EXERCISE TRAINING MODULATES APOPTOTIC SIGNALING IN THE AGING RAT HEART

A Thesis

by

HYO BUM KWAK

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Kinesiology

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Approved as to style and content by:

John M. Lawler (Chair of Committee)	Robert B. Armstrong (Member)
David M. Hood (Member)	Steve M. Dorman (Head of Department)

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ABSTRACT

Exercise Training Modulates Apoptotic Signaling

in the Aging Rat Heart. (August 2004)

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Aging is characterized by a progressive decline in cardiac function. A critical contributor to the age-related impairment in heart function is the loss of cardiac myocytes through “apoptosis”, or programmed cell death. A dramatic increase in the rate of apoptosis has been reported with aging in the rat left ventricle. In contrast, exercise training not only improves cardiac function, but also reduces the risk of heart disease. However, the ability of exercise training to modulate apoptotic signaling and apoptosis in the aging heart remains unknown. Therefore, the purpose of this study was to determine the effects of exercise training on apoptotic signaling and apoptosis in the aging heart. We hypothesized that (1) aging would increase pro-apoptotic signaling and apoptosis in the rat left ventricle, and (2) exercise training would ameliorate upregulation of Bcl-2 family-driven apoptosis in the heart. Four and 25 month old Fischer-344 rats were assigned to four groups: young control (YC), young trained (YT), old control (OC), and old trained (OT). Exercise training groups ran on a treadmill for 60 min/day at 15 m/min (15˚ incline), 5 d/wk for 12 wk. Protein expression of Bax, Bcl-2, caspase-9, and cleaved caspase-3 was measured using Western immunoblot analysis. Apoptosis (DNA

fragmentation) was assessed using a cell death detection ELISA. Bax levels in OC were dramatically higher (+176.0%) compared to YC. In contrast, exercise training resulted in a significant decrease (-53.4%) in Bax in OT compared to OC. Bcl-2 levels in OC were lower (-26.3%) compared to YC. Conversely, exercise training significantly increased Bcl-2 levels by 117.8% in OT compared to OC. Caspase-9 levels were higher (+98.7%) in OC than YC, while exercise training significantly reduced caspase-9 levels in YT (-52.6%) and OT (-76.9%), respectively. Aging resulted in a dramatic increase (+122.8%) in cleaved caspase-3 levels and a significant decrease (-32.9%) with exercise training. Finally, apoptosis (DNA fragmentation) significantly increased (+163.8%) with aging and decreased (-43.9%) with exercise training. These novel data indicate that aging increases pro-apoptotic signaling and apoptosis in the left ventricle, while exercise training is effective in diminishing pro-apoptotic signaling and apoptosis in the aging heart.

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Finally, I dedicate this thesis to my wife, Jin-Hee, who tolerated and encouraged me through this endeavor. I deeply appreciate her patience and love.

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INTRODUCTION

Aging is characterized by a progressive decline in cardiac function including stroke volume, cardiac output, blood flow, and oxygen consumption (2, 11) and an increase in susceptibility to inflammation, oxidative stress, and disease (53, 63). Impaired cardiac function, increased inflammatory cytokines, and elevated oxidative stress are commonalities between aging and cardiovascular disease. A significant reduction in the number of myocytes is now believed to contribute directly to impaired contractile function, cardiomyopathy, heart disease, and heart failure (24, 48). Indeed, the typical 70 year old man has a 30% reduction in the number of cardiac myocytes compared with young adults (19, 42).

Loss of myocytes with aging occurs through necrosis and apoptosis (i.e., programmed cell death), two distinct mechanisms leading to cell death. Necrosis results from cellular injury seen with infection or inflammatory disease, and is characterized by cellular swelling and rupturing (7, 45, 63). Cell rupture with necrosis results in the release of stress and inflammatory proteins and substrates. In contrast, apoptosis is highly regulated cell death without injury, and is characterized by cell shrinking/blebbing and condensation of the nucleus (7, 47, 63). Apoptosis is a highly regulated, programmed means of cell death or elimination that plays an essential role in governing development, growth, and repair (18, 23, 63). However, too much apoptosis results in dysfunction and disease. A dramatic increase in the rate of apoptosis has been

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reported with aging in the rat left ventricle, while the rate of necrosis remained constant (19, 24). Phaneuf and Leeuwenburgh (46) recently described age-related disruption of Bcl-2 family signaling in the rat heart. Progressive apoptosis from age in post-mitotic tissues such as the heart is dire, as lost myocytes are not replaced. Unfortunately, the mechanisms responsible for apoptotic signaling and apoptosis in the aging heart remain unclear and very limited.

In contrast, exercise training can reduce the risk of injury, oxidative stress, and inflammatory signaling from mechanical and oxidant perturbations, as long as overtraining does not occur (13, 17, 49). Also, exercise training improves cardiovascular capacity and reduces cardiovascular disease risk in both young adults and the elderly (3, 30). Exercise has the potential to reduce apoptosis through upregulation of protective stress-sensitive proteins including nuclear factor kappaB (NF-κB), insulin-like growth factor (IGF-1), and heat shock proteins (HSP90 and HSP70) (15, 36, 39). However, the mechanisms by which exercise training improves heart function and cardiovascular disease risk profile are not well delineated. Moreover, the ability of exercise training to modulate Bcl-2 family apoptotic signaling and apoptosis in the aging heart has not been explored.

