EXERCISE TRAINING REGULATION OF EXTRACELLULAR MATRIX AND REMODELING IN THE AGING RAT HEART

A Dissertation

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

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Kinesiology

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ABSTRACT

Exercise Training Regulation of Extracellular Matrix
and Remodeling in the Aging Rat Heart. (May 2008)
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Aging is characterized by a progressive impairment of cardiac structure and function. The cardiac remodeling involves loss of cardiac myocytes, reactive hypertrophy of the remaining cells, and increased extracellular matrix (ECM) and fibrosis in the aging heart. 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 ECM and remodeling in the aging heart remains unknown. Therefore, the purpose of this study was to determine the effects of exercise training on ECM remodeling in the aging heart. We hypothesized that (1) exercise training would attenuate age-related changes in left ventricle morphology including extramyocyte space and collagen contents, and (2) exercise training would ameliorate age-induced changes in ECM-related factors including MMPs, TIMPs, TNF-α, TGF-β1, and α-SMA in the heart. Three and 31 month old Fischer 344 × Brown Norway F1 hybrid rats were assigned to four groups: young sedentary (YS), young exercise-trained (YE), old

sedentary (OS), and old exercise-trained (OE). Exercise training groups walked briskly on a treadmill for 45 min/day (12˚ incline) at 20m/min (young) or 10 m/min (old), 5 d/wk for 12 wk. We found that endurance exercise training might ameliorate the age-induced increase in extramyocyte space and collagen contents of the left ventricle. Exercise training might protect against age-induced fibrosis by increasing MMP-2, MMP-14 in the soluble fraction and MMP-1, MMP-3, MMP-14 in the insoluble fraction of old rat hearts. Conversely, exercise training might reduce the fibrosis by decreasing TIMP-1 in the soluble fraction of old rat hearts. Further, exercise training reduced potential upstream pro-fibrotic mediators including TNF-α and TGF-β1 in the aging rat hearts. These results are the first to demonstrate that exercise training has a protective effect against age-induced extracellular collagen matrix remodeling in the aging heart, associated with increased MMP-1, -2, -3, -14 and decreased TIMP-1, TNF-α, and TGF-β1.

DEDICATION

For Jin Hee, Minsun, and Daniel

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CHAPTER I

INTRODUCTION

Aging is characterized by a progressive impairment of cardiac structure including increased fibrosis and reduced cardiomyocyte volume density (51), and cardiac function including stroke volume, ejection fraction, and cardiac output (51, 120). There is increasing evidence that impairment of cardiac function with aging is a result of structural remodeling (16, 51). Cardiac remodeling involves loss of cardiac myocytes, reactive hypertrophy of the remaining cells, and increased extracellular matrix (ECM) including collagens and fibrosis in the aging heart (120). Myocyte loss through apoptosis and necrosis increases with advancing age in the heart (61, 68). Fibrosis with aging is very critical in impairing heart function. Progressive upregulation in fibrosis results in increased collagen accumulation, elevated internal work, heart stiffness and contributes to diastolic or systolic dysfunction and ventricular remodeling (127). However, the precise mechanisms that lead toward increased fibrosis in the aging heart remain poorly understood.

In contrast, long-term endurance exercise training improves cardiovascular work capacity, protects stress proteins, and reduces cardiovascular disease risk (11). Despite the great importance of exercise training to attenuate aging-related alterations, the ability of exercise training to ameliorate ECM remodeling in the aging heart has not been evaluated. To date, only our lab has demonstrated protective effects of endurance

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This dissertation follows the style of *Journal of Applied Physiology*.

exercise training against increased apoptosis and cardiac remodeling in the aging heart, including accumulation of extramyocyte space (68). However, no studies exist regarding exercise training regulation of ECM remodeling in the aging heart. Moreover, the mechanisms by which exercise training protects against age-induced ECM remodeling in the heart are completely unknown.

Therefore, the objectives of this study were to (i) determine the morphological effects of treadmill exercise training on age-induced ECM remodeling in the aging left ventricle, (ii) identify regulatory mechanisms by which exercise training alters extracellular matrix turnover and remodeling in the aging left ventricle, and (iii) identify putative upstream mechanisms by which exercise training affects ECM remodeling in the aging left ventricle. To accomplish the above objectives, we hypothesized that (i) exercise training would attenuate age-related changes in left ventricle morphology by ameliorating increases in extramyocyte space and collagen contents, (ii) exercise training would attenuate age-induced increases in (a) key matrix metalloproteinases (MMPs: MMP-1, -2, -3, -9,-14) and (b) tissue inhibitors of metalloproteinases (TIMPs: TIMP-1, -2, -3, -4) in the aging left ventricle, and (iii) exercise training would attenuate age-induced increases in TNF-α, TGF-β, and myofibroblast (α-smooth muscle actin) in the aging left ventricle.

Cardiac extracellular matrix

Myocardial tissue is composed of cardiac myocytes, nonmyocytes (e.g., fibroblasts, endothelial cells, vascular smooth muscle cells, etc), and extracellular matrix (ECM) proteins (8, 23). Myocardial ECM is essential for proper cardiac structural integrity and pump function (8, 30). The ECM i) provides a scaffold for myocytes, fibroblasts, and endothelial cells, and ii) transmits mechanical forces and signals to myocardial fibers (8). The ECM also provides mechanical stability, physical strength, stiffness, ductility, and energy absorption to tissues. The ECM is essential for efficient cardiac function via myocyte alignment, regulating blood flow during contraction, and compliance. Moreover, the ECM is an important mediator of growth-related factor and in modulating the cardiac phenotype during development and hypertrophy. Therefore, the disruption of ECM homeostasis is a key factor for the progression of cardiac dysfunction (8).

The ECM in the heart is composed of collagens (e.g., fibril-forming collagens and non-fibril forming collagens), glycoproteins (e.g., fibronectins, elastin, laminins, vitronectin, etc), proteoglycans, extracellular proteases, and ECM receptors (5, 33, 47). ECM in the heart is linked to cellular cytoskeleton by transmembrane molecules, mainly integrins, which provides a physical connection between cytoskeleton and ECM proteins (5, 33, 107). The interactions among ECM, cytoskeleton, and cell through integrins might be very important during cardiac remodeling (47, 56, 102). Although glycoproteins and proteoglycans are essential in proper cardiac geometry and various functions of the ECM, including signaling and turnover of the ECM (8), the most

abundant structural components of the ECM are collagens (5), which are produced primarily by fibroblasts either on the membrane-bound ribosomes of the rough endoplasmic reticulum (ER) or placed within the ECM, respectively (64).

Cardiac fibroblasts are the principal cell type, which are approximately two thirds of myocardial cells (23, 49). Cardiac fibroblasts play an important role in cardiac development, myocardial structure, cell signaling, and electro-mechanical function of the myocardium (23). Cardiac fibroblasts grow in a complex myocardial environment, contributing to the production and deposition of most ECM proteins such as collagens and fibronectin in the cardiac interstitium (4, 79). Cardiac fibroblasts possess a variety of receptors for humoral factors and can functionally respond to mechanical stimuli (130). Conversely, cardiac fibroblasts can synthesize and release autocrine/paracrine factors (e.g., tumor necrosis factor-α, transforming growth factor-β, angiotensin II) and ECM proteins, which are thought to play a key role in ECM remodeling (6).

The ability to synthesize the ECM components depends on cell types in the heart. For example, fibroblasts and smooth muscle cells synthesize collagen types I and III and fibronectin, whereas cardiac myocytes and endothelial cells produce collagen type Ⅳ (33). In addition, laminin is produced by cardiac myoctyes, smooth muscle cells, and endothelial cells (33). Alterations in the profile of ECM proteins can play a profound influence on the form and function of heart.

Cardiac ECM remodeling and aging

The aging heart is characterized by decreased myocyte number, increased myocyte size, and increased extracellular matrix compared with younger heart (3). Cell death by apoptosis or necrosis is very critical determinant of ECM remodeling because it induces a loss of contractile tissue, reactive compensatory hypertrophy of remaining myocytes, and ECM remodeling including interstitial fibrosis (59, 109). These phenotypic changes of the myocardium during aging occur in the mainly left ventricle. For example, apoptosis, programmed cell death, is localized into the left ventricle, suggesting that it is initiated by mechanical factors (61).

Overall, myocardial remodeling is determined by the consequence of changes in cardiac myocytes and disruption of ECM homeostasis. The ECM remodeling caused by aging results in myocardial remodeling, contributing to rearrangement of normally existing structures (92, 121). The ECM remodeling also occurs in dilated cardiomyopathy (91) and myocardial infarction (77). The ECM is a fibrillar network that embeds cardiomyocytes and the whole cardiac structure. The ECM remodeling is a critical part of mortality in the elderly. In particular, aging seriously affects the myocardial structure and function, as the fundamental biological process of aging is intimately associated with an increased arterial hypertension, atherosclerosis, and decreased physical activity.

Fibrosis is a complicated tissue response that causes the excessive deposition of ECM, especially collagens (66). Fibrosis, one of the major biological determinants of cardiac remodeling, is an increased collagen content and concentration, resulting in

increased myocardial stiffness and cardiac dysfunction. Fibrosis is multifactorial, and it is resulted from aging, myocardial ischemia, inflammatory processes, hormones, vasoactive peptides, or diabetes (121). There are two different types of fibrosis, namely, reparative fibrosis and reactive fibrosis (121). Reparative fibrosis occurs as a reaction to the loss of cardiac myocytes due to apoptosis or necrosis, located mainly in interstitium. In contrast, reactive fibrosis occurs as a reaction to inflammation without the loss of cardiac myocytes, observed in primarily perivascular area (115). Reparative and reactive fibrosis usually coexists during ECM remodeling. For example, after myocardial infarction, reparative fibrosis is organized as a scar and reactive fibrosis and compensatory cardiac hypertrophy surround it (131).

