully induced with T2DM and randomly divided into different groups: (1) normal chow diet group; (2) T2DM group (HFD/STZ); (3) T2DM treated with empagliflozin group (HFD/STZ + EMPA). Empagliflozin (EMPA) was orally administered at 10 mg/kg/day for eight weeks. During this step, rats were fed a standard chow diet. Rats were fed a high-fat diet (HFD) or a control diet for 4 weeks, followed by streptozotocin (STZ) injection to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (B and C) The body and left ventricular weights are in three groups. (D) Fasting blood glucose was evaluated in three groups. (E) Plasma insulin levels were quantified across the three groups. (F–H) Plasma levels of 3-hydroxybutyric acid, acetoacetic acid, and total ketone bodies were measured in the three groups. Data are expressed as mean ± SEM from three rats.*

100 at 37 °C for 5 min. After permeabilization, the sections were blocked in 2% BSA, and then the TUNEL reaction mixture was added to the sections at 37 °C for 60 min. Images were observed under a Leica DMIRB Microscope (Deer Park, IL, USA) with LAS V4.12 software (Wetzlar, Germany).

### 2.6. Statistical analysis

The results were presented as the mean ± SEM. The Mann–Whitney U test was used to compare two independent groups. SPSS software v8.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Differences were considered statistically significant at $p < 0.05$.

## 3. Results

### 3.1. Effect of EMPA on the heart of HFD/STZ-induced diabetic rats

To elucidate the potential effects of EMPA on hypertrophy of rats heart hypertrophy, we used regular chow diet and HFD/STZ-induced diabetes in rats as an *in vivo* model (Fig. 1A). The body weight was decreased in both HFD/STZ-induced diabetic rats groups compared with standard diet group (Fig. 1B). And the left ventricular weight was reduced and fasting blood glucose was increased in HFD/STZ-induced diabetic rats groups; however, oral administration of EMPA to HFD/STZ-induced diabetic rats increased left ventricular weight and

reduced fasting blood glucose (Fig. 1C and D). Moreover, plasma insulin levels were reduced in both HFD/STZ-induced diabetic rat groups compared with the standard diet group. However, oral administration of EMPA to HFD/STZ-induced diabetic rats increased plasma insulin levels (Fig. 1E). In addition, plasma levels of 3-hydroxybutyric acid, acetoacetic acid, and total ketone bodies were elevated in HFD/STZ-induced diabetic rats treated with vehicle. EMPA treatment reduced acetoacetic acid and total ketone body levels in HFD/STZ-induced diabetic rats compared with the vehicle-treated group (Fig. 1F and G). Histological analysis revealed increased cross-sectional areas and diameters in cardiomyocytes from rats with HFD/STZ-induced diabetes. The increased cardiomyocyte cross-sectional areas and diameter in HFD/STZ-induced diabetic rats were attenuated by treating with EMPA (Fig. 2A and B). Furthermore, the protein levels of β-MHC, ANP, and BNP, three hypertrophy markers, were increased in HFD/STZ-induced diabetic rats treated with vehicle compared with the standard diet group. However, EMPA treatment decreased the levels of all three hypertrophy markers in HFD/STZ-induced diabetic rats compared with the vehicle-treated group (Fig. 2C). Additionally, WGA staining analysis revealed that the cardiomyocyte cross-sectional areas were increased in HFD/STZ-induced diabetic rats treated with vehicle compared with the standard diet group. In contrast, EMPA treatment reduced cardiomyocyte cross-sectional areas in HFD/STZ-induced diabetic rats compared with the vehicle-treated group (Fig. 2D). Moreover, the analysis of α-SMA protein level and Picro-Sirius red staining analysis demonstrated increased cardiac fibrosis in

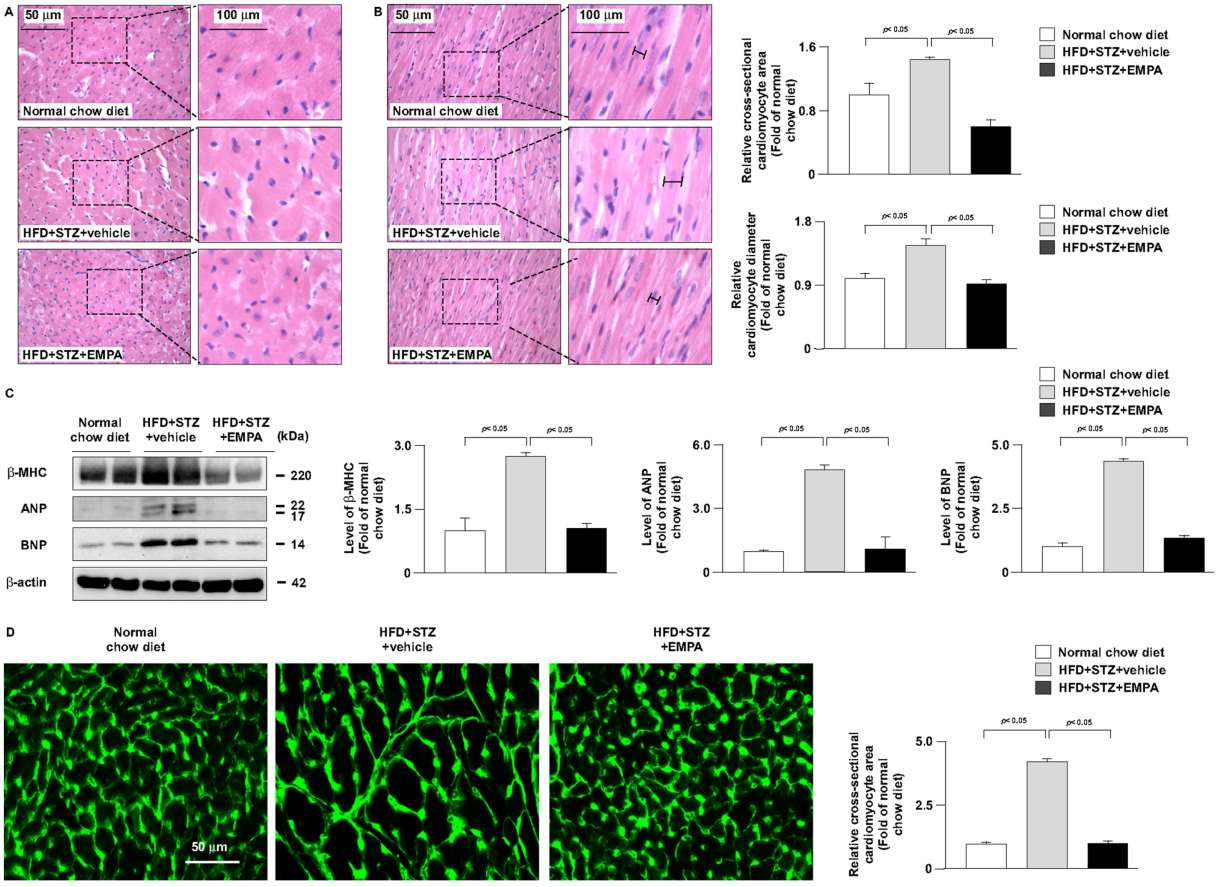


*Fig. 2.* ***Empagliflozin (EMPA) suppressed cardiomyocyte hypertrophy in HFD/STZ-induced diabetic rats.*** *Rats were fed an HFD or control diet for 4 weeks, followed by STZ injection to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (A and B) Representative histological images of myocardial sections stained with hematoxylin and eosin (H&E). (A) Cross-sectional sections of hearts and quantitative analysis of cardiomyocyte area. (B) Longitudinal sections of hearts and quantitative analysis of cardiomyocyte diameter. The black lines indicate the measured diameters. Boxes in panels A and B (left) indicate the regions illustrated by the high-magnification images (right). (C) Western blot analysis of protein levels of β-myosin heavy chain (β-MHC), atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and β-actin in the heart. (D) Representative histological images of myocardial sections stained with wheat germ agglutinin (WGA) staining. Data are expressed as mean ± SEM from three rats.*