Therefore**,** the purpose of this study was to determine the effects of exercise training on apoptotic signaling and apoptosis in the aging heart. Specifically, our hypotheses were that (1) aging would increase pro-apoptotic proteins, Bax, caspase-9, and caspase-3 and apoptosis (DNA fragmentation), and decrease anti-apoptotic protein, Bcl-2, in the rat left ventricle, and (2) exercise training would reverse age-induced

changes in apoptotic signaling (Bax, Bcl-2, caspase-9, and caspase-3) and apoptosis (DNA fragmentation).

Apoptosis and mitochondrial control

Apoptosis is regulated by genetic programming and plays an essential role in governing development, growth, and disease (18, 63). One of the most visible examples of cell death that occurs in normal animal development is the loss of the tadpole's tail (23). However, too much apoptosis or dysregulation of apoptosis results in dysfunction and disease (63).

Apoptosis is distinct from necrosis (7, 14, 47). Necrosis or "accidental cell death" is the pathological process which is caused by injury, infection or inflammatory diseases such as high blood pressure and heart attacks (20). In contrast, apoptosis or "programmed cell death" is highly regulated cell death involving DNA fragmentation, blebbing, and dismantling of the cell (23, 31).

Apoptotic signaling induces apoptosis through complex pathways (6, 18). They include (a) cytokine receptor-driven fas pathways (20), (b) mitochondrial-driven pathways (35), and (c) endoplasmic reticulum/Ca^{2+}-driven pathways (47). Among them, mitochondrial-mediated pathways including the Bcl-2 family and apoptosis inducing factor (AIF) are the best characterized, and Bcl-2 signaling is believed critical in regulating apoptosis with aging (16, 18, 35, 47). In other words, mitochondria are important sites of programmed cell death (51). A critical factor in mitochondrial-mediated apoptosis is that deficiency of survival stimuli such as growth factors increase mitochondrial permeability. Mitochondrial membrane permeability transition is regulated by a number of pro-and anti-apoptotic proteins such as Bcl-2 family of proteins (16, 18, 35).

Bcl-2 family has been classified into three functional groups (18). Group I such as Bcl-2, Bcl-X_L inhibits apoptosis. Group II such as Bax initiates apoptosis. And group III such as Bid also initiates apoptosis. In other words, Group I functions as gatekeepers at the outer surface of mitochondria, whereas Group II and III function as gatecrashers. Therefore, cell death or survival depends on the ratio of the pro- and anti-apoptotic proteins expressed. For example, high levels of Bcl-2 relative to Bax promote survival, whereas the reverse ratio promotes myonuclear and cell death (18, 44, 50). Thus the ratio of anti-apoptotic/pro-apoptotic proteins in the Bcl-2 family functions as an upstream modulator of apoptosis in the mitochondrial-mediated apoptotic pathways. The proteins of Bcl-2 family can regulate apoptosis by controlling permeability transition and the release of cytochrome c from mitochondria (35).

Caspases are a group of **c**ysteine dependent **a**spartate-**s**pecific **p**rote**ases** (40, 62). In other words, caspases are a complex cascade of protein-cleaving enzymes. Fourteen caspases have now been identified in mammals, leading to apoptotic cell death (28). Caspases play a pivotal role in apoptotic pathways and interact with the non-caspase apoptotic pathways (40, 62). Cytochrome c associates with Apaf-1 (apoptotic protease-activating factor 1) and pro-caspase-9 to form the apoptosome, which activates caspase-9 and caspase-3, resulting in apoptosis through DNA fragmentation (27, 35, 48, 58, 64).

Aging and apoptosis

Aging is characterized by a general decline of physiological function (11). Aging enhances the susceptibility to apoptosis in several types of tissues (19). It has been shown that aging is associated with increased Bax protein as well as enhanced DNA

fragmentation in brains (19). Aging-induced apoptosis may contribute to a 30% loss of cardiac myocytes outside changes noted in cardiac diseases (19, 42). Myocyte cell death including both apoptosis and necrosis increased with aging in the heart of Fischer-344 rats, mediating ventricular dysfunction and failure (24). Liu et al. (32) also reported that expression of Bcl-2 and Bax proteins both increased in the heart of elderly Fischer-344 rats under physiological conditions. In contrast, Nitahara et al. (41) showed that there was an increase in cardiomyocyte apoptosis with age from Fischer-344 rats without significant changes in Bax and Bcl-2 protein levels with age. These findings suggest that age-related changes in the expression of Bcl-2 family proteins (Bcl-2 and Bax) are still debatable. In addition, downstream caspases were not measured.

Progressive apoptosis with advancing age in post-mitotic tissues including heart, skeletal muscle, and brain is dire, because lost myocytes are not replaced. Phaneuf and Leeuwenburgh (46) recently studied markers of apoptosis in hearts (left and right ventricles) of 6-, 16-, and 24-month-old male Fischer-344 rats. Cytosolic cytochrome c was significantly increased in the 16- and 24-month-old animals compared to the 6-month-old animals, indicating increased mitochondrial pore permeability and activation of the apoptosome. Also, Bcl-2 levels were decreased with aging, whereas mitochondrial Bax levels remained unchanged.

