行政院國家科學委員會專題研究計畫 成果報告

SIRT1 activators 配合運動治療對第二型糖尿病抗老化延壽和抗心臟細胞凋亡的療效探討

研究成果報告(精簡版)

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Background. Cardiac apoptosis was found in diabetes but very limited information regarding the influence of chronic exercise on cardiac apoptosis in diabetes was available. The purpose of this study was to evaluate the influence of chronic exercise on cardiac apoptotic pathways. **Methods.** Cardiac architecture, apoptosis-related pathways and apoptotic cells in the excised hearts of three groups at 4 month of age, Wistar rats (Control, n=12) and Streptozotocin-induced diabetic rats (DM, n=9) and Streptozotocin-induced diabetic rats undergoing treadmill running exercise 1 hour daily, 5 sections per week, for 8 weeks (DM-EX, n=11), were measured by H&E stain, Western Blotting, and TUNEL assays. **Results.** Citrate synthase activity in skeletal muscle in DM-EX is significantly increased compared with sedentary group (Control and DM). Levels of apoptotic proteins, including Fas ligand, Fas death receptors, Fas-associated death domain, activated caspase-8 (Fas pathway), BAD, cytochrome c, activated caspase-9 (mitochondria pathway) t-BID (transition from Fas to mitochondria), and activated caspase-3 (Fas & mitochondria common pathway) were increased in DM group compared with control. Chronic exercise decreased diabetes-induced cardiac TUNEL-positive apoptotic cells. **Conclusions.** Chronic exercise suppressed diabetes-induced Fas-dependent and mitochondria-dependent cardiac apoptotic pathways. Chronic exercise may be one of possible therapeutic approaches for preventing cardiac apoptosis in diabetes.

Key words. Diabetes, heart, Fas-dependent, mitochondria dependent, apoptosis

Introduction:

Diabetes mellitus is a worldwide epidemic disease and the incidence and prevalence of diabetes mellitus have significantly increased in recent decades (1, 2). Diabetes is a chronic metabolic disorder associated with secondary complications in cardiovascular and other systems in humans and animals. It is recognized as an independent risk factor for cardiovascular morbidity and mortality (3). Several reports have described that early changes of cardiac echocardiographic structure and function were found in diabetic patients (4, 5). Diabetic cardiomyopathy is a seriously complications but the mechanisms relating diabetes-associated cardiac pathophysiology are still unclear including the direct metabolic consequences of diabetes on the myocardium (3, 6). In human and animal models of diabetes, diabetic cardiomyopathy is associated with excessive apoptotic cardiomyocyte death (7, 8). It is possible that this loss of cells progressively leads to interstitial fibrosis accumulation, myocardial hypertrophy, contractile impairment, and eventual heart failure diseases in diabetes (9-11).

Apoptosis, a physiological program of cellular death, may contribute to many cardiac disorders (12, 13). The occurrence of apoptosis has been reported to contribute to the loss of cardiomyocytes in cardiomyopathies, and is recognized as a predictor of adverse outcomes in subjects with cardiac diseases or heart failure (14). The ‘extrinsic’ Fas ligand or tumor necrosis factor-alpha (TNF-α) dependent (type I) apoptotic pathway and the ‘intrinsic’ mitochondrial-dependent (type II) apoptotic pathway are believed to be the major pathways directly triggering cardiac apoptosis (11, 12). Type I apoptosis is initiated by binding the Fas ligand to the Fas receptor, which results in the clustering of receptors and initiating an extrinsic pathway (11). Fas ligand and Fas-receptor are known to lead to the formation of a death-inducing signal complex starting with recruitment of the Fas-associated death domain (FADD) of the adaptor protein (11). FADD recruits and aggregate the pro form of caspase-8 and leads to its activation of caspase-8 (15) (16). Additionally, downstream protein of Fas-dependent apoptotic pathway, caspase-8, can cleave Bcl-2 homology domain 3 (BH3)-interfering domain death agonist (BID), and the cleaved BID to truncate BID (t-BID) then causes the release of mitochondrial cytochrome c, leading to the activation of pro-caspase-9(10).

The ‘intrinsic’ mitochondrial-dependent (type II) apoptotic pathway is mediated by internal factors, especially in mitochondria (11). The mitochondria is the main site

of action for members of the apoptosis-regulating protein family exemplified by Bcl-2 family, such as p-Bad, Bad, Bid, and t-Bid (11). Pro-apoptotic and anti-apoptotic Bcl2 family members can homodimerize or heterodimerize to each other, and appear to interact with and neutralize each other, so that the relative balance of these effectors strongly influences cytochrome c release (10, 11). P-Bad is an anti-apoptotic protein, prevents cytochrome c release whereas Bad and t-Bid, pro-apoptotic proteins, enhance cytochrome c release from mitochondria (11). When cytochrome c is released from mitochondria into cytosol, it is responsible for activating caspase-9, which further activates caspase-3 and executes the apoptotic program (17). The activated caspase-8 and caspase-9 cleaves pro-caspase-3, which then undergoes autocatalysis to form active caspase-3, a principle effecter execute of apoptosis. However the mechanisms about cardiac apoptotic pathway are still unclear.

It is well known that chronic exercise improves cardiac functions in humans significantly. Physical activity and exercise therapy has been regarded as an important therapeutic approach in the treatment of diabetes (9, 18, 19). And many studies already proved that the exercise training improved myocardial function in diabetes rats (19, 20). To our knowledge, the therapeutic effect of exercise training on cardiac apoptosis in diabetes is still unknown. In the current study, we hypothesized that exercise training may prevent cardiac Fas and mitochondria dependent apoptotic pathways in diabetes. The heart weight index, myocardial morphology, key components of Fas and mitochondria dependent apoptotic pathways and apoptotic activity in the excised hearts of three groups, Wistar rats (Control) and STZ-induced diabetes rats (DM), and DM after exercise training (DM-EX) were determined by heart weighing, histopathological analysis, Western blotting, and TUNEL assay.

Materials and Methods

Animal model

This study was approved by Institutional Animal Care and Use Committee of National Cheng Kung University University. All procedures followed the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH). Thirty-six male Wistar rats (8-week old), purchased from Laboratory Animal Center of National Cheng Kung University were randomly assigned to one of three groups. Wistar rats without streptozotocin injection (Control, n=12) and Wistar rats with streptozotocin injection (Streptozotocin-induced diabetic rats, DM, n=9, 3 rats excluded) and Wistar rats with streptozotocin injection undergoing exercise training for 8 weeks (DM-EX, n=11; 1 excluded).

All animals were housed in a temperature-controlled room (about 25 °C) with a 12 h dark-light cycle at Laboratory Animal Center of National Cheng Kung University. No statistical difference in body weight and blood glucose level was found in the beginning of the experiment. To induce type 1 diabetes mellitus, rats from the DM and DM-EX groups were fasted for three days before induction. Rats were generally anaesthetized by an intraperitoneal injection of sodium pentobarbital in the concentration of 45 mg/kg of body weight. After anaesthetization, femoral artery pulse was palpated to identify the position of femoral vein (femoral artery and vein are parallels nearby). Then the skin superficial to the femoral vein was cut into an incision but also the superficial connective tissues were removed. When the femoral vein was exposed, Streptozotocin (65 mg/kg of body weight), freshly prepared by dissolving in 50mM citrate buffer (pH 4.5) with the ratio of 1:65, was injected immediately into femoral vein (21). The incision then was sewed up and disinfected. To confirm the induction of type 1 diabetes, fasting blood glucose level (fasting overnight) was determined by glucose test from tail vein blood one week after STZ injection. STZ-induced rats failed to exhibit an elevated blood glucose level greater than 200 mg/dL were excluded from the study.

Exercise training

The 8 week exercise training protocol was implemented according to the study of Chen et al (22). Before the beginning of exercise training, rats in the DM-EX group had run on a horizontal treadmill (Model T510E, Diagnostic and Research Instruments Co., Taiwan) at the speed of 12 m/min for habituation as well as rats in . After one week of familiarization, all rats had run on the treadmill 60 minutes per

session, 5 sessions per week, for 8 weeks totally. During the training period, the running speed was gradually increased 3 m/min every two weeks, from 15 m/min to 24 m/min finally. In contrast, rats from sedentary groups were placed on the treadmill without running for 15 minutes each session. To avoid acute effect of exercise, all animals were sacrificed 48 hours after exercise training. Body weight and fasting blood glucose level were measured before sacrificing.