HFD/STZ rats treated with vehicle compared with the standard diet group. However, the cardiac fibrosis was decreased in HFD/STZ rats orally treated with the EMPA group compared with the vehicle group (Fig. 3A and B). These findings indicate that EMPA effectively attenuates cardiac hypertrophy and fibrosis in the T2DM rat model.

### *3.2. EMPA-activated AMPK/TFEB-mediated autophagy and antioxidant capacity in the heart of HFD/STZ-induced diabetic rats*

Autophagy has been observed in the hearts of diabetic animal models in previous studies [6,16,19]. We then investigated whether EMPA induced autophagy in the hearts of rats with HFD/STZ-induced diabetes. The results of western blot analysis suggested that EMPA induced autophagy flux, evidenced by an increase in the protein levels of p-AMPK and PP2B, coupled with the reduction in the levels of mTOR, p-TFEB, LC3, and p62 (Fig. 4A). Immunofluorescence staining suggested that EMPA promoted the nuclear translocation of TFEB while decreasing the cytosolic levels of p-TFEB as compared to the vehicle-treated group (Fig. 4B). Moreover, cardiac hypertrophy progression is often associated with dysregulated reactive oxygen species (ROS) production and oxidative stress [46]. We next explored whether EMPA decreased oxidative stress in the hearts of HFD/STZ-induced diabetic rats. Western blot analysis revealed that the administration of EMPA decreased the levels of 4-HNE (Fig. 5A). Furthermore, EMPA increased antioxidant capacity, and the protein levels of HO-1, SOD1, SOD2, and GPx in the heart of HFD/STZ-induced diabetic rats (Fig. 5B and C). These findings suggest that EMPA induces autophagy and diminishes HFD/STZ-induced diabetic cardiac oxidative stress by modulating the expression of proteins involved in autophagy and the antioxidant detoxification system.

### *3.3. EMPA attenuated cardiac apoptosis and promoted mitochondrial function in the heart of HFD/STZ-induced diabetic rats*

Cardiomyocyte apoptosis has been observed in the hearts of diabetic animal models in previous

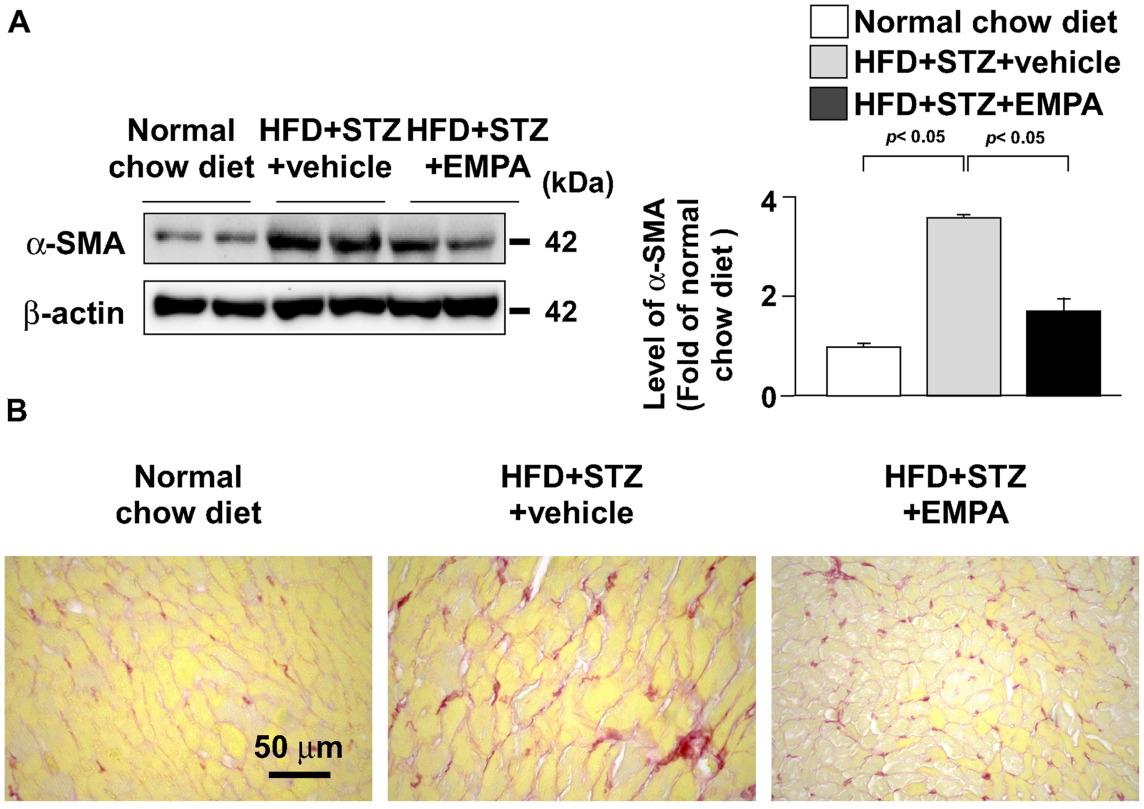


*Fig. 3.* ***EMPA attenuated cardiomyocyte fibrosis in HFD/STZ-induced diabetic rats.*** *Rats were fed an HFD or control diet for 4 weeks, followed by STZ injection to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (A) Western blot analysis of protein levels of α-smooth muscle actin (α-SMA) and β-actin in the heart. (B) Representative histological images of myocardial sections stained with Picro-Sirius red staining. Data are expressed as mean ± SEM from three rats.*