In contrast, mitotic tissues such as the liver, colon mucosa, etc. display reduced pro-apoptotic protein levels, cytochrome c contents, caspase levels, and DNA fragmentation with aging. This dysregulation of apoptotic signaling from aging is postulated as a mechanism contributing to cancer risk (57). For example, it has been

reported that caspase-3 and caspase-9 levels and activities were lower in colon mucosa in old (22-24 months) Fischer-344 rats compared to their young (4-5 months) and middle-age (12-14 months) counterparts (61). These changes were accompanied by a reduced number of apoptotic cells in the colonic mucosa of 12-14 and 22-24 month old rats compared with in 4-6 month old rats. In addition, the levels of pro-apoptotic Bak were decreased by about 50%, whereas anti-apoptotic Bcl-X_L levels were increased by about 50% in 22 month old rats compared to 4 or 13 month old rats (61). Suh et al. (57) also showed that apoptosis-induced DNA fragmentation was dramatically upregulated in the liver of young (2 months) female Fischer rats but not in the old (26 months) counterparts. Additionally, the levels of cytochrome c in livers from old (26 months) Fischer-344 rats were significantly lower than those from young (6 months) counterparts (64). These opposite results could be tissue specific and possible characteristics of mitotic but not post-mitotic tissues.

Apoptosis and heart

The heart may undergo an increase in apoptosis during ischemia-reperfusion, heart failure, doxorubicin, and aging (8, 19, 43, 49). It has been reported that a number of cardiomyopathies are associated with mitochondrial DNA damage which leads to defects in electron transport chain and the upregulation of ROS (35), resulting in the apoptosis of cardiac myocytes (60). Heart is an energetically-demanding tissue that requires a continuous production of ATP via mitochondrial respiration (50). In highly aerobic tissues such as heart, mitochondria occupy up to 25% of cell volume (31). It was also shown that tumor necrosis factor-α (TNFα) increased apoptosis via inducible

nitric oxide synthase (iNOS) expression and nitric oxide (NO) in cardiac myocytes (22, 54). In both humans and animals, the aging heart is characterized by a decrease in the total number of myocytes resulting in reactive hypertrophy of the remaining cells and increased fibrosis (42, 46).

Ischemia and reperfusion of myocardium induces apoptotic cell death (49), with direct relevance to heart disease and heart attacks. Interestingly, brief periods of acute myocardial ischemia, or "preconditioning," protects the heart from the deleterious effects of longer periods ischemia and reperfusion (38, 59). The mechanisms of protection against apoptosis due to preconditioning may be mediated by nuclear factor kappaB (NF-κB), which inhibits the susceptibility to apoptosis and can promote upregulation of anti-apoptotic Bcl-2 (10, 33). Indeed, NF-κB DNA binding activity progressively increased as a function of the pre-conditioning of ischemia (34), and Bcl-2 levels were decreased in the ischemic/reperfused heart, but increased in the preconditioned myocardium (34). In addition, overexpression of anti-apoptotic Bcl-2 was effective in reducing myocardial reperfusion injury and improving heart function (4).

Indeed, the balance between Bax/Bcl-2 is postulated as a critically important factor in the increased rate of apoptosis in cardiac myocytes (9, 48). For example, Condorelli et al. (9) indicated that left ventricular hypertrophy and left ventricular dysfunction in a rat model of chronic pressure overload were accompanied by upregulated pro-apoptotic Bax and Bax/Bcl-2 ratio, leading to cardiomyocyte apoptosis. Kang et al. (25) also found that apoptosis predominated in cardiomyocytes after

reoxygenation through a mitochondrial apoptotic pathway, and that Bcl-2 prevented reoxygenation-induced apoptosis by inhibiting the release of cytochrome c from mitochondria. In addition, Kirshenbaum & Moissac (26) concluded that the anti-apoptotic Bcl-2 protein can prevent apoptosis of ventricular myocytes.

Growing evidence shows that cardiomyocyte apoptosis also contributes to congestive heart failure. For example, Olivetti et al. (43) showed that cardiomyocyte apoptosis dramatically increased in the heart of patients with cardiac failure, although the level of Bcl-2 was about 2 times higher in the normal hearts, while the level of Bax remained unchanged. Saraste el al. (52) also showed that the number of cardiomyocytes undergoing apoptosis was significantly increased in failing human hearts compared with control myocardium, and that the expression of Bcl-2 was increased in failing hearts. Enhanced expression of Bcl-2 in the failing heart suggested that compensatory mechanisms were activated to prevent apoptosis (52). The expression of Bcl-2 protein was induced in human cardiac myocytes at the acute stage of infarction, but the expression of Bax protein was overexpressed at the old stage, which was related to myocyte death in human hearts (37).

METHODS

Animals

We used young (4 months) and old (25 months) male Fischer-344 rats. Fischer-344 rats are a common NIH aging model. Animals were purchased from the NIH colony and cared for at the Laboratory Animal Resources and Research facility at Texas A&M University in accordance with NIH and University Laboratory Animal Care Committee standards.

Experimental design and exercise training protocol

To test the ability of exercise training to ameliorate Bcl-2 family driven apoptotic signaling in the rat heart, the rats were randomly assigned to one of the following experimental groups (n=10/group): 1) young control (YC), 2) young exercise trained (YT), 3) old control (OC), and 4) old exercise trained (OT). Rats in the exercise training groups ran on a motor-driven treadmill for 60 min/day at 15 m/min (15° incline), 5 d/wk for 12 wk. The first 5 days were an acclimation period for rats to adapt to the treadmill machine without incline at 15 m/min for 10 min. Rats were gradually conditioned to perform an exercise regimen for 60 min/day at 15 m/min on a 15° incline over the first 3 wk of the 12 wk training program. This exercise protocol has previously been shown to elevate soleus muscle citrate synthase activity, a marker of mitochondrial content (55). Heart-to-body weight ratio and skeletal muscle citrate synthase activity were assessed as indicators of training effect to determine the efficacy of the exercise training regimen.