There are converging reports suggesting that myocardial fibrosis occurs in senescent hearts both in rats and humans (3, 9, 41, 80, 87). Furthermore, there is emerging evidence that indicates that aging is associated with increased cardiac fibroblasts (23). The ECM remodeling with aging including modifications of ECM protein synthesis and degradation would suggest that the aging heart might be unable to adapt to an increased load well (18, 35, 85).

Collagens in the heart

Collagens are a regulated family of ECM proteins that provide structure and optimize function of the heart (8). Presently, more than 20 collagen types have been identified in various vertebrate tissues. Collagen is the most abundant protein in ECM and forms the essential mechanical building blocks, providing tensile strength and

resisting stretch (59). The collagen is composed of three α-chains called triple helix or tropocollagen molecule. The common structure of collagens is repeating amino acid sequence (Gly-X-Y) that comprises the collagen chain. Most of collagens are present in the forms of polypeptide chains called collagen molecule or α-chain, consisting of glycine, proline, and hydroxyproline with hydroxylysine (5, 59).

In addition, mechanical integrity of the collagen fibers by the formation of intramolecular or intermolecular cross-linking plays an important role in overall cardiac structure and function (37). Collagen cross-linking is accomplished through lysyl oxidase catalyzed deaminate where lysine and hydroxyline moieties form allysine and hydroxyallysine covalent bonds (59). These collagen cross-linking causes changes in the structure and function of the collagen matrix resulting in cardiac stiffness and dysfunction in contractile properties, and is assessed by hydroxylysylpyridinoline (HP). (37). The collagens are essential for maintaining alignment of the myofibrils within the myocyte through a collagen-integrin-cytoskeleton-myofibril system (101).

Structurally, the network of collagens exists at three levels named i) endomysium surrounding individual muscle fibers, ii) perimysium surrounding groups of myocytes, and iii) epimysium surrounding the entire muscle (37). Connective tissue consists mainly of collagen, and to a much lesser extent, fibronectin, laminin, and elastic fibers (24, 32). For example, collagen and connective tissue occupy over 10% of extramyocyte space, and collagen itself occupies 4% of the space in the rabbit heart (44).

The collagens can be divided into two major classes, the fibrillar-forming and non-fibrillar-forming collagens (5, 59). Among collagens, five of collagens (I, II, III, Ⅴ,

and XI) form fibrils (59). The fibril-forming collagens provide the structural framework of tissues. In particular, collagen types I and III in myocardial collagens are predominantly interstitial collagens in the heart that surround cardiac myocytes and the coronary microcirculation, providing structural integrity for the cardiomyocytes (47, 59, 63, 92). Type I collagen type makes up approximately 85% and type III collagen 11% of total collagen in the heart (37, 59).

Although collagen types I and III coexist in the extracellular matrix, especially both in the perimysium and endomysium (37), there are some differences due to the composition of the α-chains that comprise the collagen triple-helix. Collagen type I is a hybrid, consisting of two identical α1 (I) chains and one α2 (I) chain that form the superhelix (5). Collagen type I is thick, yellow or red, strong fibers and is thought to play an essential role in providing structural stability to tissues (35). This is in part supported by the fact that the collagen type I has less distensible properties than collagen type III (35). In contrast, collagen type III contains three identical α1 (III) chains (5). Collagen type III is thin, greenish fibers, and fine reticular network in most soft connective tissue unlike the larger fibers that are derived from collagen type I molecules (35). Collagen type I provides high tensile strength and stiffness to tissues, whereas collagen type III provides high compliance to tissues (59, 83). So, the ratio of collagen type III to I has been implicated in functional properties of the heart, with a higher ratio of collagen type III to I indicating more compliant tissue and a lower ratio of collagen type III to I indicating a stiffer, less compliant tissue (59, 83).

Aging and cardiac collagens

Myocardial remodeling during aging is related with changes in the amount and organization of ECM components (121). In particular, myocardial collagens in ECM undergo remodeling with aging. A healthy arrangement of collagens provides a framework for myocyte sheath sliding, transmittance of force from myocyte to the ventricular chamber, prevents excessive stretch and damage, and preserves heart function (37). However, excessive accumulation of collagen matrix is upregulated in a number of cardiovascular diseases (26, 132). Moreover, aging also increases the rate of ventricular collagen turnover and deposition by fibroblasts called fibrosis (8, 124, 125).

Fibrosis with aging is characterized by increased collagen content (35, 54), decreased collagen solubility, and increased collagen cross-linking (124, 125). This increase in collagen deposition during aging may be thought to result from a combination of cellular events including increased collagen synthesis and decreased degradation. The collagen might become more resistant to collagenase degradation with aging (59). Excessive accumulation of collagen in the heart could lead to tissue stiffness, increase the incidence of arrhythmias, disrupt electronic communication between myocytes, and result in diastolic and systolic dysfunction and heart failure (8, 30).

Previous studies have demonstrated age-related changes in cardiac collagen concentration (19, 35, 54, 76, 85, 124, 125, 135). Debessa et al. (35) indicated that the number and thickness of Type I collagen increased from adulthood to old age in human heart. Studies in animal hearts also provided consistent evidence of an increase in myocardial collagen concentration with aging (18, 85, 89, 124, 125, 135). For example,

in the left ventricle of rat, the collagen concentration (hydroxyproline) has been shown to increase almost double from 5 to 26 months of age (125). These findings were confirmed by Nguyen et al. (85), who examined the collagen concentration in the left ventricles of Fischer 344 rats at 6, 18, and 24 months of age. Their results revealed that the collagen concentration, as determined by hydroxyproline assay, progressively increased during aging with greatest increments from 6 to 18 months, then leveling off at 24 months. Similar findings were previously described by Mays et al. (83), who found a gradual increase in collagen concentration, based on hydroxyproline levels between 2 weeks and 24 months of age. In addition, Lindsey et al. (76) recently reported that total collagen levels increased with advancing age. Taken together, the increased collagen content/concentration might be an integral part of ECM remodeling that takes place in the left ventricle consequent to the natural aging process leading to an increase in myocardial passive stiffness and impaired contractile function.

A few studies also have showed age-related increases in collagen cross-linking in cardiac muscle (124, 125). Increased collagen cross-linking could be implicated as a potential mechanism for an impaired extensibility and increased stiffness in aged heart. For example, Thomas et al. (124, 125) reported that there were significant overall aging–related increases in collagen cross-linking in the both left ventricle and septum. In addition to the heart, skeletal muscle also showed the same phenomena that collagen content (48, 65, 84) and collagen cross-linking (48, 90, 97) significantly increased from young to senescence in skeletal muscle.

Exercise and cardiac collagens

Alterations in collagen profile have been shown to occur following exercise training in heart (124, 125) and skeletal muscle (48, 65, 138). For example, Thomas et al., (124) observed that ten weeks of treadmill exercise training reduced age-induced upregulation of collagen concentration (percent collagen) in the left ventricle septum of rats. The collagen cross-linking (HP) of left ventricle free wall was significantly lower in old trained rats, compared with their sedentary counterparts (124, 125).

Conflicting results were also reported by others. Burgess et al. (17) suggested that total collagen concentration (hydroxylproline) of rat left ventricle did not change by 10 weeks of treadmill exercise training. In addition, collagen type III -to-I ratio was not altered by exercise training in the rat heart (17). Woodiwiss et al. (134) found that demonstrated that 16 weeks of habitual voluntary wheel running had no effects on myocardial collagen concentration and cross-linking in the rat left ventricle, although cardiac stiffness was reduced. Similarly, Jin et al. (57) showed that mRNA levels of collagen types I and III did not change with 13 week treadmill exercise training in the rat heart. So, based on previous findings, the role of exercise training on collagen concentration and cross-linking in the heart remains to be clarified.

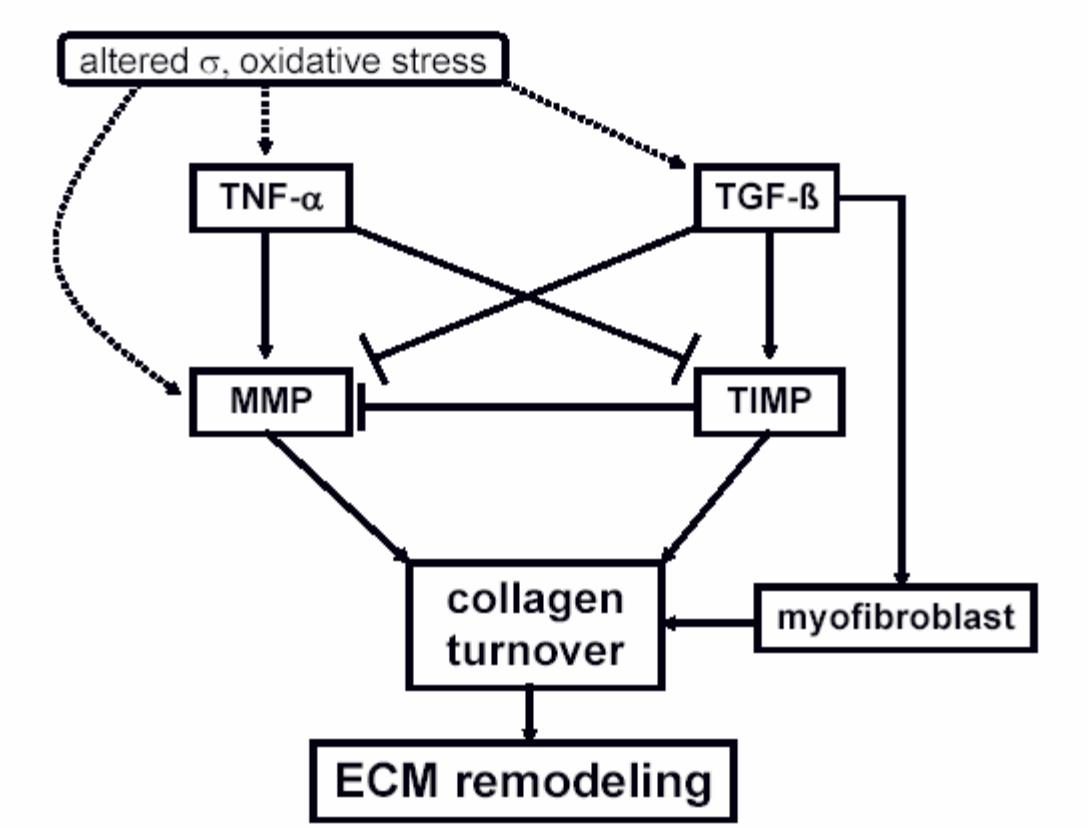

Fig. 1. Collagen ECM turnover signaling in the heart. Altered mechanical stress and oxidative stress may stimulate TNF-α, TGF-β, and MMP. TNF- α may stimulate MMP and inhibit TIMP. However, TGF-β may inhibit MMP and stimulate TIMP and myofibroblast. Finally, MMP degrades collagens, but TIMP and myofibroblast inhibit collagen degradation and promote collagen synthesis, which determine collagen ECM remodeling.