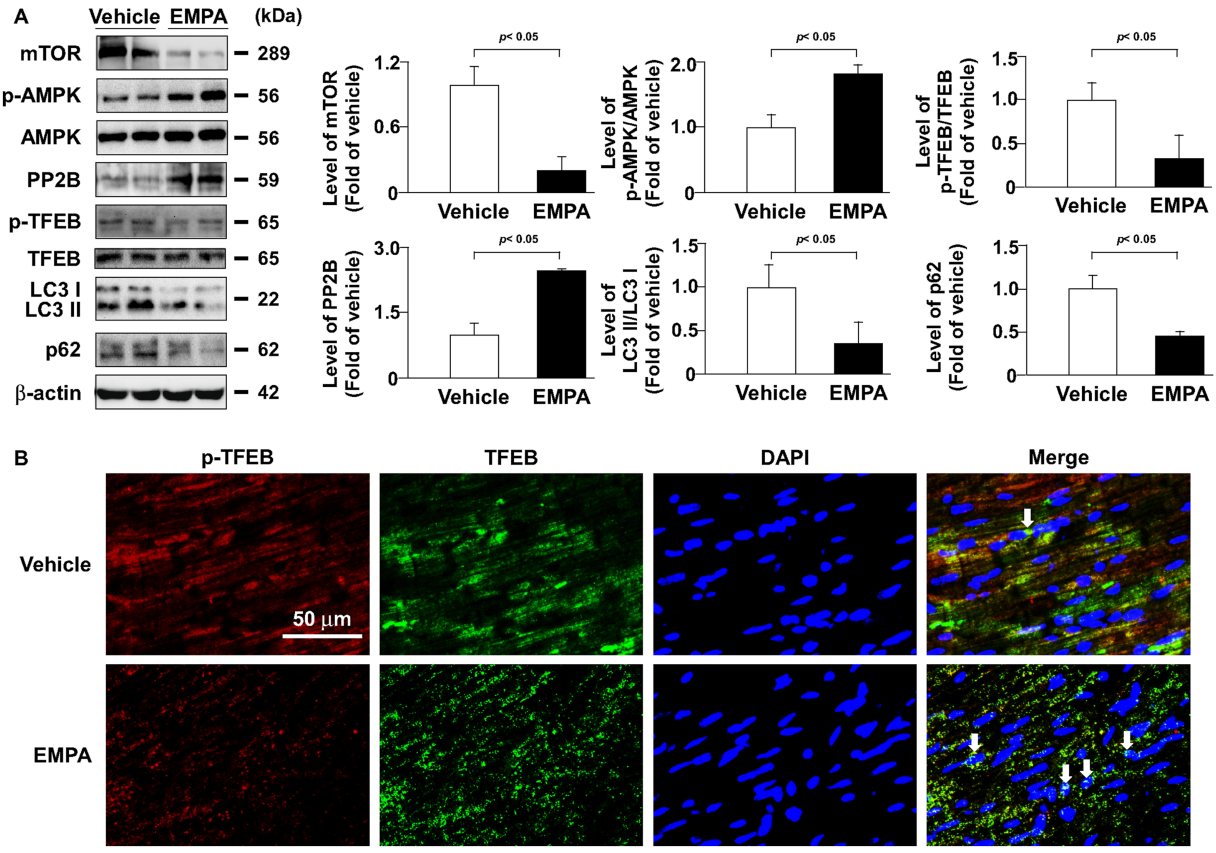


*Fig. 4. Effects of EMPA on AMP-activated protein kinase (AMPK)/transcription factor EB (TFEB)-dependent autophagy in the heart of HFD/STZ-induced diabetic rats. Rats were fed an HFD for 4 weeks, injected with STZ to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (A) Western blot analysis of protein levels of the mammalian target of rapamycin (mTOR), phosphorylated and total AMPK, protein phosphatase 2B (PP2B), phosphorylated and total TFEB, microtubule-associated protein 1 light chain 3 (LC3), sequestosome 1 (p62), and β-actin in the heart. (B) Immunohistochemistry of p-TFEB and TFEB in heart sections. Data are expressed as mean ± SEM from three rats.*

studies [37,38]. Next, we detected the effect of EMPA on cardiac apoptosis. Western blot analysis showed that EMPA inhibited caspase-3 activity with no observable change in Bcl-2 protein expression (Fig. 6A). Moreover, the administration of EMPA significantly decreased the number of TUNEL-positive cells in the heart compared with that of the vehicle group (Fig. 6B). Since mitochondrial function in the heart plays a pivotal role in cardiac energy supply [47,48], we further explored the effect of EMPA on cardiac mitochondrial function. Our results revealed that EMPA increased the number of mitochondria, as evidenced by the expression of MTCO1, a key enzyme in the mitochondrial electron transport chain. Additionally, EMPA significantly increased the cardiac ATP production compared with the vehicle group of HFD/STZ-induced diabetic rats (Fig. 7A). Immunofluorescent images corroborated these findings (Fig. 7B). Collectively, these results suggest that EMPA attenuates the diabetic cardiomyopathy by decreasing the apoptosis of cardiomyocytes and promoting mitochondrial function in the heart of HFD/STZ-induced diabetic rats (Fig. 8).

## 4. Discussion

SGLT2 inhibitors protect against metabolic stress-induced cardiomyopathy [39–42], but their molecular mechanisms remain incompletely understood. Our study provides several novel contributions advancing SGLT2i mechanism understanding: (1) TFEB-specific mechanistic pathway: While AMPK activation by SGLT2i has been reported, we first demonstrate that TFEB nuclear translocation serves as a critical downstream AMPK effector mediating cardioprotective effects of EMPA in diabetic cardiac hypertrophy. (2) Integrated pathway characterization: We uniquely demonstrate the complete AMPK-TFEB-autophagy signaling cascade *in vivo*, showing how EMPA-induced AMPK activation leads to TFEB nuclear translocation, subsequently upregulating autophagy-related and antioxidant proteins coordinately. (3) Functional outcomes