Tissue preparation

Following the exercise training period, rats were anesthetized with sodium pentobarbital (120 mg/kg) following the exercise training period in both the exercise trained and control groups. Left ventricle was chosen because it is susceptible to age-induced cell death, or apoptosis (24). Therefore, the left ventricle samples were quickly excised and immediately placed in chilled (4°C) phosphate-buffered saline. Left ventricle samples were quickly frozen in liquid nitrogen and stored at –80°C until analysis.

Homogenization procedure

The tissues were further minced into fine pieces and homogenized (20:1 w/v) in ice-cold lysis buffer (pH = 7.5; temp. = 4°C), and then diluted to 51:1 w/v. The lysis buffer contained the following: 20 mM HEPES, 350 mM NaCl, 20% glycerol, 1% Igepal-CA630, 1 mM $MgCl_2$, 0.5 mM EDTA, and 0.1 mM EGTA. DTT (10 mM) and protease inhibitor cocktail (Roche) were added fresh daily to the lysis buffer. Samples were homogenized using a motorized (Bellco Biotechnology: Vineland, NJ), ground glass on ground glass pestle and then centrifuged twice at 10,000 g and the supernatant removed for analysis. Protein concentration was measured using a BCA protein assay kit (Pierce: Rockford, IL) at 562 nm and quantified by spectrophotometry.

Western immunoblot analysis

Protein expression was determined by Western immunoblot analysis. 100 μg of protein was loaded on 10% polyacrylamide gels. The samples were electrophoresed using a Bio-Rad Protein III gel-box and then transferred onto a nitrocellulose membrane

(Bio-Rad: Hercules, CA). Membranes were blocked in 5% nonfat milk with 0.1% Tween-20 in PBS for 6 h, and then incubated overnight at room temperature with the appropriate antibodies, including Bax (1:200, Santa Cruz Biotechnology: Santa Cruz, CA), Bcl-2 (1:250, BD Transduction Laboratories: Lexington, KY), caspase-9 (1:500, Cell Signaling Technology: Beverly, MA), and cleaved caspase-3 (1:500, Cell Signaling Technology: Beverly, MA). Following three washings in PBS with 0.4% Tween-20, samples were incubated for 90 min in horseradish peroxidase (HRP)–conjugated secondary antibodies (Santa Cruz Biotechnology: Santa Cruz, CA). Enhanced chemiluminescence (ECL) detection system (Amersham: Piscataway, NJ) with Kodak hyper-film was used for detection and visualization. Densitometry of film was performed a scanner interfaced with a MacIntosh computer and the NIH Image Analysis 1.61 software program.

Apoptosis (DNA fragmentation) analysis

Apoptotic DNA fragmentation was assessed using a cell death detection ELISA (enzyme-linked immunoabsorbant assay) (Roche Molecular Biochemicals, Germany) (12). The assay is based on a quantitative sandwich-enzyme-immunoassay principle using mouse monoclonal antibodies directed against DNA and histones, respectively. DNA is cleaved between histones and released into the cytosol as mono- and oligonucleosomes during DNA fragmentation (8). This allows the specific determination of mono- and oligonucleosomes in the cytosolic fraction of samples. Twenty µl of heart samples, positive control (DNA-histone-complex), heart samples incubated in 0.1 mg/ml camptothecin (apoptosis-inducing drug) at 37°C for 3 hrs with 95% O_2 and 5% CO_2,

negative control, and background control was transferred into a streptavidin-coated microplate. Eighty µl of an Immunoreagent (mixture of Anti-histone-biotin, Anti-DNA-POD, and incubation buffer) was then added to each well. After covering the microplate with an adhesive cover, the microplate was incubated gently on a shaker for 2 h at room temperature, and then the solution was removed carefully. After 100 µl ABTS solution was pipetted to each well as substrate, the wells were incubated at room temperature 18 min until the color development for photometric analysis. Finally, we measured spectrophotometrically at 405 nm against ABTS solution as a blank (reference wavelength approx. 490 nm).

Statistical analysis

Data were analyzed with two-way ANOVAs. When appropriate, a Fisher-LSD was performed for post hoc comparisons. Statistical significance was established at $P < 0.05$.

RESULTS

Heart-to-body weight ratio

Heart-to-body weight ratio was assessed as an indicator of training effect to determine the efficacy of the exercise training regimen. 12 weeks of endurance exercise training significantly increased heart-to-body weight ratio in both young (6 months) and old (27 months) Fischer-344 rats (Fig. 1). Although no change was seen between young controls and old controls, the heart-to-body weight ratio in young exercise-trained rats was 20.7% higher when compared to young controls (Fig. 1). In addition, the heart-to-body weight ratio was 19.1% greater in old exercise-trained rats when compared to old controls (Fig. 1). The training-induced increase in the heart-to-body weight ratio suggests that cardiac hypertrophy occurred.