Regulation of cardiac collagen ECM

Collagen ECM plays an important role in cardiovascular function, and remodeling in the ECM contributes to myocardial dysfunction (47, 59). Myocardial failure and remodeling are usually characterized by collagen accumulation, collagen fibril disruption, myocyte loss via apoptosis or necrosis, and impaired rearrangement of structure (121). In particular, accumulation of collagen ECM with aging in the heart could create a mechanical environment and stress distribution that contributes diminished systolic performance, decreased compliance, and diastolic dysfunction (37).

Therefore, the balance of ECM remodeling via collagen ECM synthesis and degradation is essential for normal cardiac structure and function (59). Collagen ECM remodeling is modulated by regulatory proteins, hormonal factors, cytokines, and growth factors (8, 23). Thus an understanding of upstream ECM regulatory factors including matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and myofibroblasts is necessary for gaining new insights into managing cardiac remodeling and dysfunction with aging (Fig. 1).

Cardiac MMPs and TIMPs

This extracellular collagen matrix depends on a balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), which determines cardiac remodeling (1, 59). MMPs are an endogenous family of enzymes that degrade ECM proteins, which are responsible for ECM remodeling in a number of

physiological and pathological process (1, 127). To date, the MMP family consists of more than 20 unique proteins in vertebrates (60). Most of MMPs are inactive enzymes that are activated in the extracellular matrix. It has been shown that important MMPs highly related with myocardial remodeling are collagenases (e.g., MMP-1 and MMP-13), gelatinases (e.g., MMP-2 and MMP-9), stromelysin (e.g., MMP-3), and the membrane-type MMP (e.g., MMP-14) (63). These kinds of MMPs degrade predominantly collagen types I and III in the ECM of the heart (60, 63, 111).

MMPs are Ca^{2+}-and Zn^{2+}-dependent proteases that are usually synthesized as an inactive form or pro-MMP, which will be activated by the cleavage of an amino-terminal propeptide domain either by autoproteolysis, another MMP, or serine protease (59). For example, MMP-14 activates MMP-2, which requires TIMP-2 binding to its active place (69). MMPs in the heart are expressed primarily by fibroblasts (27) and cardiomyocytes (31). Most pro-MMPs are stored extracellular bound to different ECM components (59). Upon stimulation, activated MMPs degrade the ECM proteins including collagens, fibronectin, laminin, gelatin, and proteoglycan. Therefore, MMPs are significant regulators of ECM turnover in the heart, thus contributing to physiological function as well as pathology.

In contrast, activity of MMPs is in part regulated by endogenous inhibitors (59). TIMPs are specific MMP inhibitors in the ECM (78). The role of TIMPs is to prevent excessive ECM degradation by MMPs. There are 4 TIMPs identified in vertebrates, TIMP-1, -2, -3, and -4, acting as the natural inhibitors of active forms of all MMPs through binding to MMPs in a 1:1 ratio (30). Among them, TIMP-1, -2, and -4 are

soluble forms, whereas TIMP-3 binds to the ECM via heparan sulfate proteoglycans within the ECM (70, 136).

The balance between MMPs and TIMPs plays a critical role in the process of cardiac ECM remodeling which contributes to cardiac function (110). Based on previous findings, it appears that cardiac ECM remodeling is generally associated with enhanced MMP and reduced TIMP activities (59). However, there are differences in the studies. For example, the levels of TIMP-1 were either repressed (128) or increased (123) in dilated cardiomyopathy patients.

It has been shown that inhibition of MMP activity is beneficial during cardiac remodeling and wall stress following injury due to myocardial infarction (93, 119). For example, Rohde et al. (99) indicated that a broad range MMP inhibitor attenuated left ventricular dilatation 4 days after infarction in a mouse myocardial infarction. In addition, inhibition of MMP-9 activity attenuated left ventricular enlargement and collagen content after myocardial infarction (39). In addition, heart failure is associated with alterations in MMPs including increased MMP-3 and MMP-9, and decreased MMP-1, while MMP-2 was unchanged (123). Therefore, MMP inhibition might be a new therapeutic treatment to control cardiac dysfunction and failure (93).

Studies investigating age-related alterations of MMP and TIMP expression in the heart remain limited. A few publications indicated that MMP levels increased, and TIMP levels decreased in the rat heart with advancing age (76, 82). For example, Lindsey et al. (76) found that the levels of MMP-3, MMP-8, MMP-9, MMP-12, and MMP-14 increased, and the levels of TIMP-3 and TIMP-4 decreased in the insoluble fraction of

old mice, compared with young adult mice, suggesting that aging is associated with increased ECM degradative capacity. However, much different findings were reported by Robert et al (98). Their results indicated a 40-45% decrease in both MMP-2 and pro-MMP-1 activity and mRNA in 24-month-old rat heart, suggesting that the reduction of ECM degradation pathway by MMP allows accumulation of collagen and promotion of age-associated fibrosis. Thus, the current literature is unclear about MMP or TIMP expression with aging in the heart as both decreased (98) and increased (76) levels have been reported. In addition, most of findings did not measure MMPs or TIMPs in the insoluble fraction, associated with structural ECM proteins such as collagen (82, 98). Moreover, it is unknown if exercise training modulated age-associated effects on MMP and TIMP levels in the heart.

Cardiac TNF-α

Upstream regulators of MMPs and TIMPs include inflammatory cytokines in the heart (118, 127). It seems likely that the cytokines may lead to an imbalance in myocardial MMP/TIMP ratio resulting in altered myocardial ECM architecture and development of left ventricle remodeling and dysfunction (59, 118). Among cytokines, tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine, can increase the matrix collagen degradation by upregulating MMP activity and downregulating TIMPs (59, 118). TNF-α has a variety of different biological capacities in response to one or more different forms of environmental stress in heart failure, including LV dysfunction, cardiomyopathy, LV remodeling, abnormalities of mitochondrial energetics, increased

production of reactive oxygen, and cardiac myocyte apoptosis (81). In particular, LV remodeling by TNF-α is involved in alterations in the biology of the cardiac myocyte, progressive myocyte loss, and alterations in the extracellular matrix including synthesis and degradation of collagen matrix (81).

Significantly increased levels of TNF-α have been demonstrated in patients with dilated or ischemic cardiomyopathy, (88, 108) and in animal models of myocardial infarction (55). Furthermore, Li et al. (74) suggested that cardiac overexpression of TNF-α in transgenic mice caused increases in MMP-2 and MMP-9 activity as well as marked diastolic dysfunction. In isolated cardiac fibroblasts, TNF-α decreases collagen synthesis, increases MMP expression, and decreases TIMP expression (75, 117). In contrast, Sivasubramanian et al. (116) reported that there were significant decreases in total MMP activity and elevated TIMP-1 levels in the cardiac overexpression of TNF-α in transgenic mice, suggesting a possible mechanism for the increase in myocardial fibrosis. Mann (81) also showed that TNF-α promoted cardiac fibroblast proliferation and fibrosis. Although the mechanisms by which TNF-α affect MMP and TIMP may depend on in vitro and in vivo models, TNF-α may indeed induce an imbalance in MMP/TIMP ratio, remodeling and fibrosis in the heart.

There are controversial evidences regarding the effects of aging on TNF-α in the heart. Some findings suggest that there is no change of TNF-α with advancing age in the heart (105) and serum (103). In contrast, others indicate that TNF-α increases with aging in the vessels (34) and serum (104). So, the role of TNF-α in aging heart remains unclear.

However, there is emerging evidence in support of exercise training-induced suppression of TNF-α (7, 10, 71, 72). For example, after 12 weeks of exercise, there was a significant decrease in plasma TNF-α levels in patients with heart failure (71). These findings were confirmed by LeMaitre et al. (73), who showed that 6 weeks of bicycle exercise training in chronic stable heart failure patients attenuated the circulating levels of TNF-α and TNF-α receptor 2.