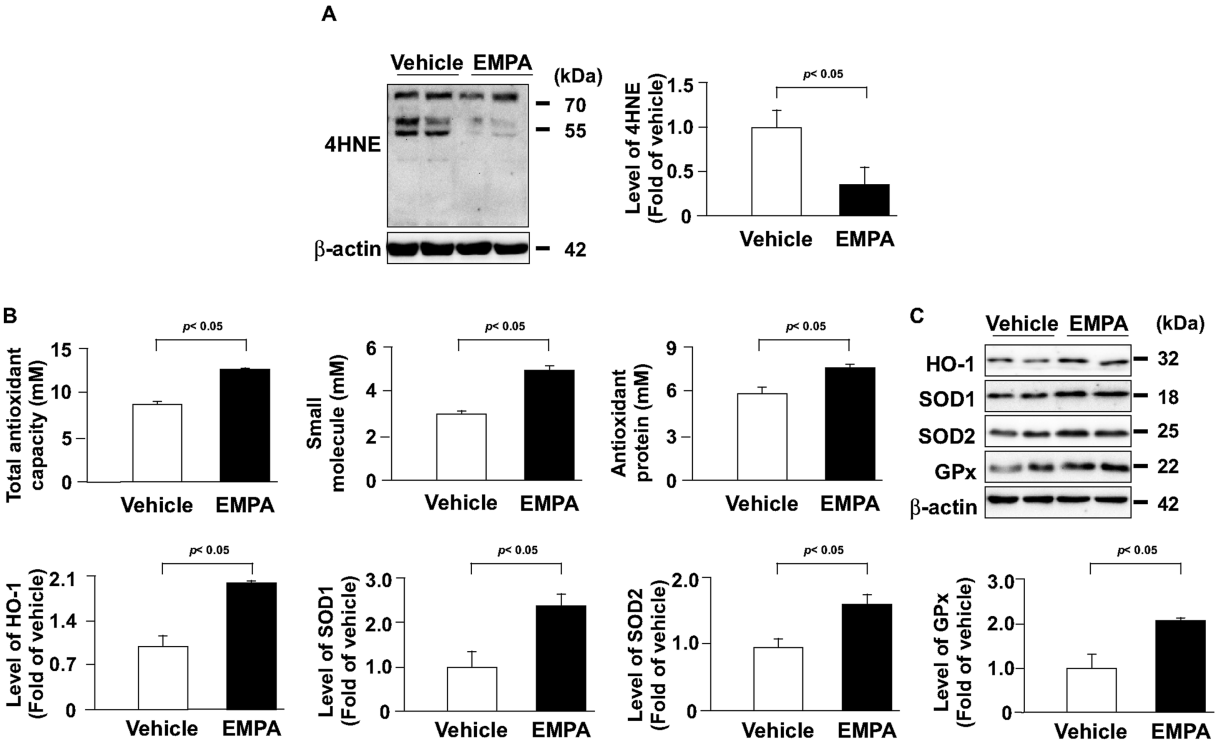


*Fig. 5. **Effects of EMPA on antioxidant activity in the heart of HFD/STZ-induced diabetic rats.** Rats were fed an HFD for 4 weeks, injected with STZ to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (A) Western blot analysis of protein levels of 4-hydroxynonenal (4-HNE) and β-actin. (B) The levels of total antioxidant capacity, antioxidant proteins, and small molecules. (C) Western blot analysis of heme oxygenase-1 (HO-1), superoxide dismutase (SOD) 1, SOD2, glutathione peroxidase (GPx), and β-actin in the heart. Data are expressed as mean ± SEM from three rats.*

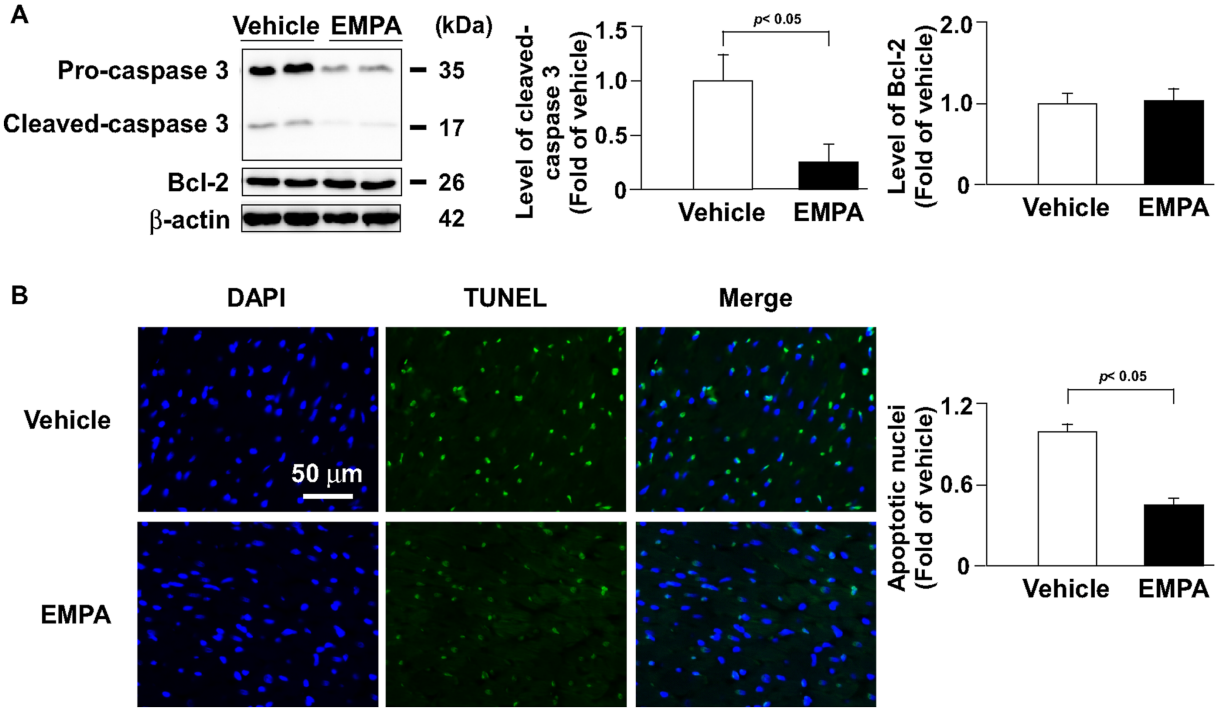


*Fig. 6. **Effects of EMPA on apoptosis in the heart of HFD/STZ-induced diabetic rats.** Rats were fed an HFD for 4 weeks, injected with STZ to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (A) Western blot analysis of caspase-3, B-cell lymphoma 2 (Bcl-2), and β-actin protein levels. (B) Cell apoptosis was evaluated using the TUNEL assay. Data are expressed as mean ± SEM from three rats.*