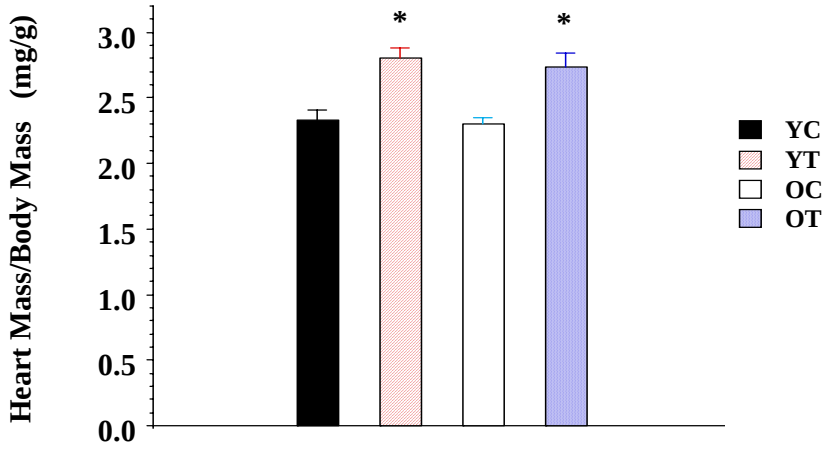

Fig. 1. Effect of aging and exercise training on heart-to-body weight ratio. There are four groups; 1) young control (YC), 2) young trained (YT), 3) old control (OC), and 4) old trained (OT). Values are means ± SE. *Significantly different from control within same age group ($P < 0.05$).

Pro-apoptotic Bax protein expression

The levels of Bax, a promoter of apoptosis, in the left ventricle of old controls were significantly higher (+176.0%) compared to the young controls (Fig. 2). In contrast, exercise training resulted in a marked decrease (-53.4%) of Bax levels in the old trained group compared to old controls (Fig. 2). However, there was no significant difference in Bax protein expression between young sedentary controls and young trained rats (Fig. 2).

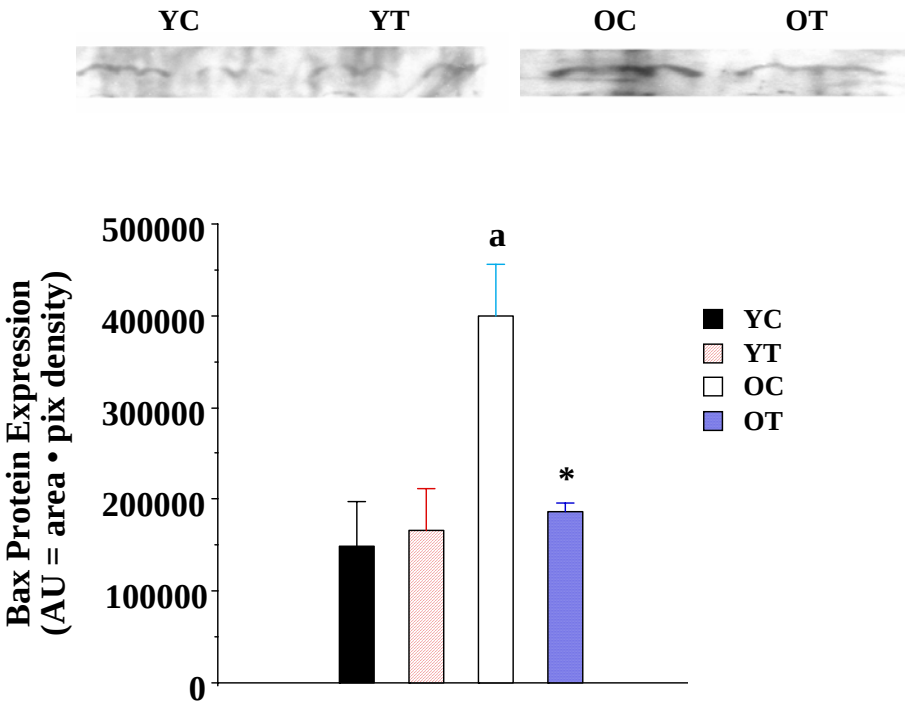

Fig. 2. Effect of aging and exercise training on Bax protein expression. Values are mean ± SE. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old control (OC) is significantly different from young control (YC) ($P < 0.05$).

Anti-apoptotic Bcl-2 protein expression

The levels of Bcl-2, an inhibitor of apoptosis, in the left ventricle of old controls were lower (-26.3%) than young controls although it was not significant (Fig. 3). Exercise training dramatically increased Bcl-2 levels by 117.8% in the old trained group compared to old controls (Fig. 3). But, there was no significant training effect on Bcl-2 protein expression in the young group (Fig. 3).