Cardiac TGF-β

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that plays an important role in cell migration, proliferation, differentiation, apoptosis, and ECM protein production (2, 8, 106, 114). TGF-β, an anti-inflammatory cytokine, is a potent stimulator of collagen synthesis (112, 118). It consists of three isoforms, TGF-β1, TGF-β2, and TGF-β3 that are structurally and functionally closely related to one another (2). The TGF-β released from platelets and leukocytes stimulates the synthesis of ECM components including collagens, fibronectin, proteoglycans, and integrins in tissue repair after injury (13). It mediates collagen synthesis through increasing transcription and decreasing collagen degradation via reduced MMPs or enhanced TIMPs, thus favoring an accumulation of ECM and especially of collagen (12, 40, 118). For example, Seeland et al. (112) suggested that the overexpression of TGF-β1 in transgenic mice resulted in increased protein expression of collagen types I and III, reduced interstitial collagenase protein activity and mRNA expression, and increased TIMP-1, -2, and -4 protein levels in the heart. Additionally, in cardiac fibroblasts, procollagen formation

was stimulated by mechanical loading and TGF-β (20). Moreover, there is an interesting evidence that indicates that the addition of TGF-β1 to cultured cardiac fibroblasts increases ECM mRNAs (42).

Acute exercise or mechanical loading may stimulate TGF-β synthesis in the heart (22), smooth muscle (50), skeletal muscle (14, 46), and circulating blood (52, 53) as a physiological response. For example, Calderone et al. (22) reported that TGF-β1 mRNA increased in the left ventricle of a voluntary exercise rat model of physiological cardiac hypertrophy. However, excessive and chronic expression of TGF-β is associated with many fibrotic diseases including cardiac fibrosis after infarction, lung fibrosis, and scarring (2, 13). TGF-β may play a role in stimulating abnormal accumulation signaling of ECM proteins in the cardiovascular diseases (58). Rosenkranz et al. (100) showed that TGF-β overexpression in the transgenic mice heart resulted in cardiac hypertrophy and fibrosis. Similarly, Brooks & Conrad (15) found that TGF-β1 deficient old mice heart exhibited a decrease in myocardial fibrosis and reduced myocardial stiffness, indicating the role of TGF-β to contribute to ECM component synthesis in the heart.

Although several findings suggested that pro-inflammatory cytokine, TNF-α can increase the collagen matrix degradation by upregulating MMP activity and downregulating TIMPs (59, 75, 88, 108, 117, 118), which is antagonistic to the role of TGF-β, Sivasubramanian et al. (116) interestingly demonstrated that TGF-β1 and TGF-β2 protein levels were significantly increased in the cardiac overexpression of TNF-α transgenic mice, providing one potential explanation for the increase in myocardial fibrosis.

Cardiac myofibroblasts

Myofibroblast is a differentiated cell type from fibroblast characterized by increased ECM protein synthesis called fibrosis formation, providing an essential role for ECM remodeling during normal and pathological wound healing (30, 96, 126, 129). Myofibroblasts as a smooth-muscle like fibroblasts might be produced from progenitor stem cells in the heart or from the circulation, and secret cytokines (e.g., TNF-α), growth factors (e.g., TGF-β), chemokines, and inflammatory mediators (96). In addition, differentiation to the myofibroblast may be induced by transforming growth factor-β1 (TGF-β1) (45, 126).

Myofibroblast expression may be not detectable in the normal healthy adult hearts, while myofibroblasts are often associated with injured heart such as myocardial infarction for wound healing (8, 94, 133). In particular, Poobalarahi et al.(94) reported that increased type I collagen synthesis by myofibroblasts was accompanied by a significant increase in collagen deposition into insoluble ECM in the heart. Accordingly, myofibroblasts appear to play a critical role in production of cardiac ECM in response to injury. In addition, differentiated myofibroblasts are unique in that they express α-smooth muscle actin (α-SMA) unlike adult fibroblasts (28, 96, 113).

Expression of α-SMA positive myofibroblasts appears to be regulated by transforming growth factor-β1 (TGF-β1) (28, 36). A similar finding was also reported that TGF-β1 promoted the conversion of myofibroblasts in vitro (45). Additionally, Kuwahara et al. (67) found that TGF-β1 function-blocking antibodies administered to pressure-overload rats prevented the myofibroblasts conversion in cardiac interstitium

and subsequent increases in mRNA of type I collagen as well as diastolic heart failure. Interestingly, Porter et al. (95) showed that tumor necrosis factor-α (TNF-α) via a TNF-R1 receptor also increased myofibroblast proliferation in human heart.

Although increased gene expression of ECM proteins by myofibroblasts is well documented, much less is known regarding effects of aging and exercise training on myofibroblasts in the heart. Only one result has been published regarding the effect of exercise training on α-smooth muscle actin in the normal heart (57). That result indicated that there was no change of mRNA levels of α-smooth muscle actin in the young (6-8 wk) rat heart after 13 week treadmill exercise training.

CHAPTER II

METHODS

Animals

Young (3 months) and old (31 months) male Fischer 344 × Brown Norway F1 hybrid (F344BNF1) rats were used at the beginning of the study. F344BNF1 rats are a preferred NIH aging model with greater longevity (33 mo) than F344 rats (24 mo), as F344BNF1 rats are less susceptible to mortality from pathology including cancer than F344 rats (86). Rats were purchased from the NIA colony and cared for at the Comparative Biology Laboratory facility at Texas A&M University in accordance with NIH and University Laboratory Animal Care Committee standards. Rats were housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle, and water and rat chow provided *ad libitum*.

Exercise training protocol

To test the exercise training regulation of ECM remodeling in the aging heart, rats in the exercise groups trained at a relative intensity of approximately 70% of maximal aerobic capacity based on previous work (29, 68). Rats walked briskly on a motor-driven treadmill for 45 min/day at 12° incline, 5 d/wk for 12 wk. Running speeds were 20 m/min (young) or 10 m/min (old) to reach the desired intensity. The first 5 days were an acclimation period for the rats to adapt to the treadmill exercise at 10 m/min for 10 min without incline. Rats were gradually conditioned to perform treadmill exercise

for 45 min/day over the first 4 wk of the 12 wk training program. Heart-to-body weight ratio and skeletal muscle citrate synthase activity were assessed as an indicator of the efficacy of the exercise training regimen. This exercise regimen has previously been shown to elevate citrate synthase activity as a marker of oxidative mitochondrial capacity in skeletal muscle (29, 68).

Experimental design

To determine whether exercise training attenuates age-induced changes in ECM remodeling and signaling in the heart, the rats were randomly assigned to one of the following experimental groups (n=10/group): 1) young sedentary controls (YS), 2) young exercise trained (YE), 3) old sedentary controls (OS), and 4) old exercise trained (OE). Rats in the exercise training groups were anesthetized with sodium pentobarbital (50 mg/kg) 48 h after the last bout of exercise training to avoid influence of the last acute exercise bout. The left ventricle was then quickly extracted, weighed, and placed in ice-cold phosphate-buffered saline (PBS) (pH=7.4). The samples were frozen in liquid nitrogen and stored at –80°C until analyses.

Homogenization procedure

Left ventricle samples were minced into fine pieces and homogenized (26:1 v/w) in ice-cold (4°C) lysis buffer solution (pH=7.40) containing the following: 20 mM HEPES, 350 mM NaCl, 20% glycerol, 1% Igepal-CA630, 1 mM $MgCl_2$, 0.1 mM DTT, 0.5 mM EDTA, 0.1 mM EGTA, and protease inhibitor cocktail (Roche Applied

Science). Minced muscle samples were homogenized using a ground glass on ground glass homogenizer (Bellco Biotechnology) at 4°C, and then isolated for soluble fraction, nucleosome fraction, and insoluble fraction as the following. The tissue homogenates were first centrifuged for 10 min at 3000 g at 4°C, and supernatant was withdrawn from the first centrifugation. The supernatant was used as the soluble fraction after second centrifugation at 10,000 g for 10 min at 4°C. The resuspended pellet from first centrifugation in complete lysis buffer was centrifuged again at 12,000 g for 30 min at 4°C. This supernatant was used as the nucleosome fraction.

For insoluble fraction, the second pellet was resuspended by boiling in SDS-PAGE sample buffer including 50 mM Tris (pH 6.8), 0.1 mM DTT, 2% SDS, 1 mM glycerol, and 1.67 mM EDTA. Total protein concentration of each fraction was measured using BCA protein assay reagent kit (Pierce) at 562 nm absorbance using spectrophotometry.

Measurement of citrate synthase activity

Citrate synthase activity with gastrocnemius and soleus skeletal muscles was measured as described previously (72). It was used as a marker of oxidative capacity in skeletal muscle and is indicative of mitochondrial density and function. In brief, reaction cocktail (1.0 ml; 0.1mM DTNB, 0.07% Triton X-100, 0.1 mM acetyl CoA in 100 mM potassium phosphate buffer with 10 mM EDTA, pH 7.40) was combined with 10 µl samples of 1:50 homogenates in a cuvette and incubated for 5 min. The substrate oxaloacetate (50 µl: 0.1 mM in buffer) was added and then the reaction was commenced.

After vortexing the final mixture, the absorbance was measured from 1 to 4 min at 412 nm using spectrophotometry. Enzyme activity was expressed as Units per g wet weight of muscle.

Measurement of TNF-α

TNF-α activity levels in left ventricle muscle tissue homogenates were quantified using an ELISA kit designed specifically for rat use (Pierce Biotechnology). In brief, following adding 50 µl of pre-treatment buffer, 50 µl of samples or standards were transferred into each well of microplate and incubated at room temperature (RT) for an hour. After three times washing, 50 µl of biotinylated antibody reagent were added to each well and incubated for an hour at RT. After three times washing, 100 µl of streptavidin-HRP reagent were added to each well. After covering the microplate, it was incubated for 30 min at RT. After washing three times, 100 µl of TMB substrate solution were added to each well, and developed at RT for 10 min. After that, the reaction was stopped by adding 100 µl of stop solution. Finally, the absorbance on a plate reader at 450 nm minus 550 nm was measured.