Cardiac characteristics

The hearts of Wistar rats and diabetes rats were excised and cleaned with PBS. The left ventricle were separated and weighed. The right tibias were also separated and tibia lengths were measured by the electronic digital venire caliper to adjust the whole heart weight. The ratios of the total heart weight to body weight, the left ventricle weight to body weight, the left ventricle weight to the whole heart weight, the whole heart weight to tibia length, and the left ventricle weight to tibia length were calculated.

Tissue Extraction

Cardiac tissue extracts were obtained by homogenizing the left ventricle samples in a lysis buffer at a ratio of 100 mg tissue/1ml buffer for 1 min. The homogenates were placed on ice and then centrifuged at 12,000 *g* for 40 min. The supernatant was collected and stored at -80°C for further experiments.

Assay of citrate synthase activity

Citrate synthase is one of the main oxidative enzymes in mitochondria. Previous studies have proven that citrate synthase activity increased significantly after chronic exercise. Therefore an increase in citrate synthase activity is commonly used as a surrogate index for the effectiveness of exercise training. Soleus muscles (weight ~0.1g) isolated from four groups were immediately stored at -80℃ until analyzed. When measuring, the defrosted muscle samples were homogenized by homogenizer (Model 398, Biospec Products Inc, Mexico) with quintuple volumes of Tris buffer (0.1 mol/L) containing Triton X-100 (0.1%). After homogenizing, the samples were centrifuged by centrifuge (Kendro Laboratory Products, Langenselbold, Germany) at the speed of 13000 rpm for 15 minutes, and then the supernatants were collected. The 50μl of supernatant was added into cuvette which had already contained 100μl of DTNB (1mM), 30μl of acetyl-CoA (10mM), and 770μl of H2O. The absorption at 412 nm of wave length was measured by spectrophotometer (Beckman Instruments, Inc.,

DU640B, CA, USA) for 10 minutes to measure the possible acetyl-CoA deacylase activity. With the addition of 50µl of oxaloacetate (10mM), the measurement of enzymatic activity was begun also by spectrophotometeric assay for 5 minutes. Every sample tested twice and averaged. The result was expressed as micromoles of substrate utilized per minute per gram of wet tissue.

Electrophoresis and Western Blot

Protein concentration of cardiac tissue extracts was determined by the Lowry protein assay. Protein samples (40µg/lane) were separated on a 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) with a constant voltage of 75 V. Electrophoresed proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, 0.45µm pore size) with a transfer apparatus (Bio-red). PVDF membranes were incubated in 5% milk in TBS buffer. Primary antibodies including Fas ligand, Fas receptor, FADD, BID, t-BID, BAD, p-BAD, cytochrome *C*, caspase-8, caspase-9, caspase-3 and α-tubulin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were diluted to 1:500 in antibody binding buffer overnight at 4°C. The immunoblots were washed three times in TBS buffer for 10 min and then immersed in the second antibody solution containing goat anti-mouse IgG-HRP, goat anti-rabbit IgG-HRP, or donkey anti goat IgG-HRP (Santa Cruz) for 1 hour and diluted 500-fold in TBS buffer. The immunoblots were then washed in TBS buffer for 10 min three times. The immunoblotted proteins were visualized using an enhanced chemiluminescence ECL western Blotting luminal Reagent (Santa Cruz, CA, USA) and quantified using a Fujifilm LAS-3000 chemiluminescence detection system (Tokyo, Japan).

Hematoxylin-eosin staining (H&E staining) and Terminal Deoxynucleotide Transferase-mediated dUTP Nick End Labeling (TUNEL)

After the hearts were excised, the hearts were soaked in formalin, dehydrated through graded alcohols, and embedded in paraffin wax. In heart tissues, the 0.2-µm thick paraffin sections were cut from paraffin-embedded tissue blocks. The tissues sections were deparaffinized by immersing in xylene, and rehydrated. For Hematoxylin-eosin staining, the slices were then dyed with hematoxylin and eosin. After gently rinsing with water, each slide was dehydrated through graded alcohols. Finally, they were soaked in xylene twice. Photomicrographs were obtained using Zeiss Axiophot microscopes. For TUNEL assay, the sections were incubated with proteinase K, washed in phosphate-buffered saline, incubated with permeabilisation

solution, blocking buffer, and then washed two times with PBS. The terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP for 60 min at 37 °C from an apoptosis detection kit (Roche Applied Science, Indianapolis, IN, USA) was used for detection. Then added the DAPI (4,6–diamidino-2-phenylindole) 5mins and the nucleus position were fluoresced by blue light at 340 / 380 nm. TUNEL-positive nuclei (fragmented DNA) were fluoresced by bright green light at 450-500 nm. The mean number of TUNEL-positive cells were counted for at least 5-6 separate fields x 2 slices x 3 regions of the left ventricle (upper, middle, lower) excised from six rat hearts in each group. All counts were performed by at least two independent individuals in a blinded manner.

Statistical Analysis

The all data of weight index, blood sugar, protein levels, and the percentage of TUNEL positive cells were compared among the control, DM, and DM-EX groups using one-way analysis of variance (ANOVA) with pre-planned contrast comparison. In all cases, $P<0.05$ was considered significant.

Results

Body weight and cardiac characteristics

DM and DM-EX weighed were less than the Control. The other index of whole heart weight (WHW), left ventricular weight (LVW), WHW/body weight, LVW/body weight, LVW/WHW, WHW/tibia and LVW/tibia length showed no different amount three groups, Control, DM, DM-EX. And the blood sugar increased in DM and decreased in DM-EX (Table 1).

Assay of citrate synthase activity

Exercise training for 8 weeks significantly increased the citrate synthase activity of soleus muscle in the DM-Ex group as compared with the Control, DM groups ($P<0.05$), as shown in Table 1. These results indicated that our exercise program was effective.

Hematoxylin-eosin staining (H&E staining)

To investigate whether there were changes in cardiac architecture, we did a histopathological analysis of ventricular tissue stained with hematoxylin and eosin. After viewing 400 X magnified images, we found that the ventricular myocardium in the Control group showed normal architecture with normal interstitial space, but the abnormal myocardial architecture and the increased interstitial space were observed in the DM group. These myocardial architecture abnormalities in the DM-EX group became normal than those in the DM group (Fig 1).

Upstream components of cardiac Fas receptor dependent apoptotic pathways

To investigate the upstream components of cardiac Fas receptor dependent apoptotic signaling pathways in diabetic rats after exercise training, we measured the protein levels of Fas-dependent pathway in hearts excised from control, DM, and DM-EX groups. Compared with the control group, the protein levels of Fas ligand, Fas receptor and FADD (Fig 2), were significantly increased in the DM group but were not changed in the DM-EX group. The protein levels of Fas ligand, Fas receptor and FADD (Fig 2), in the DM-EX group were significantly lower than those in the DM group.

Downstream components of cardiac Fas receptor dependent apoptotic pathways

In order to identify the downstream components of cardiac Fas receptor (caspase-8 and 3) dependent apoptotic pathways, the caspase-8 and 3 were measured by Western blotting in the hearts excised from the control, DM, and DM-EX groups. Compared to the control group, the protein products of activated caspase-8 and 3 were

increased in the DM groups but not changed in the DM-EX group (Fig 3, 4). The protein level of activated caspase-8 and 3 in the DM-EX group were significantly lower than those in the DM group (Fig 3, 4).

Upstream components of cardiac mitochondria dependent apoptotic pathways

To investigate the upstream components of cardiac mitochondria dependent apoptotic signaling pathways in diabetic rats after exercise training, we measured the protein levels of mitochondria-dependent pathway in hearts excised from the control, DM, and DM-EX groups. Compared with the control group, the protein levels of t-BID (Fig 5), BAD (Fig 6), and cytochrome *C* (Fig 6) were significantly increased in the DM group but were not changed in the DM-EX group. The protein levels of t-BID (Fig 5), BAD (Fig 6), and cytochrome *C* (Fig 7) in the DM-EX group were significantly lower than those in the DM group.