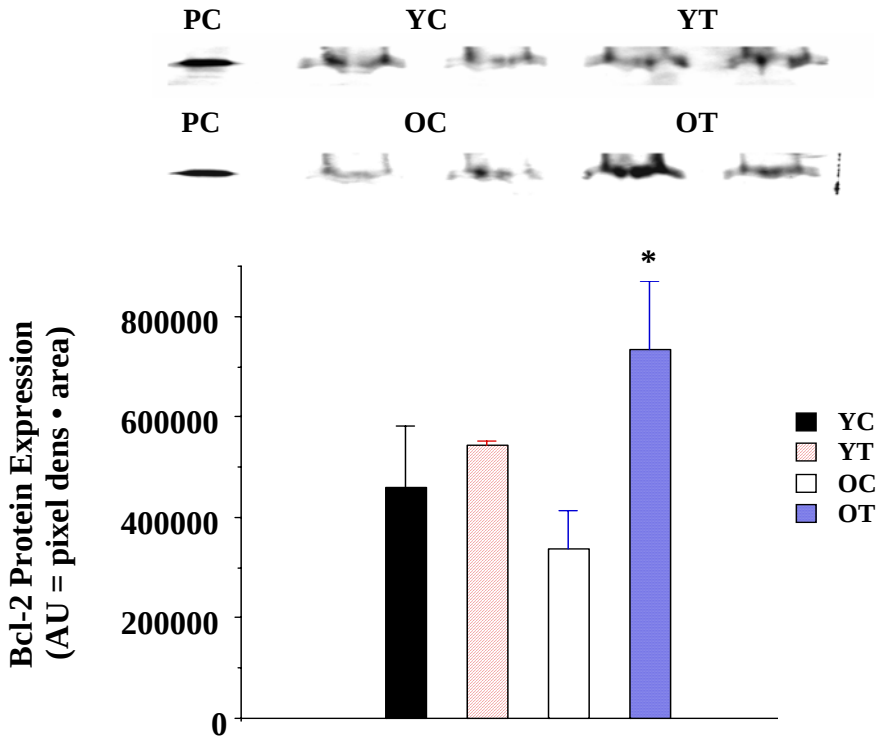

Fig. 3. Effect of aging and exercise training on Bcl-2 protein expression. Values are mean ± SE. *Significantly different from control within same age group ($P < 0.05$).

Bax/Bcl-2 ratio

There was a significant increase (+272.5%) in Bax/Bcl-2 ratio with aging (Fig. 4). In contrast, we found a marked decrease (-78.9 %) in Bax/Bcl-2 ratio with exercise training in the left ventricle from old rats (Fig. 4). But, there was no significant training effect on Bax/Bcl-2 ratio in young groups (Fig. 4).

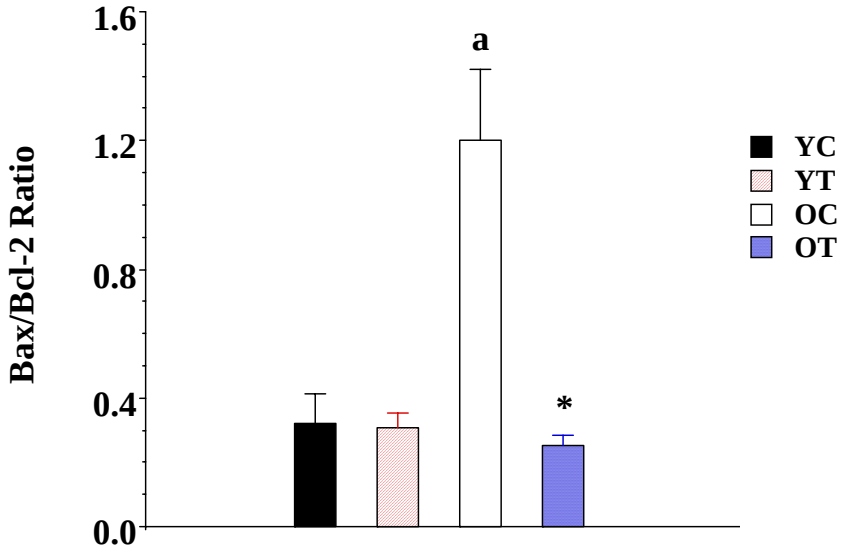

Fig. 4. Effect of aging and exercise training on Bax/Bcl-2 ratio. Values are mean ± SE. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old control (OC) is significantly different from young control (YC) ($P < 0.05$).

Pro-apoptotic caspase-9 protein expression

The levels of caspase-9 in the left ventricle of old controls were significantly higher (+98.7%) compared to the young controls (Fig. 5). Exercise training resulted in a significant decrease (-52.6%) of caspase-9 levels in the young trained group compared to young controls (Fig. 5). Importantly, caspase-9 levels were markedly reduced (-76.9%) by exercise training in the old trained group compared to old controls (Fig. 5).

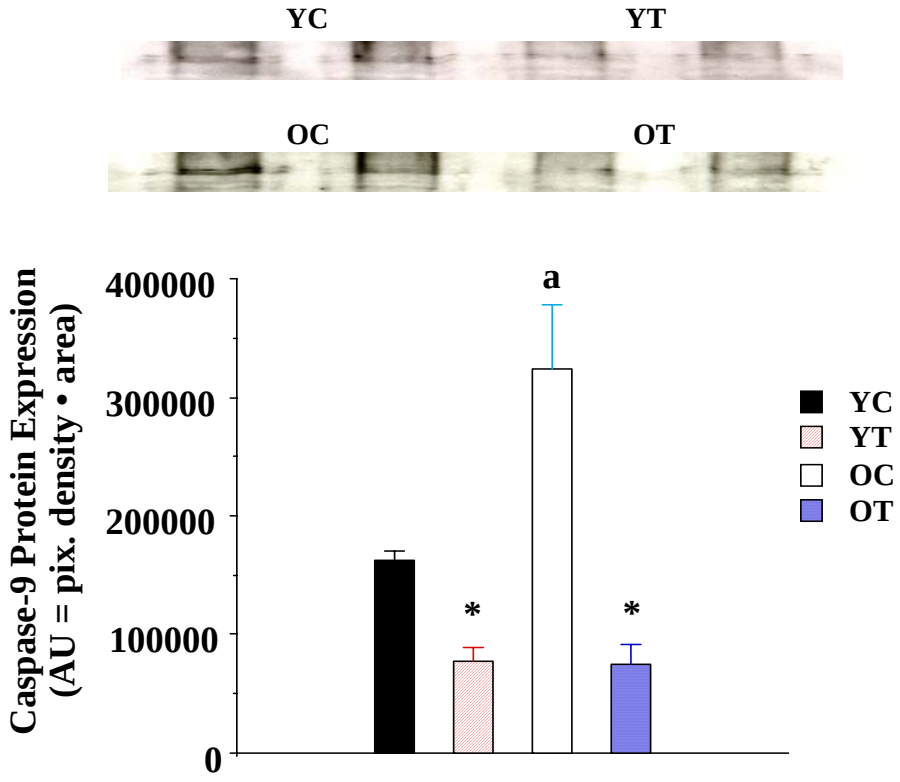

Fig. 5. Effect of aging and exercise training on caspase-9 protein expression. Values are mean ± SE. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old control (OC) is significantly different from young control (YC) ($P < 0.05$).