Western immunoblot analysis

Protein levels were determined via Western immunoblot analysis. Separating gel (375 mM Tris-HCl; pH=8.8; 0.4% SDS; 10% acrylamide) and stacking gel (125 mM Tris-HCl; pH=6.8; 0.4% SDS; 10% acrylamide) solutions were made, and polymerization then initiated by TEMED and ammonium persulfate. Separating and

stacking gels were then quickly poured into a Bio-Rad Protein III gel-box (Bio-Rad). Thirty μg of protein from soluble fraction and insoluble fraction of left ventricle in sample buffer (125 mM Tris-HCl; pH=6.8 with 2% SDS, 30 mM DTT, 25% glycerol) were loaded into the wells of the 10% polyacrylamide gels, and electrophoresed at 150V. The gels were transferred at 30V overnight onto a nitrocellulose membrane (Bio-Rad). Equal loading and transferring of proteins to the membrane in each lane were confirmed through Ponceau-S staining. Membranes were blocked in 5% nonfat milk in PBS with 0.1% Tween-20 for 6 hours. After blocking, membranes were incubated at room temperature in PBS for 12 hours with the appropriate primary antibodies including collagen type I (1:200, Santa Cruz), collagen type III (1:1000, Santa Cruz), MMP-1 (1:1000, Calbiochem), MMP-2 (1:2000, Santa Cruz), MMP-3 (1:5000, Chemicon), MMP-9 (1:8000, Chemicon), MMP-14 (1:5000, Chemicon), TIMP-1 (1:1000, Chemicon), TIMP-2 (1:2000, Calbiochem), TIMP-3 (1:2000, Cedarlane), TIMP-4 (1:1000, Chemicon), TGF-β1 (1:250, R & D), and α-SMA (1:1000, Sigma). Following three washings in PBS with 0.4% Tween-20, horseradish peroxidase (HRP)–conjugated secondary antibodies, an enhanced chemiluminescence (ECL) detection system (Amersham) and Kodak film were used for visualization. The membranes were stripped and re-probed with GAPDH (1:4000, Advanced Immunochemical), β-actin (1:800, Cell Signaling), and α-tubulin (1:200, Santa Cruz) antibodies to verify equal loading among lanes. Densitometry (as area × grayscale relative to lane background) was performed using a scanner interfaced with a microcomputer and the NIH Image J software program.

Morphological analysis

Morphological analysis and tissue sectioning were conducted as described previously (68). Briefly, cross-sections of left ventricle were cut (8 μm thick) in a cryostat (Shandon) pre-cooled to -20°C at halfway between the apex and atria, placed on slides and air-dried for 30 min. For assessment of fiber cross-sectional area, fiber number, and extramyocyte space, cross-sections were rinsed three times in PBS and stained with two drops of hematoxylin, incubated for 1 min at room temperature. Hematoxylin was chosen for visualization of morphology, identification of nuclei number, location, and extramyocyte area. Stained sections were then rinsed with PBS and air dried before mounting in Vectamount medium (Vector Laboratories). Mounted muscle cross-sections were then dried overnight prior to analyses. Finally, left ventricle cross-sectional images were visualized and captured using a microscope (Axiophot2, Carl Zeiss) at a magnitude of 200X.

Masson's trichrome staining

Extramyocyte connective tissue was estimated via collagen staining using an adaptation of the Masson's trichrome technique. Briefly, 8 μm frozen left ventricle cross-sections were cut at -16°C and placed on a slide. After a 20 min drying period, slides were placed in a Columbia jar and fixed overnight at room temperature in Bouin's solution. Slides were then rinsed in distilled water for 3 min, the running tap water for 5 min. Cross sections were then stained in Weigert's hematoxylin for 15 min, and washed in distilled water, then running tap water for 5 min. Muscle fibers were then stained with

Biebrich scarlet-acid fuschin for 15 min, then washed in dH_2O for 5 min. After differentiation in phosphomolybdic-phosphotungstic acid solution for 15 min, sections were transferred directly into aniline blue solution for 12 min. The sections were differentiated in 1% acetic acid solution for 3 min, dehydrated in 95% and 100% ethanol, then cleared in xylene. In this techniques left ventricle muscle fibers were stained bright red and collagen fibers bold blue. Images were captured on a Zeiss Axio-Vision-series microscope and software, and quantified using the NIH Image J program.

Statistical analysis

Data were analyzed with two-way ANOVAs (aging × exercise training) to determine the existence of mean differences for age and exercise effects. When appropriate, a Fisher's LSD was performed for post hoc comparisons. All values were presented as mean ± SEM. Statistical significance was established at $P < 0.05$.

CHAPTER III

RESULTS

Body weight, heart weight, heart-to-body weight ratio, and citrate synthase activity

For body weight (BW), after 12 weeks of treadmill exercise training, both aging and training effects were observed, with old animals being significantly heavier than their younger cohorts, and exercise rats being significantly lighter than sedentary counterparts in both young and old groups (Table 1). In particular, although BW of old sedentary rats increased (+19.2 g) after 12 weeks of exercise training periods as they grew, BW of exercise groups was markedly reduced (-67.2g) after exercise training.

Heart weights (HW) from the old groups (OS, OE) were also significantly heavier than those of younger animals (YS, YE) (Table 1). For example, the HW of old sedentary group was 61.8% higher than younger sedentary group. But there was no absolute hypertrophy of the heart in either exercise trained groups compared with age-matched controls.

12 weeks of endurance exercise training significantly increased Heart-to-body weight ratio (HW/BW ratio) in the young groups (Table 1). Although no change was seen between young controls and old controls, the HW/BW ratio in young exercise-trained rats was 11.7% higher when compared with young sedentary controls. Even if the body weight of old exercise group significantly decreased after exercise training, there was no change in HW/BW ratio in old exercise-trained rats due to slightly reduced heart weight compared with old controls.

We found that citrate synthase activity of soleus muscle (type I muscle) in old sedentary controls was significantly lower (-23.03%) compared to the young sedentary controls (Table 1). Exercise training resulted in a significant increase (+32.45%) in citrate synthase activity of soleus muscle in the young trained group compared to young sedentary controls. However, there was no significant difference in citrate synthase activity of soleus muscle between old sedentary and old exercise groups.

Extramyocyte space, connective tissue, and morphology

Left ventricle cross sections were stained with hematoxylin for visualization of morphology and extramyocyte space. We focused on the left ventricle as age-related cell loss and apoptosis are more prevalent in left ventricle than other parts of the heart (61). We observed dramatic remodeling of the left ventricles (Fig. 2) from the old sedentary groups when compared with left ventricle sections from the young sedentary animals. Remarkably, left ventricle cross sections from the old exercise trained group exhibited less age-related remodeling than sedentary rats of matched age (Fig. 2).

Aging increased the amount of connective tissue and fat in the left ventricles as quantified by extramyocyte space. The percentage of extramyocyte space was dramatically higher in the old sedentary rats (37.3±2.9%) compared with left ventricles in young sedentary animals (9.6±1.0%) (Fig. 3). In addition, not only was the amount of apparent fibrosis higher in old sedentary hearts, but also the geometry of connective tissue area in the aging sedentary heart was more weblike (Fig. 2).

Exercise training did not alter extramyocyte space in left ventricles from young rats (8.2±0.4% vs. 9.6±1.0%). However, exercise training did retard age-induced elevation of extramyocyte space in the left ventricle, with 22.4±4% of left ventricle area comprising connective tissue, down from 37.3±2.9% in old sedentary groups (Fig. 3). In addition, the geometric pattern of extramyocyte area was more linear and regular in the old exercise group than the old sedentary heart (Fig. 2). In other words, exercise training resulted in a pattern in the old exercise groups that was more similar to young sedentary hearts than hearts from old sedentary animals.

Masson's trichrome staining revealed greater accumulation of collagen in the OS group than YS, as seen from the blue staining (Fig. 4). There was also significant remodeling and altered geometry to a more "web-like" appearance. This occurred in the myocardium primarily in the 60% of the area towards the endocardial surface. Left ventricular samples from old rats that had exercised for 12 weeks exhibited less connective tissue and altered geometry (Fig. 4).

Table 1. Body weight, heart weight, HW-to-BW ratio, and citrate synthase activity in the soleus of young sedentary, young exercised, old sedentary, and old exercised rats.

Groups	BW (g)		HW (g)	HW/BW (mg/g)	CS (μmol/g/min)
	before	after			
YS	311 ± 9	380 ± 11	0.92 ± 0.05	2.40 ± 0.06	32.17 ± 0.75
YE	310 ± 7	336 ± 6 (*)	0.90 ± 0.02	2.68 ± 0.07 (*)	42.61 ± 0.96 (*)
OS	597 ± 38 (a)	616 ± 20 (a)	1.48 ± 0.04 (a)	2.41 ± 0.09	24.76 ± 0.91 (a)
OE	616 ± 20 (a)	549 ± 18 (a) (*)	1.37 ± 0.04 (a)	2.50 ± 0.08	23.60 ± 0.87 (a)

Data are expressed as mean ± SEM. BW, body weight; HW, heart weight; CS, citrate synthase activity; YS, young sedentary group; YE, young exercise group; OS, old sedentary group; OE, old exercise group. [a]Statistically significant change relative to young sedentary group ($P<0.05$); *Significant change relative to age-matched group ($P<0.05$).