Downstream components of cardiac mitochondria dependent apoptotic pathways

In order to identify the downstream components of cardiac mitochondria (caspase-9 and 3) dependent apoptotic pathways, the caspase-9 and 3 were measured by Western blotting in the hearts excised from the control, DM, and DM-EX groups. Compared to the control group, the protein products of activated caspase-9 and 3 were increased in the DM groups but not changed in the DM-EX group (Fig 8, 9). The protein level of activated caspase-9 and 3 in the DM-EX group were significantly lower than those in the DM group (Fig 8, 9).

TUNEL-positive apoptotic cells of cardiac tissues

In order to view the apoptotic activity in cardiac tissues, the apoptotic cells and total cells were measured by TUNEL assay and DAPI staining respectively in the hearts excised from the control, DM, and DM-EX groups. Viewing images magnified 400 X, we observed that the left ventricles of the DM groups stained with TUNEL assay had a greater number of TUNEL-positive cardiac cells than those in the control; group whereas the number of TUNEL-positive cardiac cells was similar between control and DM-EX group. Decreases in number of TUNEL-positive cardiac cells were found in the DM-EX group, compared with DM group (Fig 10).

Discussion

Major findings

Our main findings can be summarized as follows: Our main findings can be summarized as follows: (1) the myocardial architecture abnormalities in diabetic rats after exercise training became normal than those in the diabetic rats sowed in body weight and blood sugar. And whole heart weight, left ventricular weight, whole heart weight normalized by tibia length and body weight, left ventricular weight normalized by tibia length and body weight in both groups were no difference. (2) The activity of the cardiac Fas receptor-dependent and mitochondria-dependent apoptotic pathways in diabetic rats were significantly decreased after exercise training, the evidence for which is based on decreases in Fas ligand, Fas receptor, FADD, activated caspase-8, and activated caspase-3, compared with the sedentary hypertensive group and mitochondria related proteins, t-Bid, Bad, cytochrome C, activated caspase-9 and activated caspase-3. (3) After integrating our current findings into previously proposed apoptotic theories, we draw a hypothesized diagram in Fig 10, which suggest that cardiac Fas receptor-dependent and mitochondria-dependent pathways were increased in diabetes and suppressed by exercise training. Our findings imply that exercise therapy could be one of possible therapeutic approaches to prevent delirious cardiac apoptosis in diabetes mellitus.

Experimental limitation

The benefit of exercise may be involved in multiple factors such as anti-hypertension, weight loss, anti-diabetes, and normalized lipid profiles (23). Exercise improved heart function successfully and further attenuated age-induced elevation in BAX/Bcl-2 ratio, and inhibit apoptosis in rat heart(24). Therefore, the underlying mechanism how exercise prevent cardiac apoptosis in diabetes remain unknown. Any therapeutic effect of exercise training on cardiomyopathic changes and cardiac apoptosis noted in the current study cannot be isolated and attributed to any specific factor, such as weight loss, blood pressure changes or other unclear factors. However, the current study can differentiate therapeutic effects of exercise training on cardiomyopathic changes and cardiac apoptosis.

Cardiac changes

While in the recently studies, exercise training has been shown to protect the myocardium dysfunction(25, 26). However, the current study can differentiate therapeutic effects of sedentary diabetes and diabetes with chronic exercise training.

The current study showed that exercise training improved myocardial structure. The balance between cell death and cell survival is a tightly controlled process, especially in terminally differentiated cells, such as the cardiomyocytes. Therefore, prevention of cardiac apoptosis and cardiac cell death in heart health is very critical issues in the diabetes-associated heart diseases.

Cardiac Fas receptor- and mitochondrial-dependent apoptotic pathways

This study also represents the first to report the prevention from an increase in one of major cardiac apoptotic pathways, Fas receptor-dependent and mitochondria -dependent apoptotic pathway, in diabetes after exercise training. The Fas receptor-dependent apoptotic pathway is mediated by Fas ligand, Fas-associated death domain (FADD) and activation of caspase-8 and 3, which in turn induces cardiac myocyte apoptosis. And The mitochondria-dependent apoptotic pathway is mediated by t-Bid, Bad, cytochrome *c* and activation of caspase-9 and 3, which in turn induces cardiac myocyte apoptosis (11). In the current study, exercise training was found to significantly prevent from more activated Fas receptor-dependent and mitochondria-dependent apoptotic pathway observed in sedentary diabetes, as evidenced by increases in cardiac Fas ligand, Fas-associated death domain (FADD), t-BID, BAD, cytochrome *C*, activated caspase-9, activated caspase-8 levels and activated caspase-3 levels in diabetes rats hearts but no increase after exercise training. Therefore, our findings strongly suggest that exercise training in diabetes could prevent cardiac Fas receptor-dependent and mitochondria-dependent apoptotic pathways.

Hypothesized clinical application

Diabetes related cardiomyopathies may progressively develop in heart failure. Diabetes is considered as a major risk factor for the development of heart failure, even when other known risk factors for heart failure are excluded. Since cardiac tissues are difficult to be extracted from diabetic human hearts, the current diabetic animal model under exercise training should provide an important explanation how clinical exercise prescription prevents heart failure or apoptosis-related cardiac diseases in diabetic humans. Since diabetes will enhance cardiac apoptosis and exercise training can prevent this progression, diabetes should be highly aware of the possibility of progressive development in cardiac abnormality as well as the importance of exercise therapy and modified lifestyle. Based on the current evidence from animal study showing that exercise training did prevent the major apoptotic pathways and prevent

cardiac apoptosis in diabetic rats, we might further hypothesize EXERCISE IS A MEDICINE of cardiac apoptosis in diabetes mellitus. Besides, further questions is raised what underlying mechanism suppress cardiac Fas-dependent and mitochondria-dependent apoptotic pathways when exercise training undergo as well as what exercise intensity, exercise duration, and exercise frequency are minimal requirement to prevent cardiac apoptosis in the certain degree of diabetes mellitus. Of course, further studies are required to elucidate specific mechanisms responsible for this therapeutic effect in diabetes and further clinical studies are required to clarify the possible therapeutic application in diabetic human.

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Table 1. Cardiac characteristics of Control, DM group and DM with exercise training

	Control	DM	DM-EX
Number of animals	9	6	8
Body weight (BW),g	403.08±36.47	300.11±29.03***	324.63±26.8***
Whole heart weight (WHW),g	1.15±0.24	1.00±0.24	1.00±0.11
Left ventricular weight (LVW),g	0.84±0.17	0.72±0.17	0.73±0.08
WHW/ BW($\times10^4$)	28.48±6.03	33.41±8.00	30.70±3.46
LVW/ BW($\times10^4$)	20.76±4.12	23.97±5.68	22.46±2.52
LVW/ WHW	0.73±0.023	0.72±0.02	0.73±0.01
WHW/ Tibia($\times10^3$) ,g/mm	28.27±5.14	25.61±5.76	25.52±2.64
LVW / Tibia($\times10^3$) ,g/mm	20.62±3.52	18.37±4.10	18.67±1.90
Blood suger (mg/dL)	70.14±13.75	324.13±82.81***	289.25±58.52***
citrate synthase activity (μmol/min/g wet wt)	1.91±0.01	1.88±0.07	2.15±0.08 *#

Values are means ± SD among Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX). $^{*}P<0.05$, $^{**}P<0.01$ significant differences between Control and DM or Control and DM-EX group. $^{\#}P<0.05$ significant differences between DM group and DM-EX group

Fig 1. H&E stain

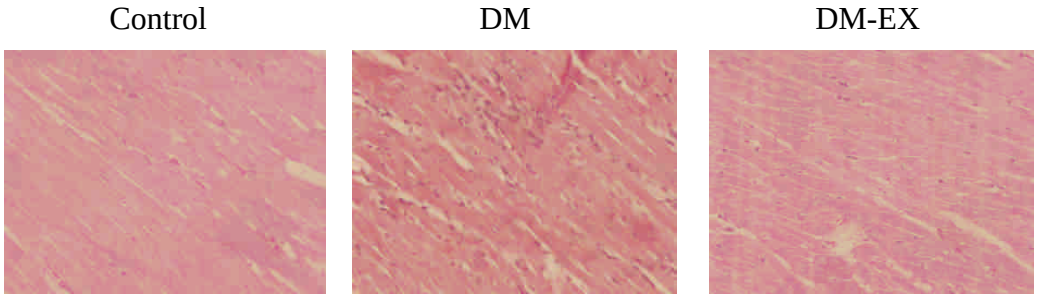


Fig 1. Representive histopathological analysis of cardiac sections from left ventricles was performed with Hematoxylin and eosin (H&E) staining in Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX). The images of cardiac architecture were magnified by 400 times. (n=3~4 in each group).