Pro-apoptotic cleaved caspase-3 protein expression

Caspase-3 activation occurs when pro-caspase-3 is cleaved into 19 kDa and 17 kDa subunits. In young rats, the 17 kDa subunit was undetectable in both young control (YC) and young trained (YT). However, age increased the protein expression of the 17 kDa subunit. Moreover, the 19 kDa subunit levels were also increased in the old groups. Exercise training resulted in a reduction in the 17 kDa subunit levels. Total cleaved caspase-3 levels in the left ventricle of old controls were significantly higher (+122.8%) than young controls (Fig. 6). Exercise training decreased (-32.9%) cleaved caspase-3 protein expression in old trained group compared to old controls (Fig. 6). But, exercise training had no effect on left ventricles in the young age group (Fig. 6).

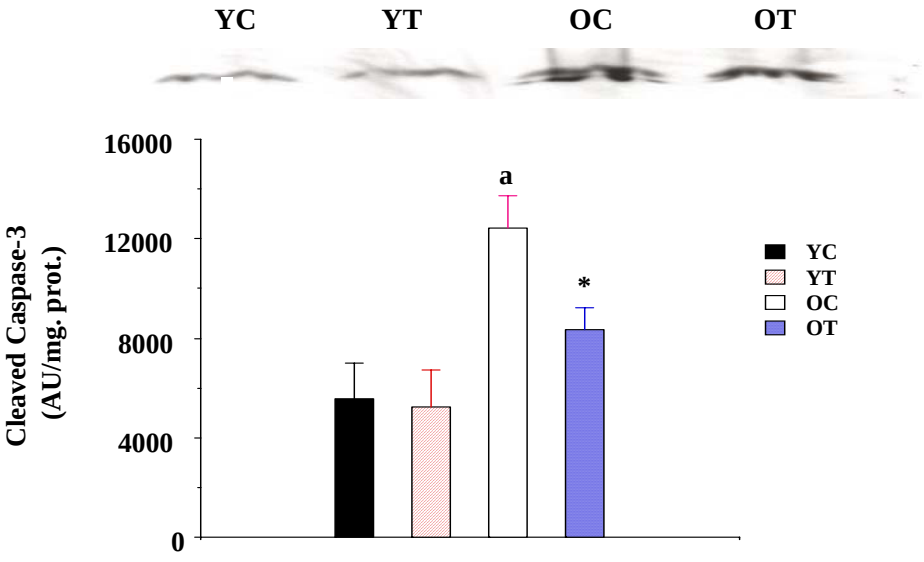

Fig. 6. Effect of aging and exercise training on total cleaved caspase-3 protein (17 kDa and 19 kDa). Values are mean ± SE. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old control (OC) is significantly different from young control (YC) ($P < 0.05$).

Apoptosis (DNA fragmentation)

We found that the apoptosis (DNA fragmentation) in the left ventricle of old controls was dramatically higher (+163.8%) compared to the young controls (Fig. 7). Exercise training resulted in a significant decrease (-43.9%) in DNA fragmentation in the old trained group compared to old controls (Fig. 7). However, there was no significant difference in DNA fragmentation between young controls and young trained rats (Fig. 7).

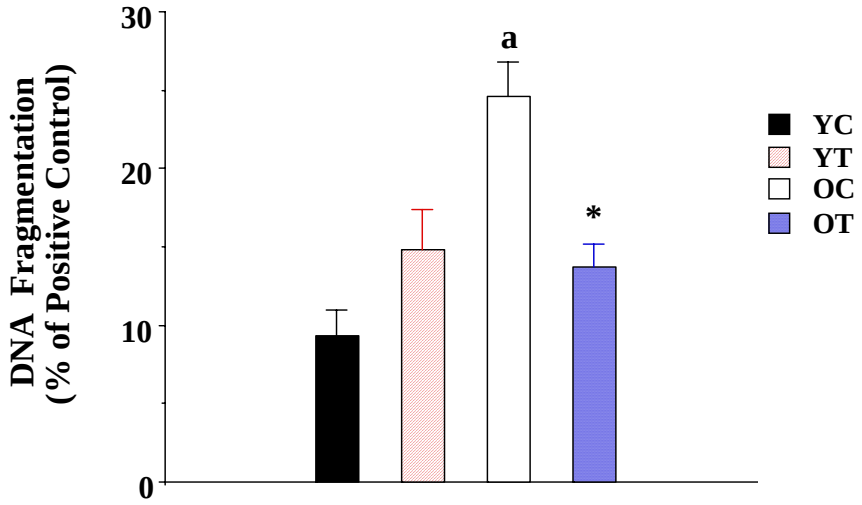

Fig. 7. Effect of aging and exercise training on DNA fragmentation. Values are mean ± SE. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old control (OC) is significantly different from young control (YC) ($P < 0.05$).