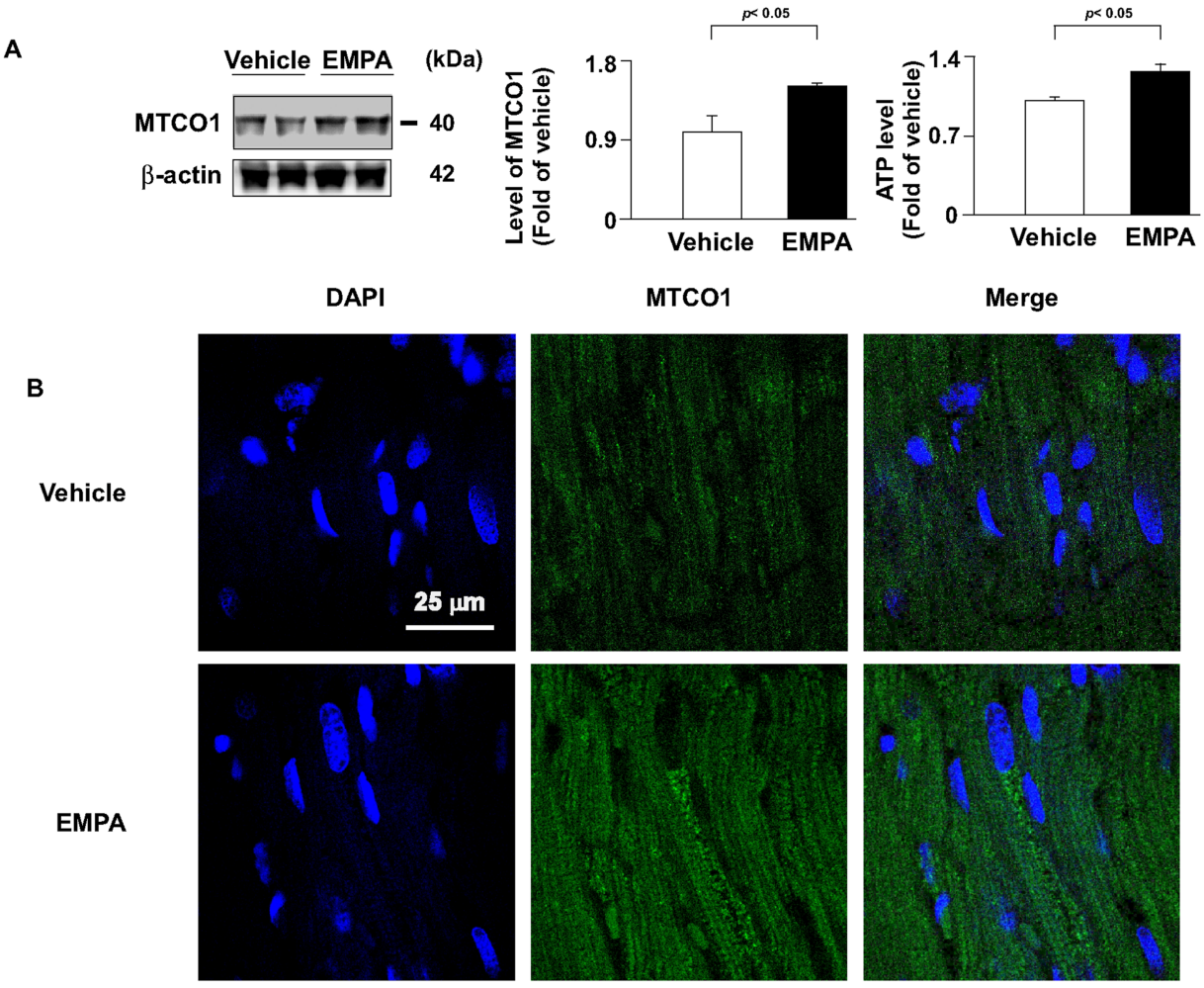


*Fig. 7.* ***Effects of EMPA on mitochondria in the heart of HFD/STZ-induced diabetic rats.*** *Rats were fed an HFD for 4 weeks, injected with STZ to induce diabetes, and then treated daily with EMPA (10 mg/kg) for 8 weeks. (A) Western blot analysis of cytochrome c oxidase subunit I (MTCO1) and β-actin protein levels. (B) Immunohistochemistry of MTCO1 in heart sections. Data are expressed as mean ± SEM from three rats.*

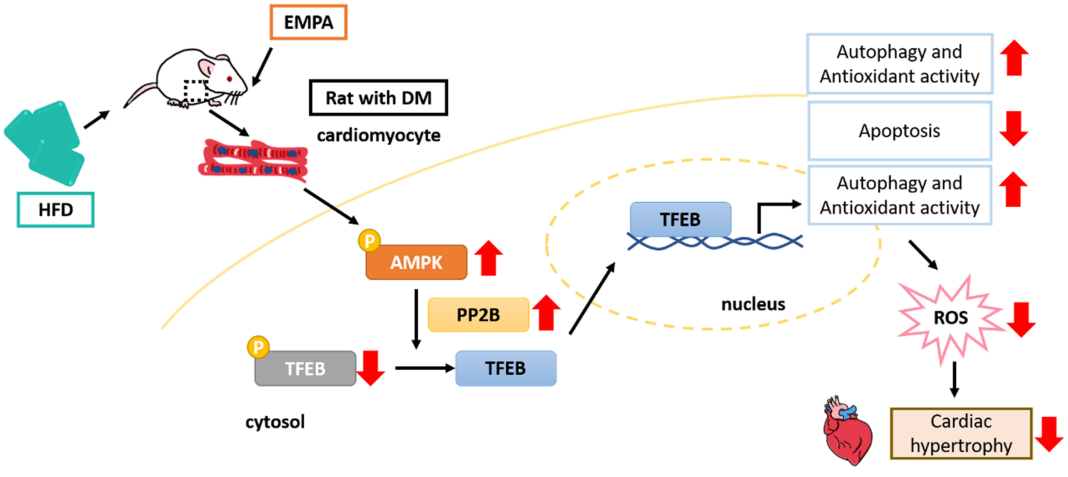


*Fig. 8.* ***Schematic illustration of the molecular mechanisms by which EMPA activates TFEB to reduce oxidative stress and cardiac hypertrophy in HFD/STZ-induced diabetic rats.*** *EMPA induces TFEB activation, which upregulates the expression of autophagy- and antioxidant-related proteins, increases the number of mitochondria, and alleviates oxidative stress, improving cardiac hypertrophy in HFD/STZ-induced diabetic rats.*

linking: We provide direct evidence connecting TFEB nuclear translocation to improved mitochondrial function, reduced apoptosis, and decreased cardiac fibrosis—a mechanistic chain not previously established for SGLT2i treatment. (4) Clinical translational relevance: Identifying TFEB as a key mediator provides a specific therapeutic target guiding combination therapy development or novel interventions beyond current SGLT2i approaches.

Using a diabetic cardiac hypertrophy rat model, we investigated the molecular actions of EMPA. Our findings provide new evidence supporting cardioprotective effects and regulatory pathways. Specifically, empagliflozin attenuated HFD/STZ-induced cardiac hypertrophy by reducing oxidative stress and enhancing antioxidant capacity, aligning with Wang et al.'s findings [49]. Additionally, we observed activation of the AMPK−TFEB−autophagy pathway, which has been reported to play protective roles in metabolic disorders [50−53]. These results suggest EMPA confers protective effects against diabetic cardiomyopathy by modulating redox homeostasis and autophagy. Furthermore, Marfella et al. reported elevated SGLT2 protein levels in diabetic patients compared to non-diabetic patients [54]. Based on our findings and theirs, EMPA may confer cardioprotective effects through both glucose-dependent and -independent mechanisms. However, the mechanisms by which SGLT2 inhibitors regulate redox homeostasis and autophagy in diabetic hearts require further investigation.

Autophagy is crucial for maintaining physiological function but is often impaired in diabetic hearts [15,16,20,21]. Increasing autophagy activity with rapamycin attenuates angiotensin II-induced chronic heart failure [55]. Our study found EMPA treatment increased autophagic flux activity in diabetic hearts, evidenced by decreased LC3II and p62 protein levels, markers of autophagy pathway activation. EMPA treatment increased phosphorylated AMPK levels, which are crucial molecules regulating autophagy and redox homeostasis in the pathogenesis of metabolic diseases [50−53]. TFEB activity is modulated by AMPK phosphorylation [50,52]. Under physiological conditions, TFEB resides in the cytoplasm; however, conditions such as starvation, lysosomal dysfunction, or oxidative stress trigger the nuclear translocation of TFEB, stimulating the transcription of target genes, including those related to autophagy and antioxidants [51−53]. TFEB phosphorylation at S211 is also regulated by mTOR signaling [56]. Chen et al. demonstrated that AMPK activation leads to RPTOR phosphorylation, thereby inhibiting mTORC1 activity and promoting TFEB nuclear translocation and autophagy activation, which aligns with our observations [57]. Given the essential roles of these regulators, targeting the AMPK-TFEB pathway may have therapeutic value for managing cardiac hypertrophy-induced metabolic stress.