Fig 2.

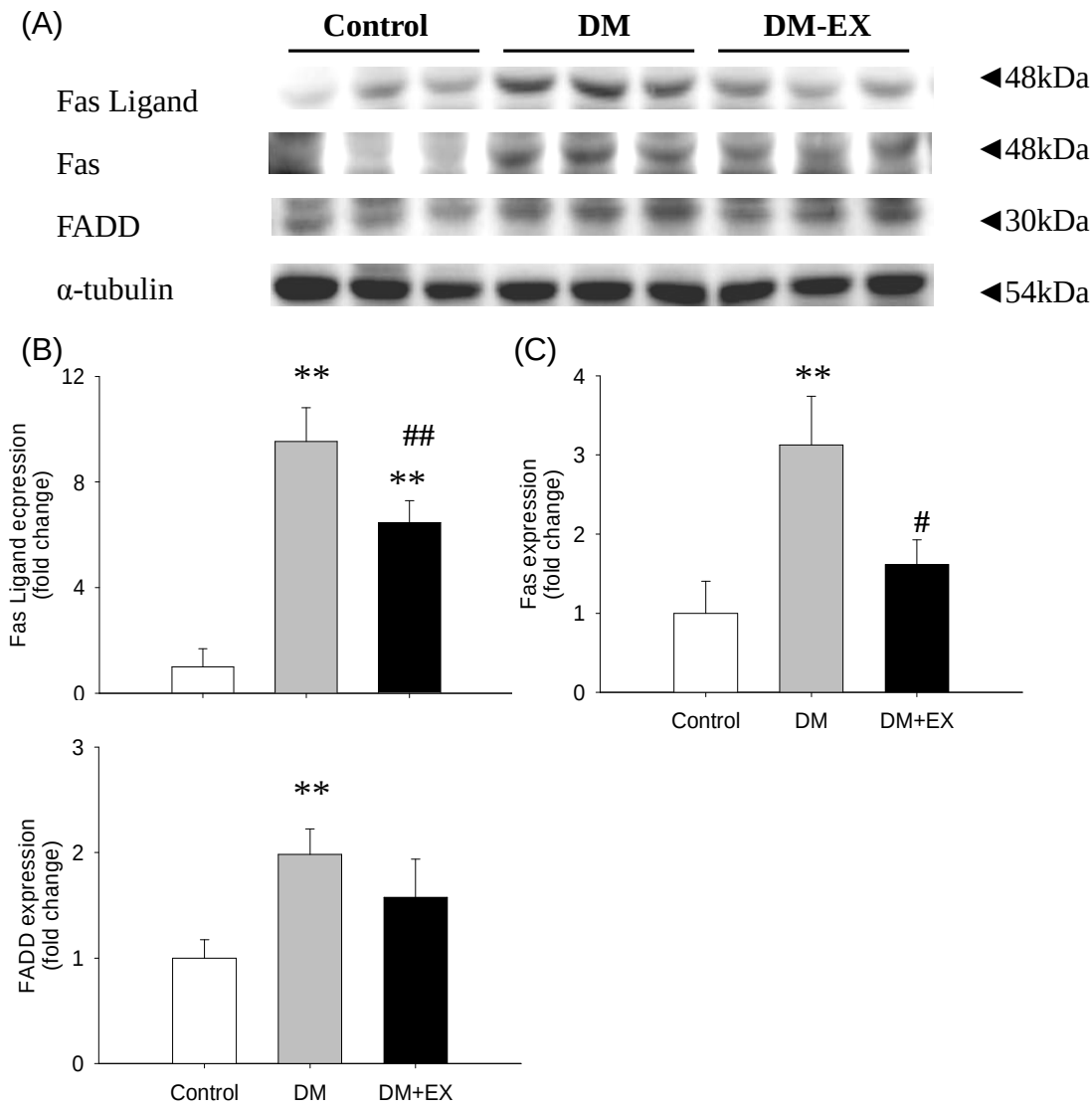


Fig 2. (A) The representive protein products of Fas ligand, Fas receptor and Fas-associated death domain (FADD) extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in Fas ligand/α-tubulin and Fas receptor/α-tubulin and FADD/α-tubulin and mean values±SD (n=6 in each group). **P*<0.05, ***P*<0.01, significant differences from Control group. $^{\#\#}P$<0.01 significant differences between DM group and DM-EX group.

Fig 3.

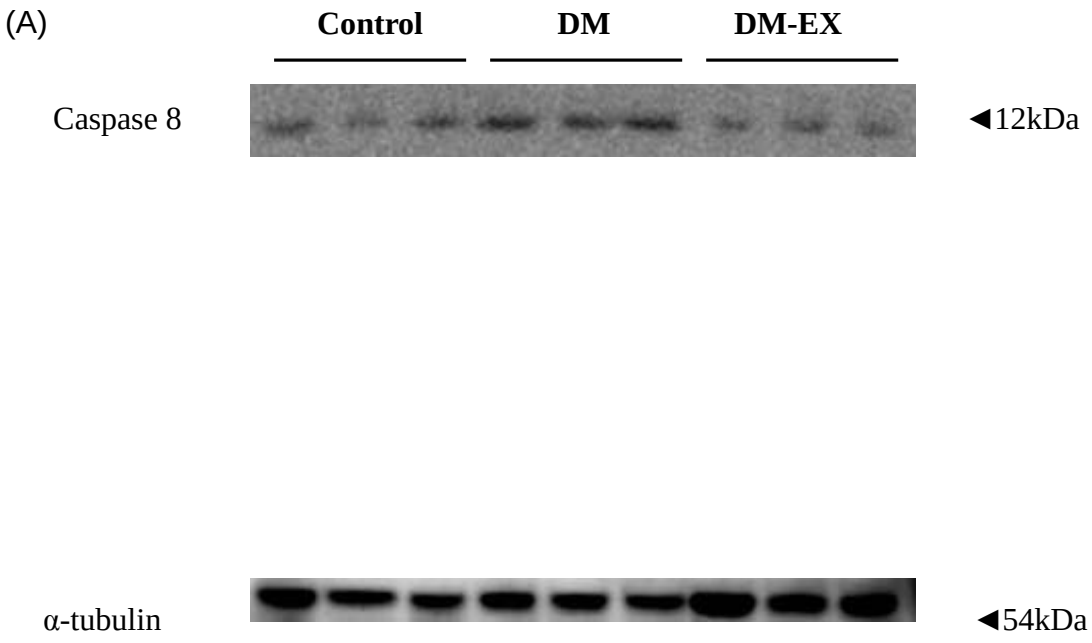


Fig 3. (A) The representive protein products of Caspase-8 extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in Caspase-8 /α-tubulin and mean values±SD (n=6 in each group). *P<0.05, **P<0.01, significant differences from Control group. ##P<0.01 significant differences between DM group and DM-EX group.

Fig 4.