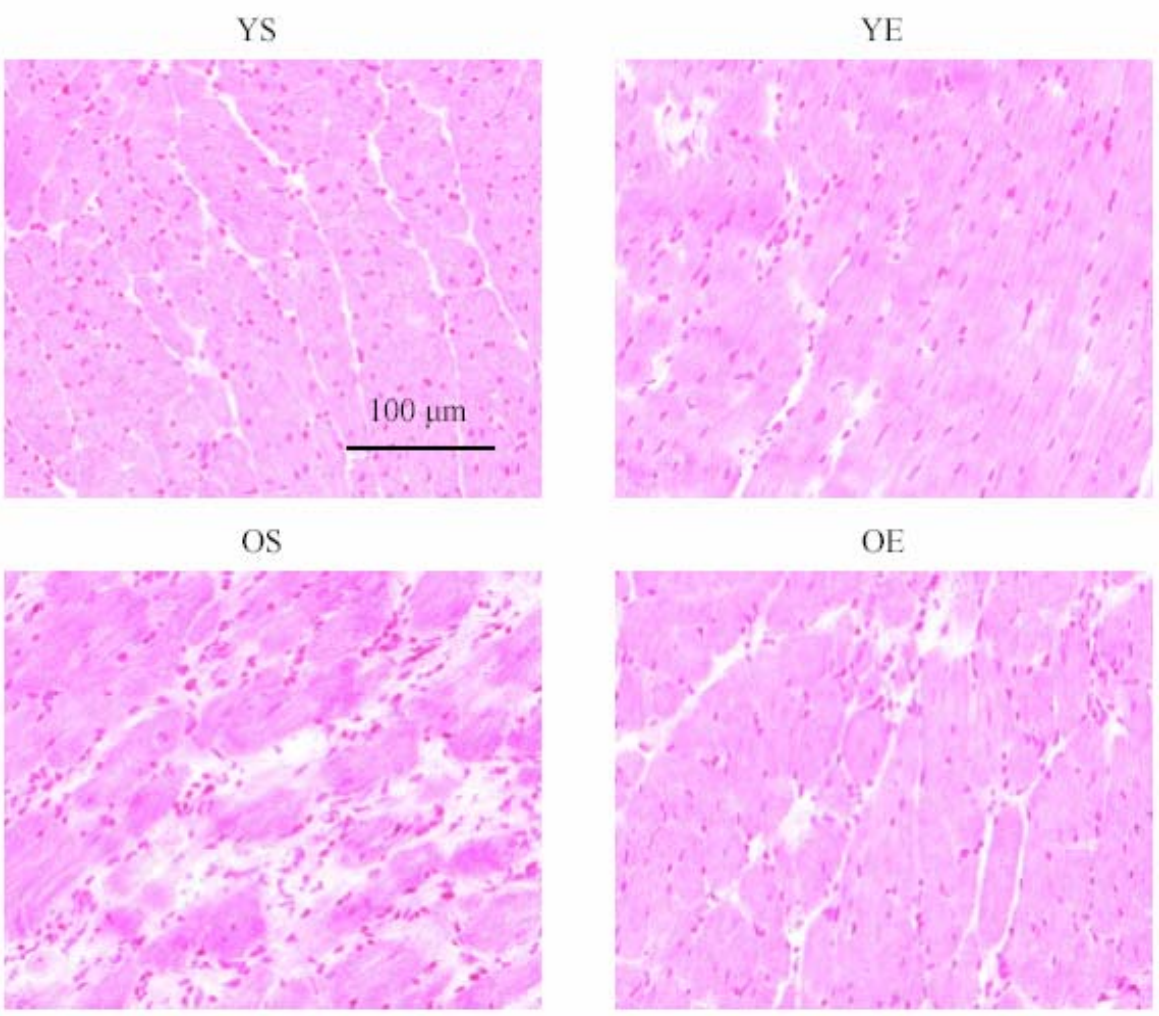

Fig. 2. Hematoxylin-stained cross sections of left ventricles with aging and exercise training. The left ventricle cross sections (×200 resolution with 100-μm calibration bar) were stained with hematoxylin from the following groups: young (6 months) sedentary controls (YS), young exercise-trained (YE), old (34 months) sedentary controls (OS), and old exercise-trained (OE).

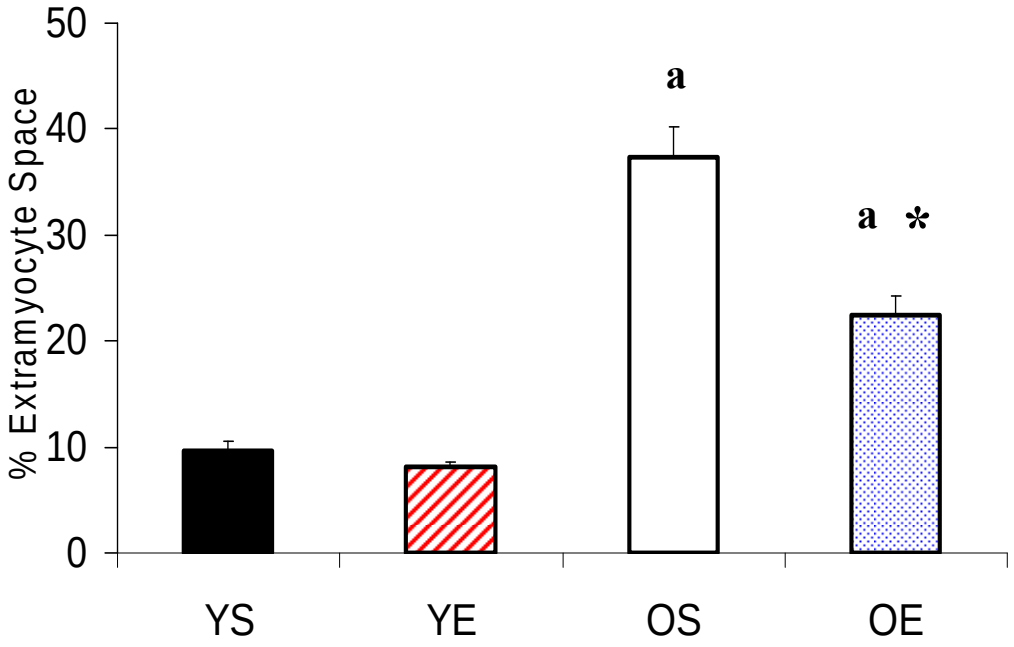

Fig. 3. Effect of aging and exercise training on percent (%) of extramyocyte space. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

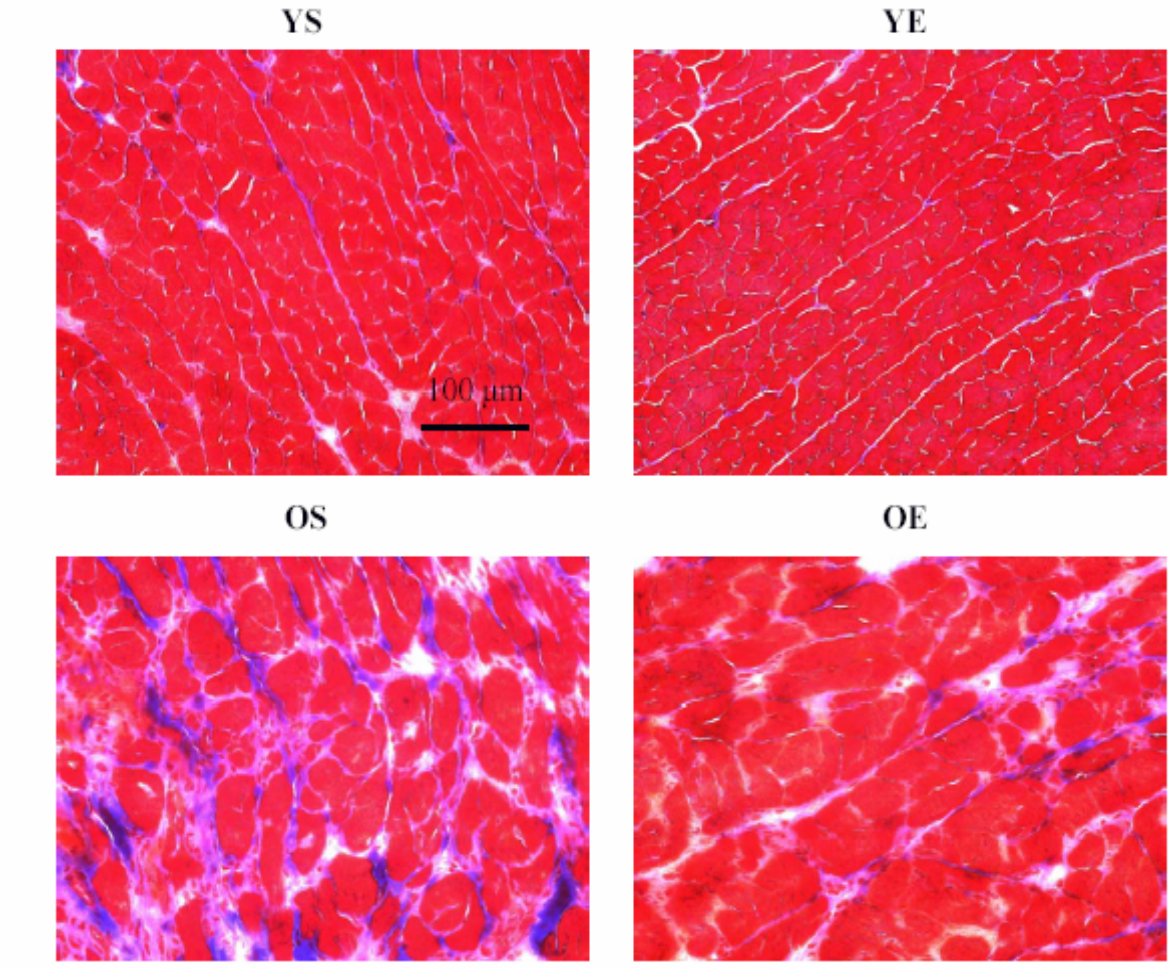

Fig. 4. Masson's trichrome-stained cross sections of left ventricles with aging and exercise training. The left ventricle cross sections (×160 resolution with 100-μm calibration bar) were stained with Masson's trichrome from the following groups: young (6 months) sedentary controls (YS), young exercise-trained (YE), old (34 months) sedentary controls (OS), and old exercise-trained (OE).