Oxidative stress, an imbalance between pro-oxidants and antioxidants, is dysregulated in various diseases [58−60]. Numerous studies indicate that dysregulated ROS signaling plays crucial roles in human disease development [58,60]. Targeting ROS pathways with antioxidants has therapeutic potential for preventing oxidative stress-mediated metabolic disorders [58−60]. Our data suggest EMPA-induced enhancement of antioxidant capacity partly explains its beneficial cardiac effects. EMPA treatment may confer protection against oxidative stress, thereby alleviating the progression of diabetic cardiac hypertrophy by activating the AMPK-TFEB pathway and stimulating the upregulation of antioxidant proteins.

Apoptosis plays a pivotal role in pathological cardiac hypertrophy development [38,61]. Previous research indicates cardiomyocyte apoptosis contributes to cardiac dysfunction and pathological hypertrophy [38,62]. Our findings suggest EMPA administration reduced cardiac apoptosis and caspase-3 activity, consistent with observations that attenuating apoptosis effectively improves cardiac hypertrophy [37,63]. Zhao et al. highlighted that under lipopolysaccharide-induced stress, p27 protein can trigger autophagy, enabling myocardial cells to evade apoptosis [64]. In our study, EMPA induced autophagy and suppressed apoptosis in diabetic rat hearts, consistent with previous studies on the complex interplay between autophagy and apoptosis.

Mitochondria are crucial for cardiac function, supplying over 95% of myocardial ATP [65]. Previous studies suggest that mitochondrial dysfunction is implicated in the progression of cardiac hypertrophy [46,47]. Diabetes-associated hyperglycemia contributes to mitochondrial dysfunction by reducing mean mitochondrial size and count [46,47]. High glucose levels shift mitochondrial energy production from fatty acid oxidation to alternative substrates, resulting in reduced ATP production and ultimately leading to cardiac apoptosis [46,47]. Many studies indicate TFEB-mediated signaling critically improves mitochondrial function. Kim et al. demonstrated that TFEB nuclear translocation enhances mitophagy and mitochondrial biogenesis, ameliorating inflammatory liver injury [66]. Similarly, Zhu et al. reported that TFEB-mediated

autophagy attenuates mitochondrial dysfunction and acute kidney injury [67]. These findings align with our results, showing that EMPA administration significantly increases mitochondrial numbers and ATP production in diabetic hearts, suggesting improved mitochondrial function that protects cardiomyocytes from apoptosis. Although numerous studies indicate that other SGLT2 inhibitors exert beneficial effects [68,69], Kim et al. demonstrated that EMPA was associated with a lower risk of cardiovascular mortality [69].

Despite persistent hyperglycemia in the EMPA-treated group, markers of oxidative stress and apoptosis were significantly reduced. We observed the upregulation of key antioxidant enzymes (HO-1, SOD1, SOD2, GPx) and a decrease in the expression of cleaved caspase-3, suggesting direct cytoprotective effects independent of glycemic control. Our findings align with studies indicating that the cardioprotective effects of SGLT2 inhibitors extend beyond glucose lowering, as evident in non-diabetic settings [70,71]. Nevertheless, our study has several limitations. While multiple complementary techniques consistently implicated AMPK and TFEB in EMPA-conferred protection against cardiac hypertrophy and fibrosis, we did not perform direct functional validation, such as AMPK or TFEB knockdown and inhibition. This limitation should be addressed in future studies to definitively confirm the mechanistic role of AMPK–TFEB signaling in EMPA-mediated cardiac protection.

In conclusion, our findings elucidate the regulatory mechanisms of EMPA, indicating AMPK-TFEB pathway activation stimulates autophagy- and antioxidant-related proteins, reduces cardiac apoptosis, and improves mitochondrial function. This mechanism may underlie the protective effects of EMPA on redox homeostasis deregulation, ultimately mitigating cardiac hypertrophy. Our study reveals new molecular mechanisms underlying the protective effects of SGLT2 inhibitors against diabetic cardiac hypertrophy, contributing to a better understanding of regulatory mechanisms and the identification of new therapeutic targets.

## Author contributions

Conceptualization, C.-H W. and T.-S.L.; methodology, M.-C.H., R.-W C., C.-H.C., M.-C. W.; W.-H. C.; formal analysis, M.-C.H.; R.-W C.; investigation, M.-C.H., C.-H.C.; data curation, M.-C.H., R.-W C.; writing—original draft preparation, M.-C.H., C.-H W. and T.-S.L.; supervision, C.-H W. and T.-S.L.; funding acquisition, M.-C. W., C.-H W., and T.-S.L. All authors have read and approved the published version of the manuscript.

## Ethics approval and consent to participate

The Animal Care and Use Committee of National Taiwan University approved the study protocol (No. 20210323).

## Availability of data and materials

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

## Consent for publication

Not applicable.

## Conflicts of interest statement

The authors declare that they have no conflict of interest.

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## References

[1] Glovaci D, Fan W, Wong ND. Epidemiology of diabetes mellitus and cardiovascular disease. Curr Cardiol Rep 2019; 21:21.
[2] Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence trends of type 1 and type 2 diabetes among youths, 2002-2012. N Engl J Med 2017;376:1419–29.
[3] Writing Group Members, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al., American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Executive summary: heart disease and stroke statistics–2016 update: a report from the American heart association. Circulation 2016;133:447–54.
[4] Tentolouris A, Eleftheriadou I, Athanasakis K, Kyriopoulos J, Tsilimigras DI, Grigoropoulou P, et al. Prevalence of diabetes mellitus as well as cardiac and other main comorbidities in a representative sample of the adult Greek population in comparison with the general population. Hellenic J Cardiol 2020;61:15–22.
[5] Matheus AS, Tannus LR, Cobas RA, Palma CC, Negrato CA, Gomes MB. Impact of diabetes on cardiovascular disease: an update. Int J Hypertens 2013;2013:653789.