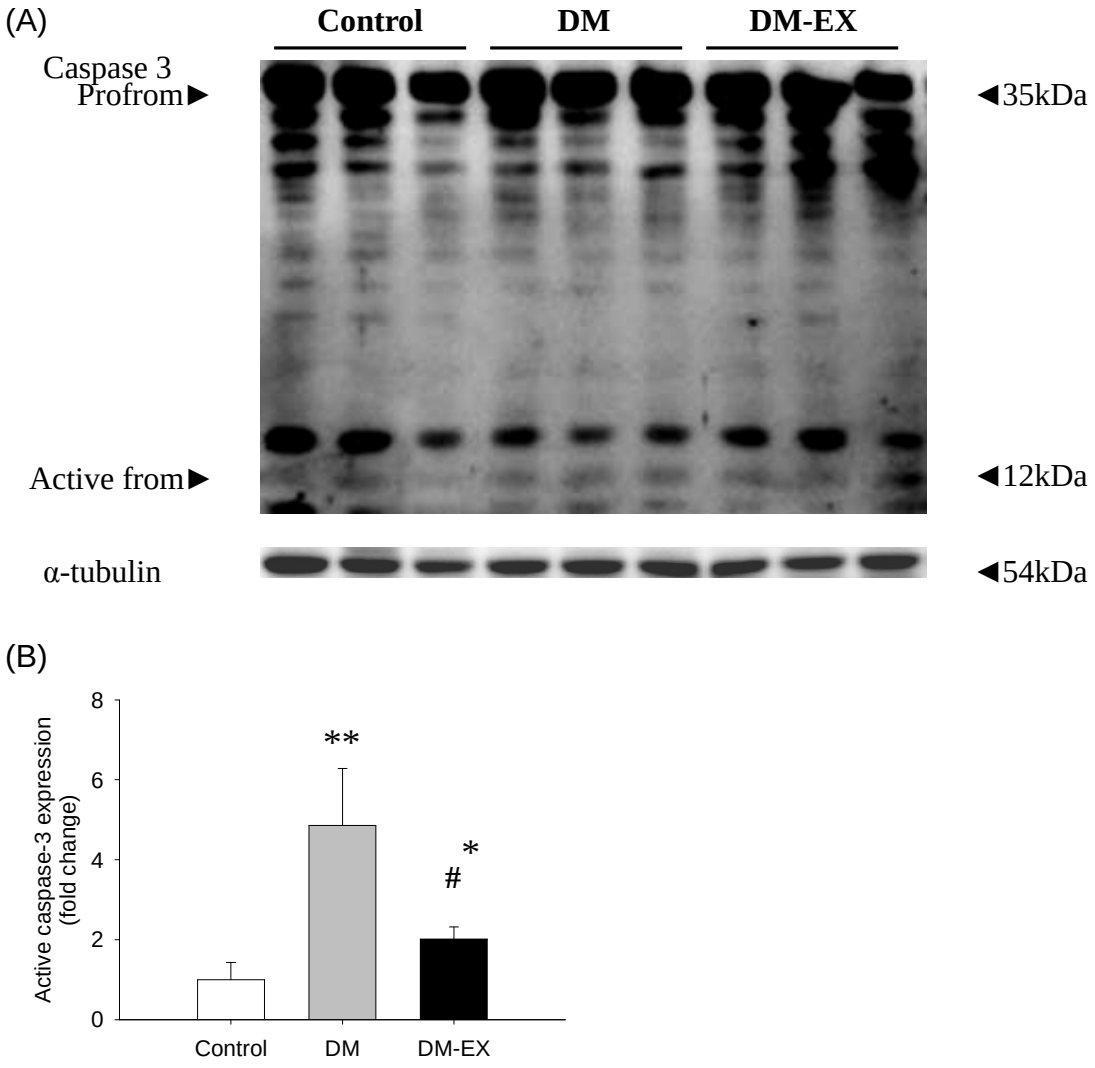


Fig 4. (A) The representive protein products of Caspase-3 extracted from the left ventricles of excised hearts in the 3 wistar rats (Control), 3 streptozotocin-induced diabetes rats (DM) and 3 streptozotocin-induced diabetes rats after exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in Caspase-3/α-tubulin and mean values±SD (n=6 in each group). *P<0.05, **P<0.01, significant differences from Control group. ##P<0.01 significant differences between DM group and DM-EX group.

Fig 5.

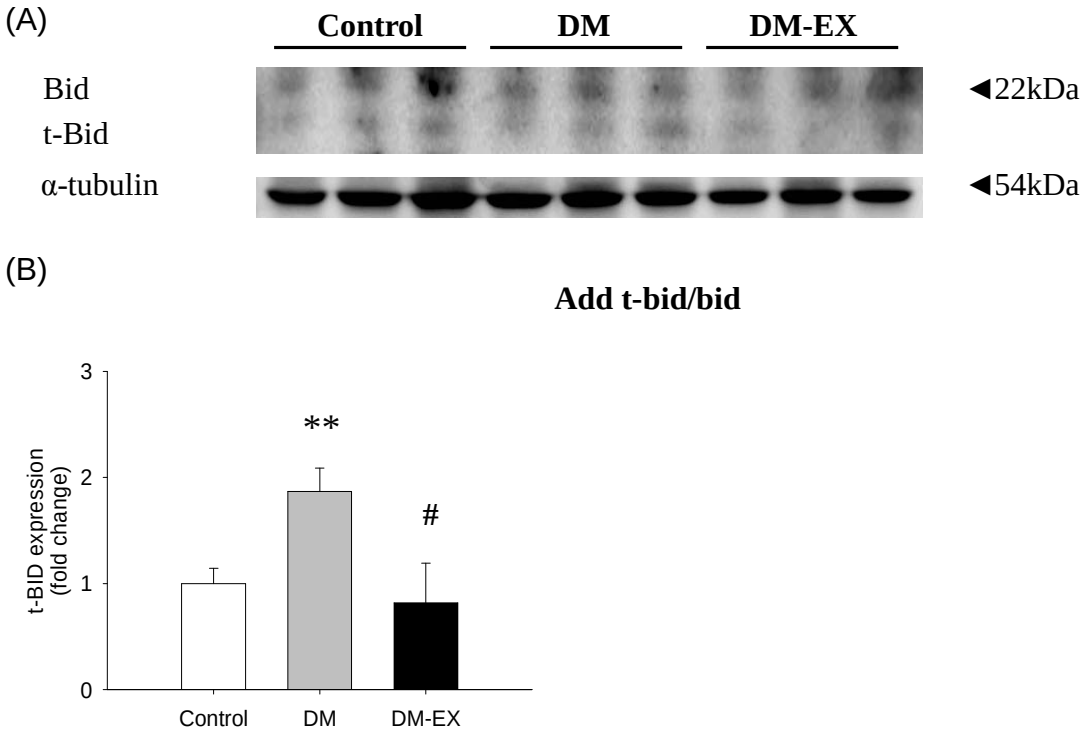


Fig 5. (A) The representive protein products of BH3 interacting domain death agonist (BID) and truncated BID (t-BID) extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in t-BID/α-tubulin and mean values±SD (n=6 in each group). ***P*<0.01, significant differences from Control group. $^{\#}P$<0.05 significant differences between DM group and DM-EX group.

Fig 6.