DISCUSSION

The purpose of this study was to determine whether exercise training modulates apoptotic signaling and apoptosis in the aging rat heart. There are four major findings in the present study. First, pro-apoptotic protein levels of Bax, caspase-9, and cleaved caspase-3 and apoptosis (DNA fragmentation) were higher in old sedentary rat heart compared to young controls. Endurance exercise training reversed the elevation of pro-apoptotic signaling and apoptosis. Second, anti-apoptotic protein Bcl-2 levels in old sedentary rat heart were lower compared to young controls. However, endurance exercise training dramatically increased the anti-apoptotic protein Bcl-2 expression in old trained rats compared to old controls. Third, the changes of Bax/Bcl-2 ratio with exercise training were consistent with those of caspase-9, cleaved capspase-3, and DNA fragmentation in the old groups. Fourth, there were no significant differences between young controls and young trained rats in apoptotic signaling and apoptosis except caspase-9. To our knowledge, these are the first data which indicate that exercise training has a protective effect against pro-apoptotic signaling in the aging heart.

The results of this present study indicate that the Bax/Bcl-2 ratio was significantly upregulated with age and markedly reduced by exercise training (Fig. 4). These data imply that aging heart is more susceptible to apoptosis than young heart, and that chronic exercise training exerts anti-apoptotic action in the aging heart. These data are consistent with recent reports showing that aging is associated with increased Bax protein as well as decreased Bcl-2 (19, 32). Moreover, in the present study, the exercise training effect of both decreased Bax and increased Bcl-2 proteins of the aging heart in

the old group is especially powerful and intriguing, since Bax/Bcl-2 ratio is a critical regulator of mitochondrial membrane pore permeability. Indeed, the Bax/Bcl-2 ratio in the left ventricle was reduced by a remarkable 4.7 fold due to long-term exercise training, presumably leading to reduced DNA fragmentation. Thus, our data indicate that mitochondrial Bcl-2 family signaling is an important site of regulating apoptosis with aging and exercise training.

The increased Bax/Bcl-2 ratio with aging heart was directly related to increases in downstream caspase signaling including caspase-9 and cleaved caspase-3, which promote DNA fragmentation and cell death. Similarly, there are consistent reports that caspase-9 and caspase-3 activities are increased in aging rat liver (64). In contrast, exercise training markedly decreased caspase-9 and cleaved caspase-3 levels as well as the Bax/Bcl-2 ratio in the aging heart, which presumably led to the decreased DNA fragmentation. These results are consistent with the hypotheses that aging increases Bcl-2 family pro-apoptotic signaling in the heart, and exercise training in the aging heart results in amelioration of age-induced changes in the mitochondrial-mediated apoptotic pathways. In particular, in the present study, cleaved caspase-3 showed similar patterns to DNA fragmentation in the effect of aging and exercise training. Caspase-3 may be an essential hallmark of apoptosis.

Aging is generally characterized by a decline of cardiac function and an upregulation of oxidative stress (2, 11, 53), which are major contributors to cell death through mitochondrial dysfunction (12, 53). Previous work has demonstrated that aging promotes the susceptibility to apoptosis in rat heart (19, 24, 41, 42) and skeletal muscle

(12). Similar to these reports, apoptosis (DNA fragmentation) in the present study was dramatically upregulated in the aging heart.

While aging heart was vulnerable to apoptosis, exercise training was effective in diminishing apoptosis (DNA fragmentation) in the aging heart (Fig 7), which indicated the beneficial effect of long-term exercise training on apoptosis in the aging heart. Over the past several years, many studies have revealed the effects of exercise training mostly on cardiac function (2, 5, 21). In fact, these are the first data to demonstrate that exercise training reduces pro-apoptotic signaling and DNA fragmentation in the aging left ventricle. Furthermore, these data imply that exercise training is associated with significantly reduced cardiac apoptosis and improved cardioprotection in old rats compared with young rats in terms of Bcl-2 family mitochondrial apoptotic signaling.

Additionally, although apoptosis (DNA fragmentation) was dramatically increased with aging in this present study, there was no significant changes in heart-to-body weight ratio between young sedentary rats and old sedentary rats (Fig 1) because the aging process of the heart in humans and animals is characterized by a significant loss of myocytes and reactive hypertrophy of the remaining cells (42, 46). Thus, loss of cardiac myoctyes doesn't necessarily cause a reduction in mass. In addition, with aging there is an increase in heart connective tissue, increasing wall stiffness/thickness, and losing elasticity of the heart (1, 29). However, cardiac myocytes that hypertrophy often have poor contractile function due to increased left ventricle wall thickness (29).

Based upon present novel findings, further investigation as future directions is required to determine the effects of exercise training on the upstream cell protective

mechanisms in the aging heart. For example, exercise training can promote cell-survival proteins including NF-kB, ERK, IGF-1, Akt, and HSPs in heart (49, 56, 59), which may be potential upstream regulators of age-induced apoptosis in the aging heart.

SUMMARY AND CONCLUSIONS

In summary, the purpose of this study was (a) to determine whether aging affects apoptotic signaling, and (b) to identify the effects of exercise training on aging-induced changes in apoptotic signaling in heart. The results of the present study demonstrated that pro-apoptotic protein levels such as Bax, Bax/Bcl-2 ratio, caspase-9, and cleaved caspase-3 and apoptosis (DNA fragmentation) were significantly increased with aging heart. However, 12 weeks of endurance exercise training reversed the elevation of apoptotic signaling and apoptosis (DNA fragmentation). These novel findings indicate that aging heart has increased apoptotic signaling, while endurance exercise training ameliorated apoptotic signaling in the aging heart.

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