[6] Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. Circ Res 2018;122:624−38.
[7] Tan Y, Zhang Z, Zheng C, Wintergerst KA, Keller BB, Cai L. Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: preclinical and clinical evidence. Nat Rev Cardiol 2020;17:585−607.
[8] Marfella R, D'Onofrio N, Trotta MC, Sardu C, Scisciola L, Amarelli C, et al. Sodium/glucose cotransporter 2 (SGLT2) inhibitors improve cardiac function by reducing JunD expression in human diabetic hearts. Metabolism 2022;127: 154936.
[9] Bhattacharjee N, Dua TK, Khanra R, Joardar S, Nandy A, Saha A, et al. Protocatechuic acid, a phenolic from Sansevieria roxburghiana leaves, suppresses diabetic cardiomyopathy via stimulating glucose metabolism, ameliorating oxidative stress, and inhibiting inflammation. Front Pharmacol 2017;8:251.
[10] Nagoshi T, Yoshimura M, Rosano GM, Lopaschuk GD, Mochizuki S. Optimization of cardiac metabolism in heart failure. Curr Pharm Des 2011;17:3846−53.
[11] Rosano GM, Fini M, Caminiti G, Barbaro G. Cardiac metabolism in myocardial ischemia. Curr Pharm Des 2008; 14:2551−62.
[12] Aragno M, Mastrocola R. Dietary sugars and endogenous formation of advanced glycation endproducts: emerging mechanisms of disease. Nutrients 2017;9:385.
[13] Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev 2010;90:207−58.
[14] Nakamura A, Terauchi Y. Impaired insulin secretion and related factors in East Asian individuals. J Diabetes Investig 2022;13:233−5.
[15] Li L, Xu J, He L, Peng L, Zhong Q, Chen L, et al. The role of autophagy in cardiac hypertrophy. Acta Biochim Biophys Sin (Shanghai) 2016;48:491−500.
[16] Kobayashi S, Liang Q. Autophagy and mitophagy in diabetic cardiomyopathy. Biochim Biophys Acta 2015;1852: 252−61.
[17] Mellor KM, Bell JR, Young MJ, Ritchie RH, Delbridge LM. Myocardial autophagy activation and suppressed survival signaling are associated with insulin resistance in fructose-fed mice. J Mol Cell Cardiol 2011;50:1035−43.
[18] Chen Y, Hua Y, Li X, Arslan IM, Zhang W, Meng G. Distinct types of cell death and the implication in diabetic cardiomyopathy. Front Pharmacol 2020;11:42.
[19] Dewanjee S, Vallamkondu J, Kalra RS, John A, Reddy PH, Kandimalla R. Autophagy in the diabetic heart: a potential pharmacotherapeutic target in diabetic cardiomyopathy. Ageing Res Rev 2021;68:101338.
[20] Glazer HP, Osipov RM, Clements RT, Sellke FW, Bianchi C. Hypercholesterolemia is associated with hyperactive cardiac mTORC1 and mTORC2 signaling. Cell Cycle 2009;8: 1738−46.
[21] Guo X, Lin H, Liu J, Wang D, Li D, Jiang C, et al. 1,25-Dihydroxyvitamin D attenuates diabetic cardiac autophagy and damage by vitamin D receptor-mediated suppression of FoxO1 translocation. J Nutr Biochem 2020;80:108380.
[22] Steingrímsson E, Tessarollo L, Reid SW, Jenkins NA, Copeland NG. The bHLH-Zip transcription factor Tfeb is essential for placental vascularization. Development 1998; 125:4607−16.
[23] Puertollano R, Ferguson SM, Brugarolas J, Ballabio A. The complex relationship between TFEB transcription factor phosphorylation and subcellular localization. EMBO J 2018; 37:e98804.
[24] Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, et al. A gene network regulating lysosomal biogenesis and function. Science 2009;325:473−7.
[25] Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, et al. TFEB links autophagy to lysosomal biogenesis. Science 2011;332:1429−33.
[26] Trivedi PC, Bartlett JJ, Perez LJ, Brunt KR, Legare JF, Hassan A, et al. Glucolipotoxicity diminishes cardiomyocyte TFEB and inhibits lysosomal autophagy during obesity and diabetes. Biochim Biophys Acta 2016;1861:1893−910.
[27] Settembre C, De Cegli R, Mansueto G, Saha PK, Vetrini F, Visvikis O, et al. TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop [published correction appears in Nat Cell Biol. 2013 Aug;15: 1016]. Nat Cell Biol 2013;15:647−58.
[28] Abdul-Ghani MA, DeFronzo RA. Dapagliflozin for the treatment of type 2 diabetes. Expet Opin Pharmacother 2013;14:1695−703.
[29] Himsworth HP. The relation of glycosuria to glycaemia and the determination of the renal threshold for glucose. Biochem J 1931;25:1128−46.
[30] DeFronzo RA, Norton L, Abdul-Ghani M. Renal, metabolic, and cardiovascular considerations of SGLT2 inhibition. Nat Rev Nephrol 2017;13:11−26.
[31] van Baar MJB, van Ruiten CC, Muskiet MHA, van Bloemendaal L, Ijzerman RG, van Raalte DH. SGLT2 inhibitors in combination therapy: from mechanisms to clinical considerations in type 2. Diabetes Management Diabetes Care 2018;41:1543−56.
[32] Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev 2011;91:733−94.
[33] Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. Circulation 2016;134:752−72.
[34] Gerich JE, Bastien A. Development of the sodium-glucose co-transporter 2 inhibitor dapagliflozin for the treatment of patients with type 2 diabetes mellitus. Expet Rev Clin Pharmacol 2011;4:669−83.
[35] Wright EM. Renal Na(+)-glucose cotransporters. Am J Physiol Ren Physiol 2001;280:F10−8.
[36] Hummel CS, Lu C, Loo DD, Hirayama BA, Voss AA, Wright EM. Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. Am J Physiol Cell Physiol 2011;300:C14−21.
[37] Chen S, Li Y, Fu S, Li Y, Wang C, Sun P, et al. Melatonin alleviates arginine vasopressin-induced cardiomyocyte apoptosis via increasing Mst1-Nrf2 pathway activity to reduce oxidative stress. Biochem Pharmacol 2022;206: 115265.
[38] Oldfield CJ, Duhamel TA, Dhalla NS. Mechanisms for the transition from physiological to pathological cardiac hypertrophy. Can J Physiol Pharmacol 2020;98:74−84.
[39] Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. EMPA-REG OUTCOME investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373:2117−28.
[40] Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al., CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 2017;377:644−57.
[41] Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. DECLARE−TIMI 58 investigators—dapagliflozin and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2019;380:347−57.
[42] McGuire DK, Shih WJ, Cosentino F, Charbonnel B, Cherney DZI, Dagogo-Jack S, et al. Association of SGLT2 inhibitors with cardiovascular and kidney outcomes in patients with type 2 diabetes: a meta-analysis. JAMA Cardiol 2021;6:148−58.
[43] Shao Q, Meng L, Lee S, Tse G, Gong M, Zhang Z, et al. Empagliflozin, a sodium glucose co-transporter-2 inhibitor, alleviates atrial remodeling and improves mitochondrial function in high-fat diet/streptozotocin-induced diabetic rats. Cardiovasc Diabetol 2019;18:165.
[44] Skovsø S. Modeling type 2 diabetes in rats using high-fat diet and streptozotocin. J Diabetes Investig 2014;5:349−58.