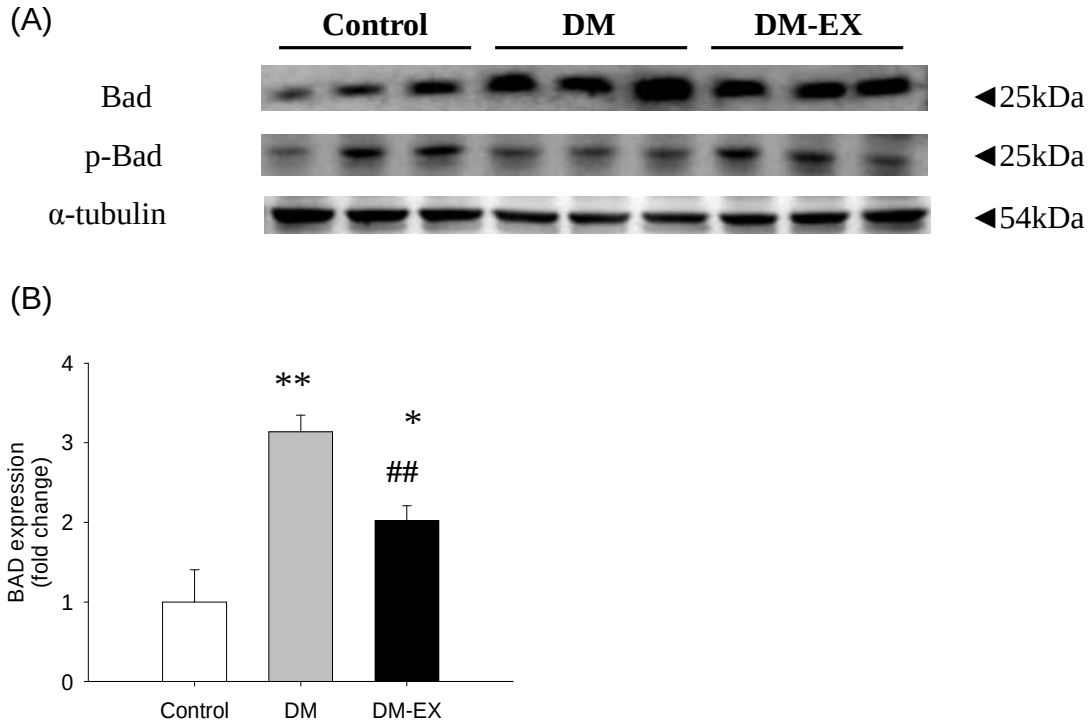


Fig 6. (A) The representive protein products of Bcl-2-associated death promoter (BAD) and phosphates Bcl-2-associated death promoter (p-BAD) extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in BAD/α-tubulin and mean values±SD (n=6 in each group). *P<0.05, **P<0.01, significant differences from Control group. $^{\#\#}P$<0.01 significant differences between DM group and DM-EX group.

Fig 7.

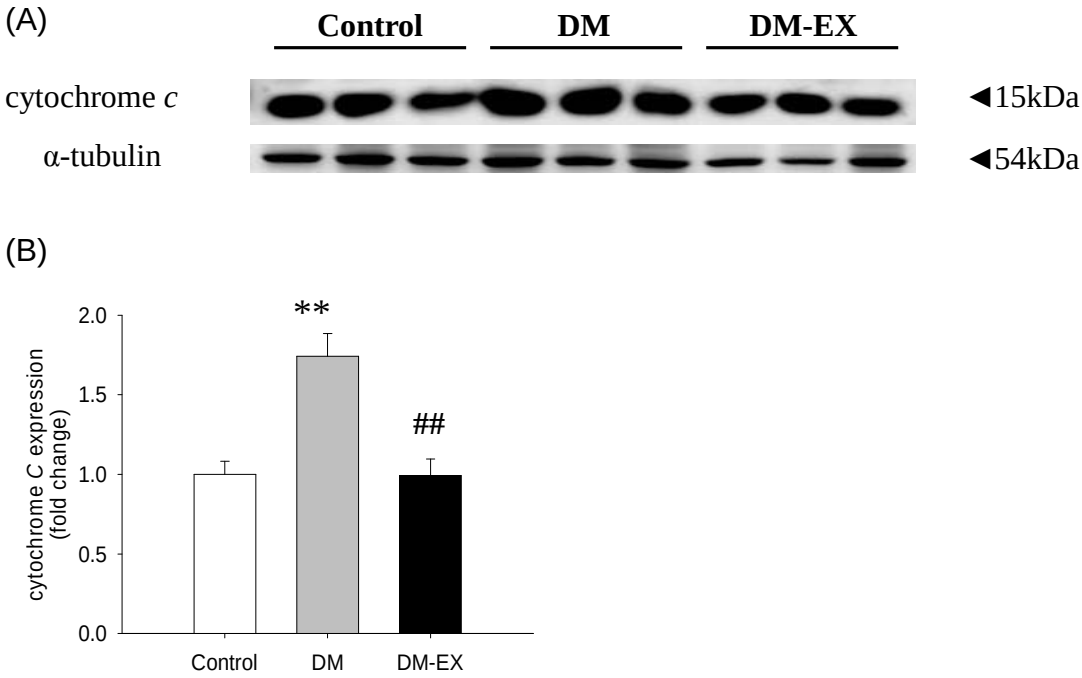


Fig 7. (A) The representive protein products of Cytochrome *C* extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in Cytochrome *C* /α-tubulin and mean values±SD (n=6 in each group). $^{**}P<0.01$, significant differences from Control group. $^{\#\#}P<0.01$ significant differences between DM group and DM-EX group.

Fig 8.

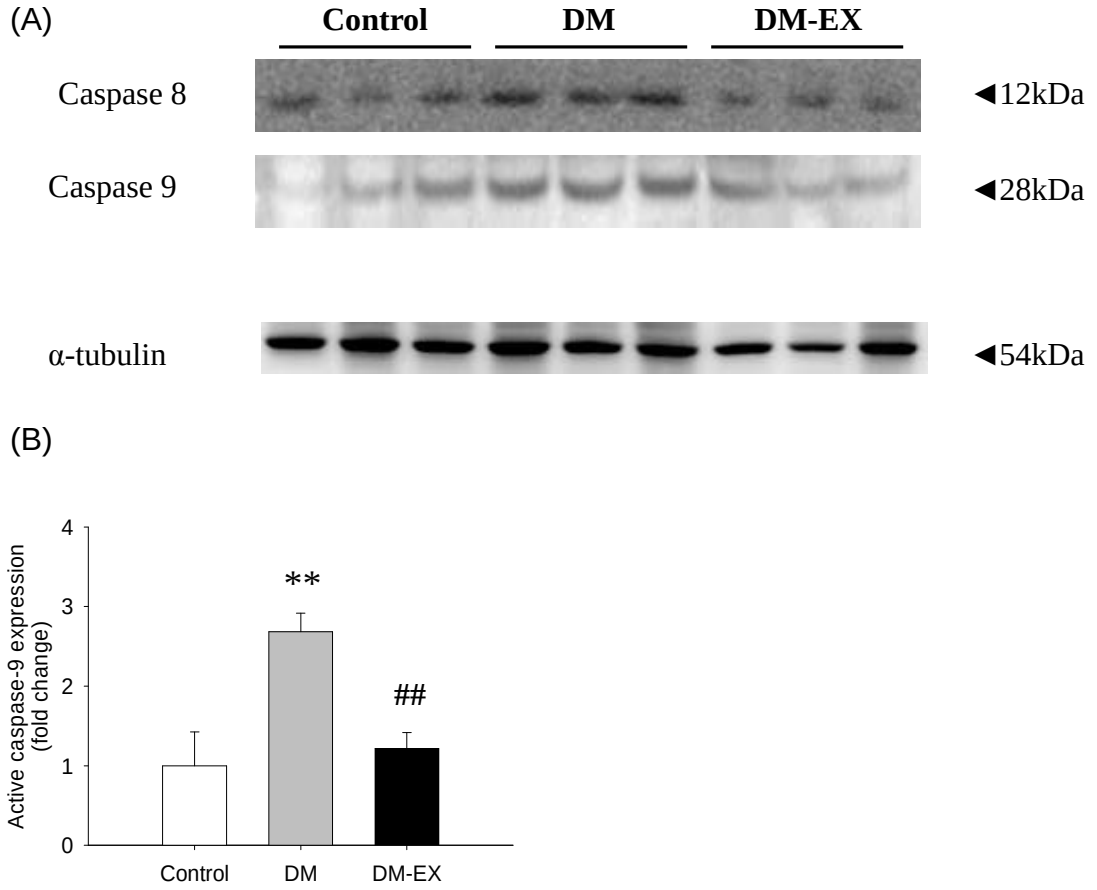


Fig 8. (A) The representive protein products of Caspase-9 extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in Caspase-9/α-tubulin and mean values±SD (n=6 in each group). ***P*<0.01, significant differences from Control group. $^{\#\#}P$<0.01 significant differences between DM group and DM-EX group.

Fig 9.