Pro-MMP-1 protein levels

There were no aging and exercise training effects on pro-matrix metalloproteinase-1 (pro-MMP-1) protein levels in the soluble fraction of left ventricle tissues (Fig. 5). Consistently, aging did not affect the protein levels of pro-MMP-1 in the insoluble fraction of left ventricle. But, there was an increased trend ($p=0.063$) in old trained group compared to old controls (Fig. 6)

Active MMP-1 protein levels

There were also no aging and exercise training effects on active MMP-1 protein levels in the soluble fraction of left ventricle tissues (Fig. 7). However, the protein levels of active MMP-1 in the insoluble fraction of old controls were significantly lower (-12.0%) compared to the young controls (Fig. 8). In contrast, exercise training resulted in a significant increase (+12.4%) of active MMP-1 protein levels in the old trained group compared to old controls (Fig. 8). However, there was no significant difference in active MMP-1 insoluble protein levels between young sedentary controls and young trained rats (Fig. 8).

Pro-MMP-2 protein levels

The protein levels of pro-MMP-2 in the soluble fraction of left ventricle of old controls were significantly lower (-8.9%) than young controls (Fig. 9). However, exercise training significantly decreased pro-MMP-2 soluble protein levels by 7.6% in the young trained group compared to young sedentary controls (Fig. 9).

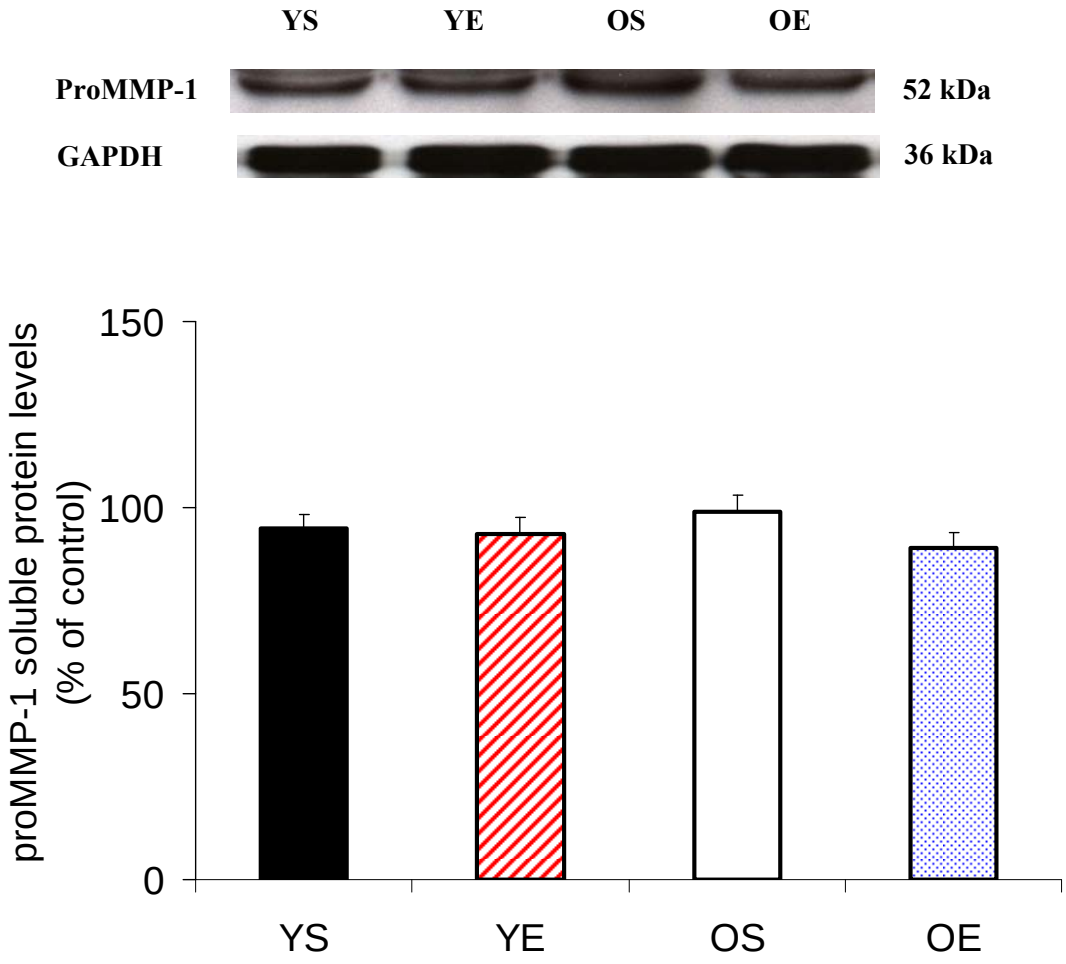

Fig. 5. Effect of aging and exercise training on pro-MMP-1 protein levels in the soluble fraction. Values are mean ± SEM.

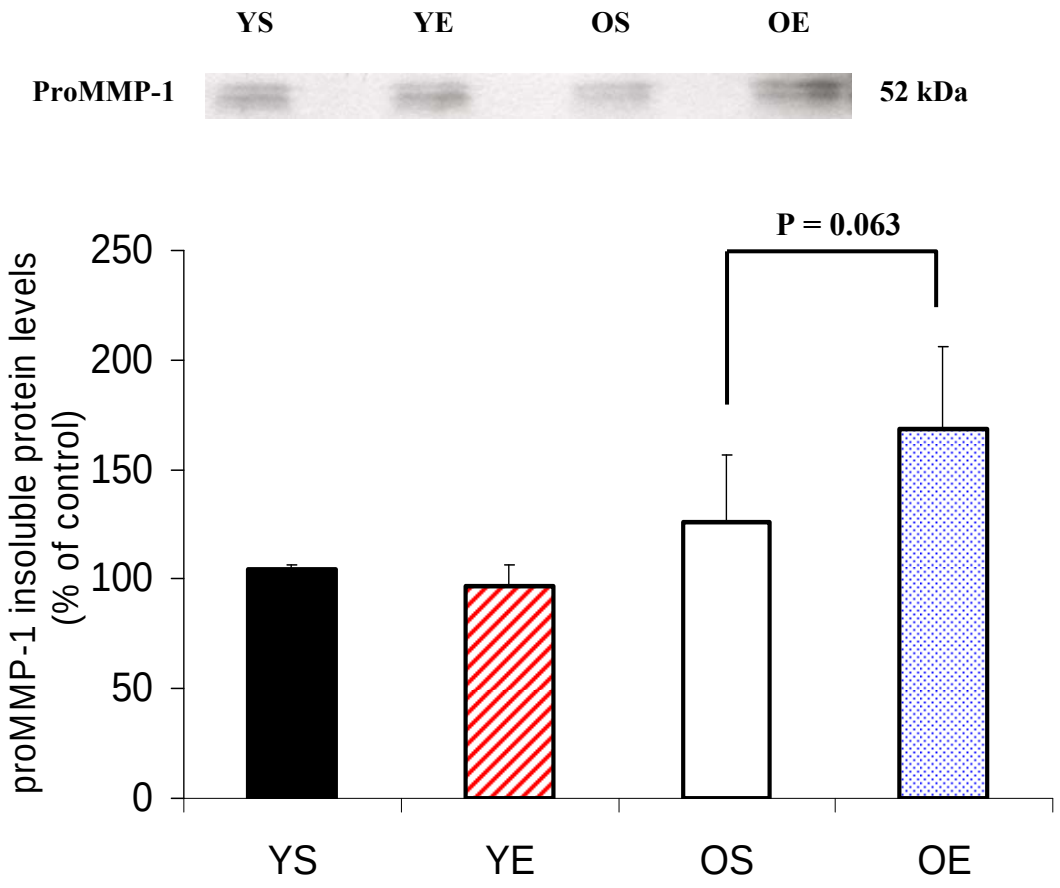

Fig. 6. Effect of aging and exercise training on pro-MMP-1 protein levels in the insoluble fraction. Values are mean ± SEM.