[45] Hussein AM, Eid EA, Taha M, Elshazli RM, Bedir RF, Lashin LS. Comparative study of the effects of GLP1 analog and SGLT2 inhibitor against diabetic cardiomyopathy in type 2 diabetic rats: possible underlying mechanisms. Biomedicines 2020;8:43.
[46] Ramachandra CJA, Cong S, Chan X, Yap EP, Yu F, Hausenloy DJ. Oxidative stress in cardiac hypertrophy: from molecular mechanisms to novel therapeutic targets. Free Radic Biol Med 2021;166:297−312.
[47] Facundo HDTF, Brainard RE, Caldas FRL, Lucas AMB. Mitochondria and cardiac hypertrophy. Adv Exp Med Biol 2017;982:203−26.
[48] Dabravolski SA, Sadykhov NK, Kartuesov AG, Borisov EE, Sukhorukov VN, Orekhov AN. The role of mitochondrial abnormalities in diabetic cardiomyopathy. Int J Mol Sci 2022;23:7863.
[49] Wang J, Huang X, Liu H, Chen Y, Li P, Liu L, et al. Empagliflozin ameliorates diabetic cardiomyopathy via attenuating oxidative stress and improving mitochondrial function. Oxid Med Cell Longev 2022;2022:1122494.
[50] Chen CH, Hsia CC, Hu PA, Yeh CH, Chen CT, Peng CL, et al. Bromelain ameliorates atherosclerosis by activating the TFEB-mediated autophagy and antioxidant pathways. Antioxidants (Basel) 2022;12:72.
[51] Paquette M, El-Houjeiri L, C Zirden L, Puustinen P, Blanchette P, Jeong H, et al. AMPK-Dependent phosphorylation is required for transcriptional activation of TFEB and TFE3. Autophagy 2021;17:3957−75.
[52] Wu C. Betulinic acid inhibits pyroptosis in spinal cord injury by augmenting autophagy via the AMPK-mTOR-TFEB signaling pathway. Int J Biol Sci 2021;17:1138−52.
[53] Cao S, Wang C, Yan J, Li X, Wen J, Hu C. Curcumin ameliorates oxidative stress-induced intestinal barrier injury and mitochondrial damage by promoting Parkin-dependent mitophagy through AMPK-TFEB signal pathway. Free Radic Biol Med 2020;147:8−22.
[54] Marfella R, Scisciola L, D'Onofrio N, Maiello C, Trotta MC, Sardu C, et al. Sodium-glucose cotransporter-2 (SGLT2) expression in diabetic and non-diabetic failing human cardiomyocytes. Pharmacol Res 2022;184:106448.
[55] Gao G, Chen W, Yan M, Liu J, Luo H, Wang C, et al. Rapamycin regulates the balance between cardiomyocyte apoptosis and autophagy in chronic heart failure by inhibiting mTOR signaling. Int J Mol Med 2020;45:195−209.
[56] Napolitano G, Esposito A, Choi H, Matarese M, Benedetti V, Di Malta C, et al. mTOR-dependent phosphorylation controls TFEB nuclear export. Nat Commun 2018;9:3312.
[57] Chen M, Liu G, Fang Z, Gao W, Song Y, Lei L, et al. Buddleoside alleviates nonalcoholic steatohepatitis by targeting the AMPK-TFEB signaling pathway. Autophagy 2025;21:1316−34.
[58] Vendrov AE, Sumida A, Canugovi C, Lozhkin A, Hayami T, Madamanchi NR, et al. NOXA1-dependent NADPH oxidase regulates redox signaling and phenotype of vascular smooth muscle cells during atherogenesis. Redox Biol 2019;21:101063.
[59] Di Marco E, Gray SP, Chew P, Koulis C, Ziegler A, Szyndralewiez C, et al. Pharmacological inhibition of NOX reduces atherosclerotic lesions, vascular ROS, and immune-inflammatory responses in diabetic Apoe(-/-) mice. Diabetologia 2014;57:633−42.
[60] Chen CH, Zhao JF, Hsu CP, Kou YR, Lu TM, Lee TS. The detrimental effect of asymmetric dimethylarginine on cholesterol efflux of macrophage foam cells: role of the NOX/ROS signaling. Free Radic Biol Med 2019;143:354−65.
[61] Li X, Lan Y, Wang Y, Nie M, Lu Y, Zhao E. Telmisartan suppresses cardiac hypertrophy by inhibiting cardiomyocyte apoptosis via the NFAT/ANP/BNP signaling pathway. Mol Med Rep 2017;15:2574−82.
[62] Teiger E, Than VD, Richard L, Wisnewsky C, Tea BS, Gaboury L, et al. Apoptosis in pressure overload-induced heart hypertrophy in the rat. J Clin Investig 1996;97:2891−7.
[63] Cheng Y, Shen A, Wu X, Shen Z, Chen X, Li J, et al. Qingda granule attenuates angiotensin II-induced cardiac hypertrophy and apoptosis and modulates the PI3K/AKT pathway. Biomed Pharmacother 2021;133:111022.
[64] Zhao X, Qi H, Zhou J, Xu S, Gao Y. P27 protects cardiomyocytes from sepsis via activation of autophagy and inhibition of apoptosis. Med Sci Monit 2018;24:8565−76.
[65] Nguyen BY, Ruiz-Velasco A, Bui T, Collins L, Wang X, Liu W. Mitochondrial function in the heart: the insight into mechanisms and therapeutic potentials. Br J Pharmacol 2019;176:4302−18.
[66] Kim HJ, Joe Y, Rah SY, Kim SK, Park SU, Park J, et al. Carbon monoxide-induced TFEB nuclear translocation enhances mitophagy/mitochondrial biogenesis in hepatocytes and ameliorates inflammatory liver injury. Cell Death Dis 2018;9:1060.
[67] Zhu L, Yuan Y, Yuan L, Li L, Liu F, Liu J, et al. Activation of TFEB-mediated autophagy by trehalose attenuates mitochondrial dysfunction in cisplatin-induced acute kidney injury. Theranostics 2020;10:5829−44.
[68] Madonna R, Biondi F, Alberti M, Ghelardoni S, Mattii L, D'Alleva A. Cardiovascular outcomes and molecular targets for the cardiac effects of Sodium-Glucose Cotransporter 2 Inhibitors: a systematic review. Biomed Pharmacother 2024;175:116650.
[69] Kim DJ, Sheu WH, Chung WJ, Yabe D, Ha KH, Nangaku M, et al., EMPRISE East Asia Study Group. Empagliflozin is associated with lower risk of cardiovascular events and all-cause mortality in routine care in East Asia: results from the EMPRISE study. J Diabetes Investig 2023;14:417−28.
[70] Khiali S, Taban-Sadeghi M, Sarbakhsh P, Khezerlouy-Aghdam N, Rezagholizadeh A, Asham H, et al. SGLT2 inhibitors' cardiovascular benefits in individuals without diabetes, heart failure, and/or chronic kidney disease: a systematic review. J Clin Pharmacol 2023;63:1307−23.
[71] Matthews J, Herat L, Schlaich MP, Matthews V. The impact of SGLT2 inhibitors in the heart and kidneys regardless of diabetes status. Int J Mol Sci 2023;24:14243.