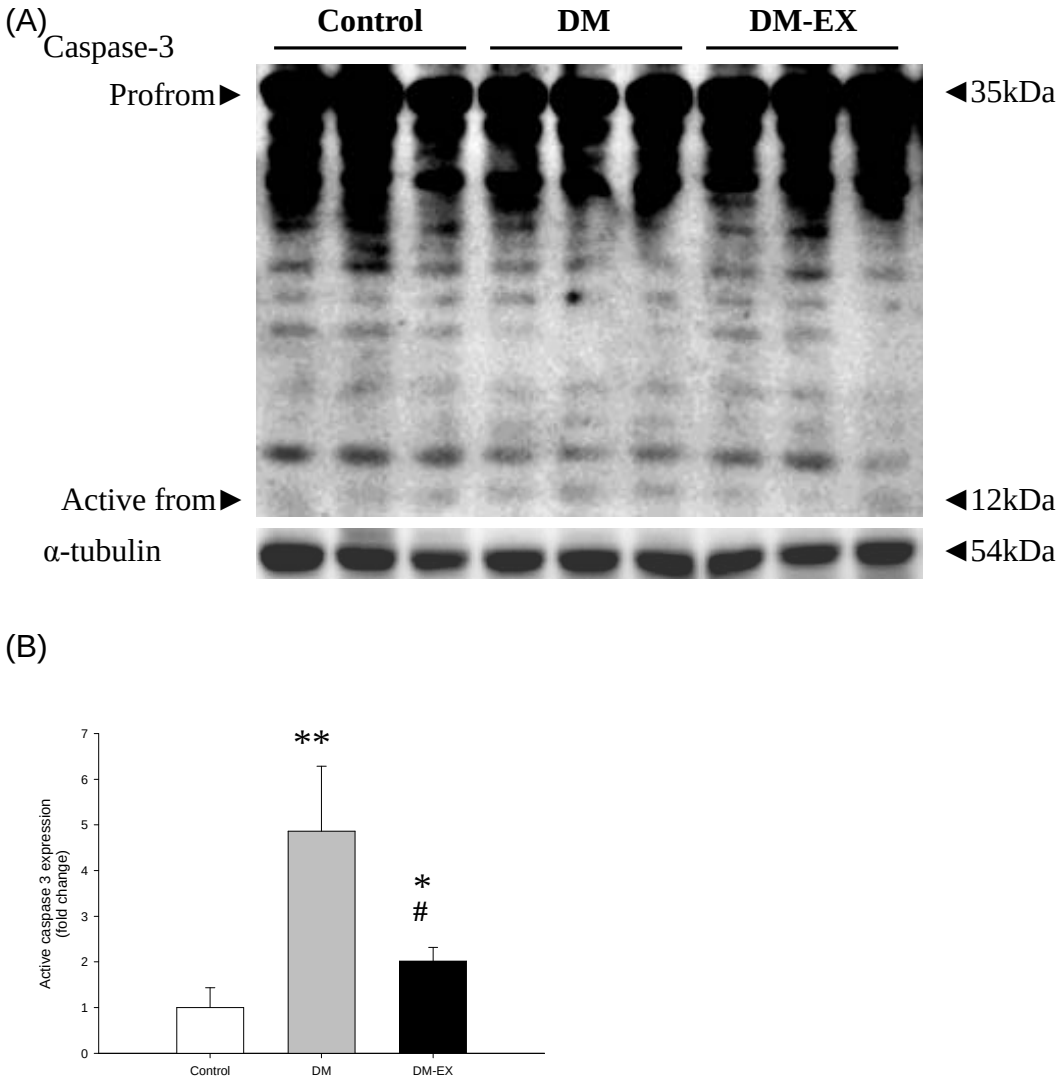


Fig 9. (A) The representive protein products of Caspase-3 extracted from the left ventricles of excised hearts in 3 Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by Western Blotting analysis. α-tubulin was use as a internal control. (B) Bars represent the relative fold changes of protein quantification relative to Control group in Caspase-3/α-tubulin and mean values±SD (n=6 in each group). **P*<0.05, ***P*<0.01, significant differences from Control group. $^{\#}$*P*<0.05 significant differences between DM group and DM-EX group.

Fig 10. TUNEL

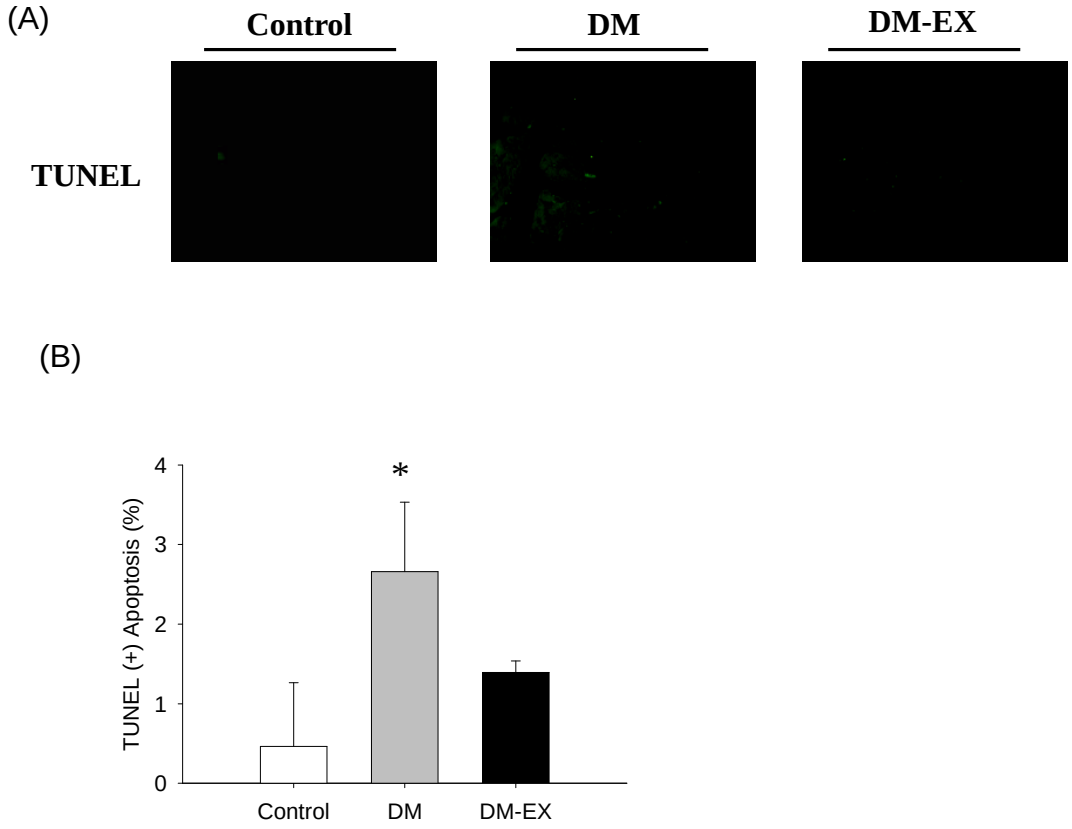
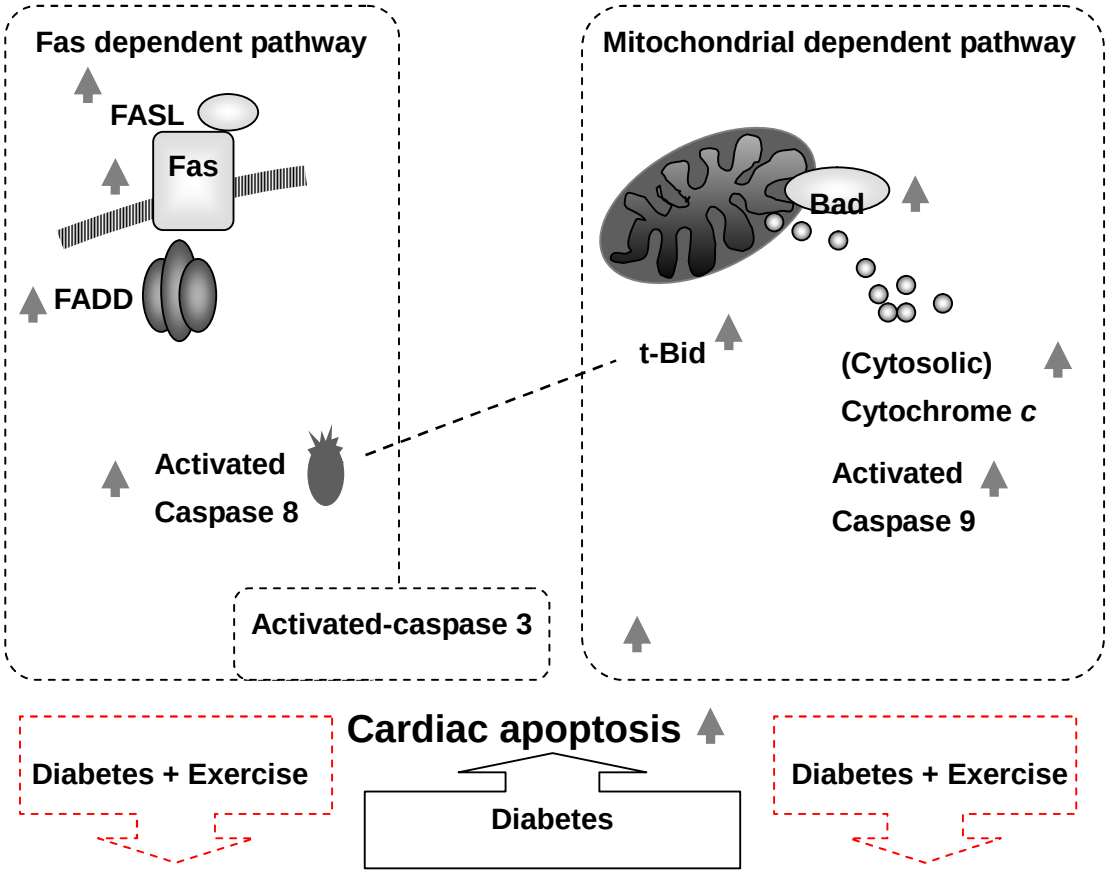


Fig 10. (A) Representive stained apoptotic cells of cardiac sections from left ventricle in Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with exercise training (DM-EX) were measured by staining with TUNEL assay with dark background (right panels, green spots). The relative background tissues were shown in left panels. The images were magnified by 400 times. (B) Bars present the percentage of TUNEL positive cells relative to total cells. *P<0.05, **P<0.01, significant differences from Control group. ##P<0.01 significant differences between DM group and DM-EX group.

Fig 11.


Fas dependent pathway
FASL
Fas
FADD
Activated
Caspase 8
Activated-caspase 3
Mitochondrial dependent pathway
Bad
t-Bid
(Cytosolic)
Cytochrome c
Activated
Caspase 9
Cardiac apoptosis
Diabetes
Diabetes + Exercise
Diabetes + Exercise

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

99　年 7 月 23 日

附件三

報告人姓名	李信達	服務機構及職稱	中國醫藥大學物理治療學系
會議時間地點	XI International Conference on Obesity - ICO 2010 - Stockholm, Sweden99 年 7 月 11 日至 99 年 7 月 15 日	本會核定補助文號	0990505FD24001 電子文 NSC 98-2314-B-039-002
會議名稱	（中文）國際肥胖會議 （英文）International Conference on Obesity		
發表論文題目	（中文）肥胖與老化加乘心臟纖維化 （英文）The coexistence of obesity and aging additively increase TGF-beta and MMP related cardiac fibrosis		

一、參加會議經過

會議摘要：

中國醫藥大學物理治療學系教授 李信達於2010年 7月 15 至Stockholm, Sweden參加XI International Conference on Obesity - ICO 2010 ，發表The coexistence additively increase TGF-beta and MMP related cardiac fibrosis此次會議的主題是”Gobal Obesity research”，因為個人對肥胖研究主題的興趣所以對第二型糖尿病也深感興趣。這一次參加會議主題自己參加會議的主題設定在肥胖和第二型糖尿病患者導致心血管異常的主題。在2010年International Conference on Obesity會議裡聽取最新肥胖和的臨床醫療報告、臨床研究、基礎研究、幾場主題研討報告會。如 “Metabolic control by brain “，”Early determinants of obesity “，“Inflammation in human obesity “，” obesity research - Towards new biomarkers”，會議中吸收了不少新知，也對最新肥胖的運動治療稍做了一些深入探討。

二、與會心得

心臟肥大主要是由心肌細胞肥大所造成，心肌細胞肥大可分為生理性肥大和病理性心肌肥大。運動員強而有力的心臟多為生理性肥大，而病理性心肌肥大包括向心性肥大和離心性肥大，很多研究發現肥胖的病人長久下來常發展成離心性的左心室肥大，過度離心式的細胞肥大會導致心臟細胞的損傷，而此細胞損傷的過程與細胞的進入心肌凋亡化現象和心臟纖維化有關。肥胖的患者心臟肥大的詳細機轉仍不是很清楚，肥胖的患者長期承受較高血壓、血液量、心輸出量，可能容易造成心臟肥大，因此有比較高心臟衰竭的機會，研究也顯示肥胖者有較高的機會發生心臟衰竭和猝死。心臟肥大進而導致心臟衰竭是臨床醫學上的重要問題，肥胖者心臟肥大的發展與多方面的因素有關，包括左心室的負荷程度、高血壓的嚴重度和罹患高血壓的時間、血量增加的多少、肥胖時間的長短、和胰島素阻抗的大小。高血壓也是決定左心室肥大的重要因素，研究顯示在高血壓肥胖患者平均左心室內徑呈現擴張性的增加，平均左心室直徑增加，平均左心室大小比非高血壓肥胖患者或相對較瘦者明顯增加許多。肥胖時間大於15年者比較肥胖時間小於15年者呈現較明顯的左心室肥大。肥胖老鼠的實驗中發現肥胖症會慢慢發展心臟肥大與心臟凋亡(apoptosis)，心臟肥大常隨著老化或肥胖漸趨嚴重，心臟的肥大慢慢會導致心臟功能不全，甚至慢慢導致肥胖症的心臟凋亡。細胞凋亡是一種程序性細胞死亡，凋亡過程中細胞縮小，DNA被切成片段，比起細胞壞死，細胞凋亡是更常見的細胞死亡形式。由於成年後哺乳類動物的心臟被認為缺乏再生能力或至少再生能力是很差的，甚至出生後不久即無法再生新的心肌細胞，因此心肌細胞凋亡後，就容易慢慢發展成心臟衰竭甚至死亡。經動物實驗證實心臟凋亡的兩大途徑death receptor-dependent及mitochondria- dependent已明顯發現在肥胖狀態被誘發凋亡，在此研究中發現肥胖鼠在不需特別缺氧、傷害、梗塞的狀態下，心肌細胞已走向肥胖症的病理性肥大與肥胖症的心肌細胞凋亡。至於為甚麼導致心臟的纖維化越老越嚴重，原因眾多，仍需進一步探討和研究。運動對肥胖症的好處多多，別忘了時時提醒自己和大家動起來保持健康。

三、考察參觀活動(無是項活動者省略): 無

四、建議: 多提倡運動風氣

五、攜回資料名稱及內容: 摘要論文集

六、其他: 無

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：李信達	計畫編號：98-2314-B-039-002-
計畫名稱：SIRT1 activators 配合運動治療對第二型糖尿病抗老化延壽和抗心臟細胞凋亡的療效探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		1
國外	論文著作	期刊論文	0	0	100%	篇	1
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

Background. Cardiac apoptosis was found in diabetes but very limited information regarding the influence of chronic exercise on cardiac apoptosis in diabetes was available. The purpose of this study was to evaluate the influence of chronic exercise on cardiac apoptotic pathways. Chronic exercise suppressed diabetes-induced Fas-dependent and mitochondria-dependent cardiac apoptotic pathways. Chronic exercise may be one of possible therapeutic approaches for preventing cardiac apoptosis in diabetes.