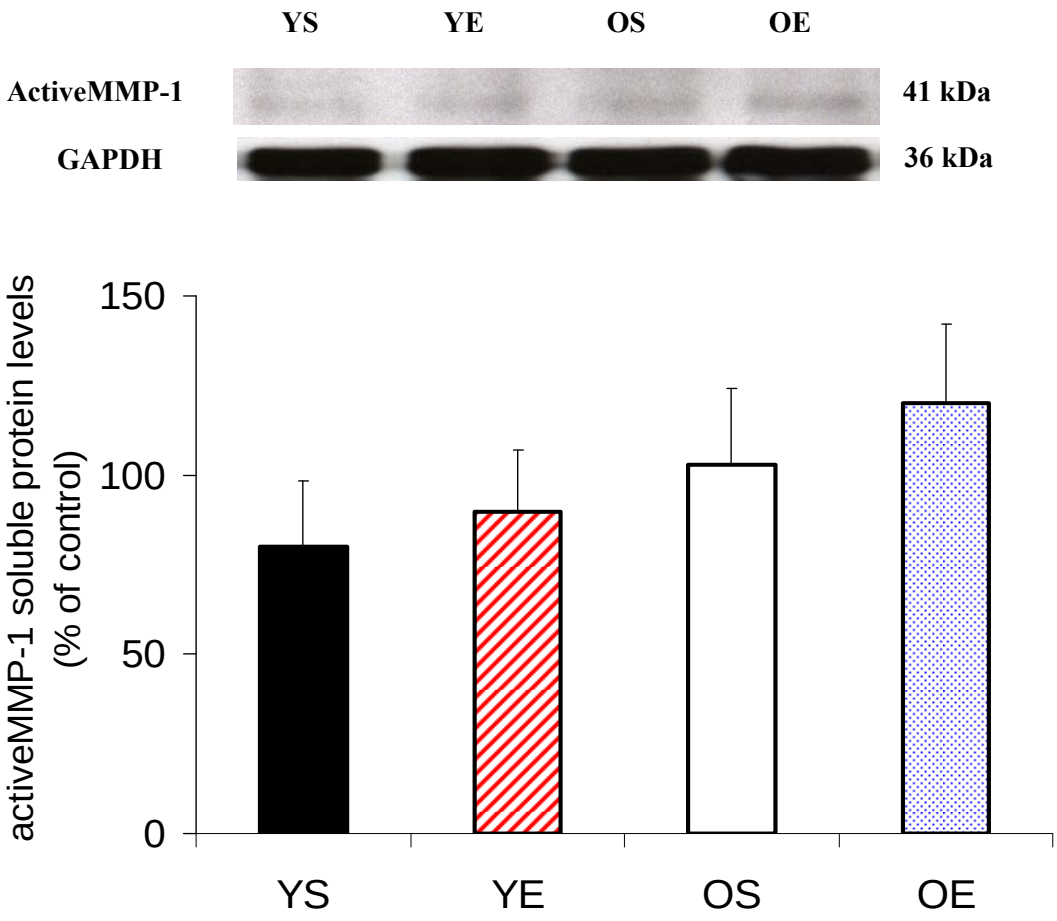

Fig. 7. Effect of aging and exercise training on active MMP-1 protein levels in the soluble fraction. Values are mean ± SEM.

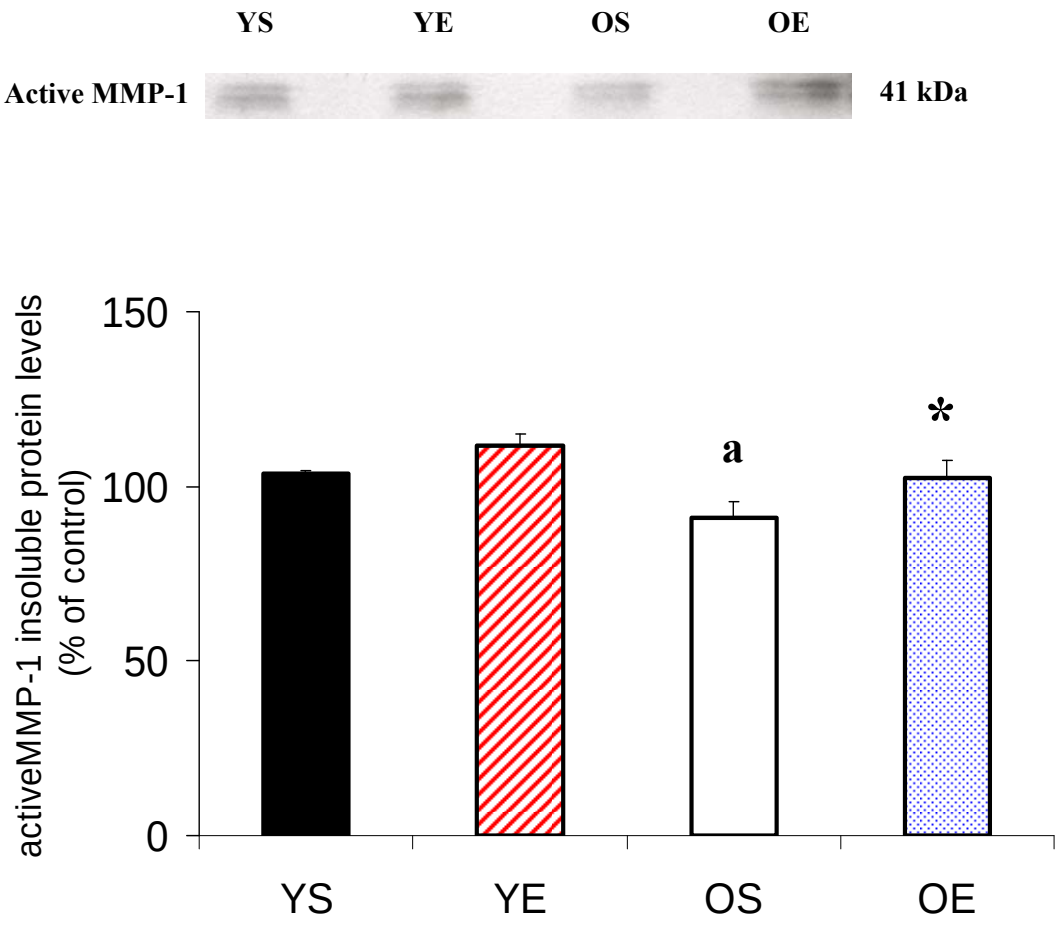

Fig. 8. Effect of aging and exercise training on active MMP-1 protein levels in the insoluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

But, there was no significant training effect on pro-MMP-2 soluble protein levels in the old group (P=0.116; Fig. 9). In addition, in the insoluble fraction of left ventricle tissues, there were no aging and exercise training effects on pro-MMP-2 protein levels (Fig. 10).

Active MMP-2 protein levels

The protein levels of active MMP-2 in the soluble fraction in the left ventricle of old controls were significantly lower (-36.3%) than young controls (Fig. 11). In contrast, we found a significant increase (+24.0 %) in active MMP-2 protein levels with exercise training in the soluble fraction of left ventricle from old rats (Fig. 11). But, there was no significant training effect on active MMP-2 protein levels in young groups (Fig. 11). The active MMP-2 proteins in the insoluble fraction of left ventricle were undetectable in both young and old groups.

Pro-MMP-3 protein levels

The protein levels of pro-MMP-3 in the soluble fraction in the left ventricle of old controls were dramatically higher (+209.6%) than young controls (Fig. 12). Although it was not significant, there was an increased trend in pro-MMP-3 soluble protein levels with exercise training in old groups (P=0.176; Fig. 12). In addition, we did not find training effect on pro-MMP-3 protein levels of the soluble fraction in the young groups (Fig. 12).

In the insoluble fraction of left ventricle tissues, there was no aging effect on pro-MMP-3 protein levels. Exercise training resulted in a significant increase (+38.3%) of pro-MMP-3 protein levels in the old trained group compared to old controls (Fig. 13).

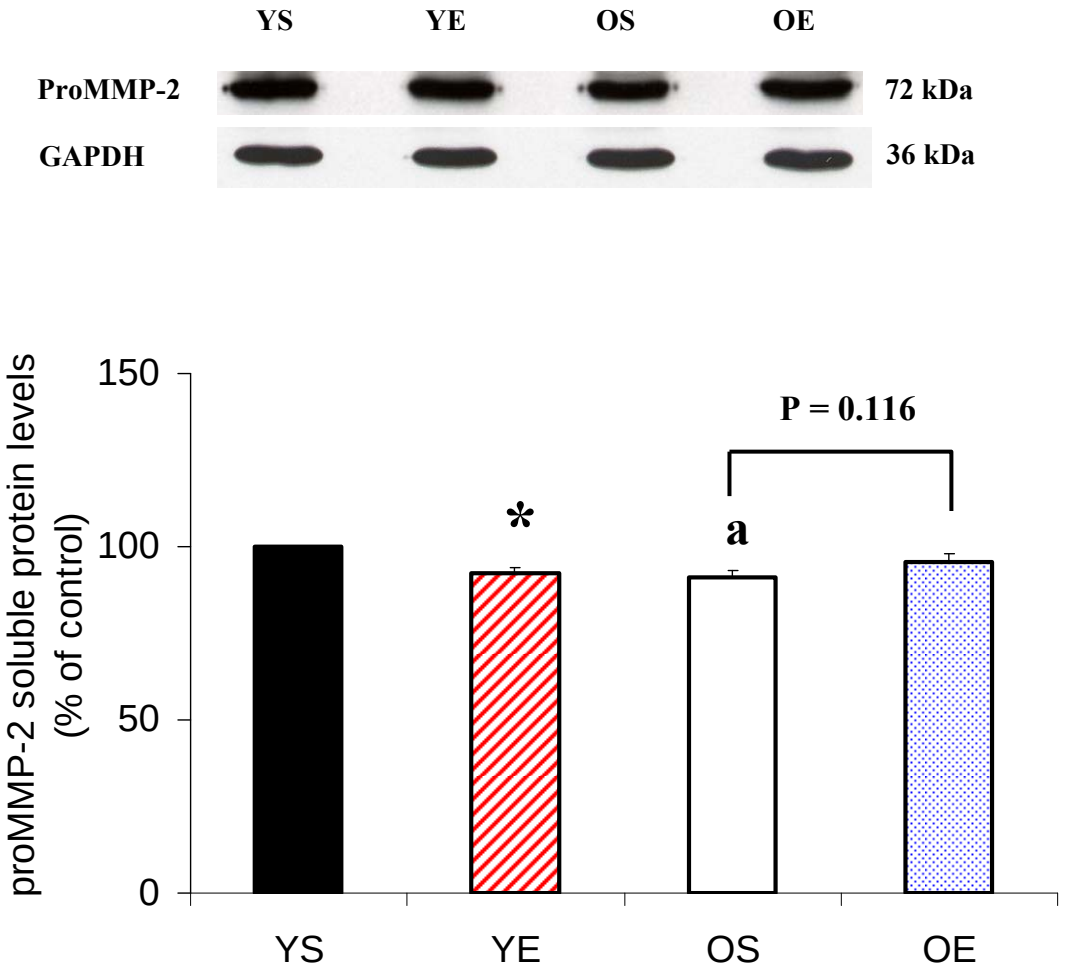

Fig. 9. Effect of aging and exercise training on pro-MMP-2 protein levels in the soluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

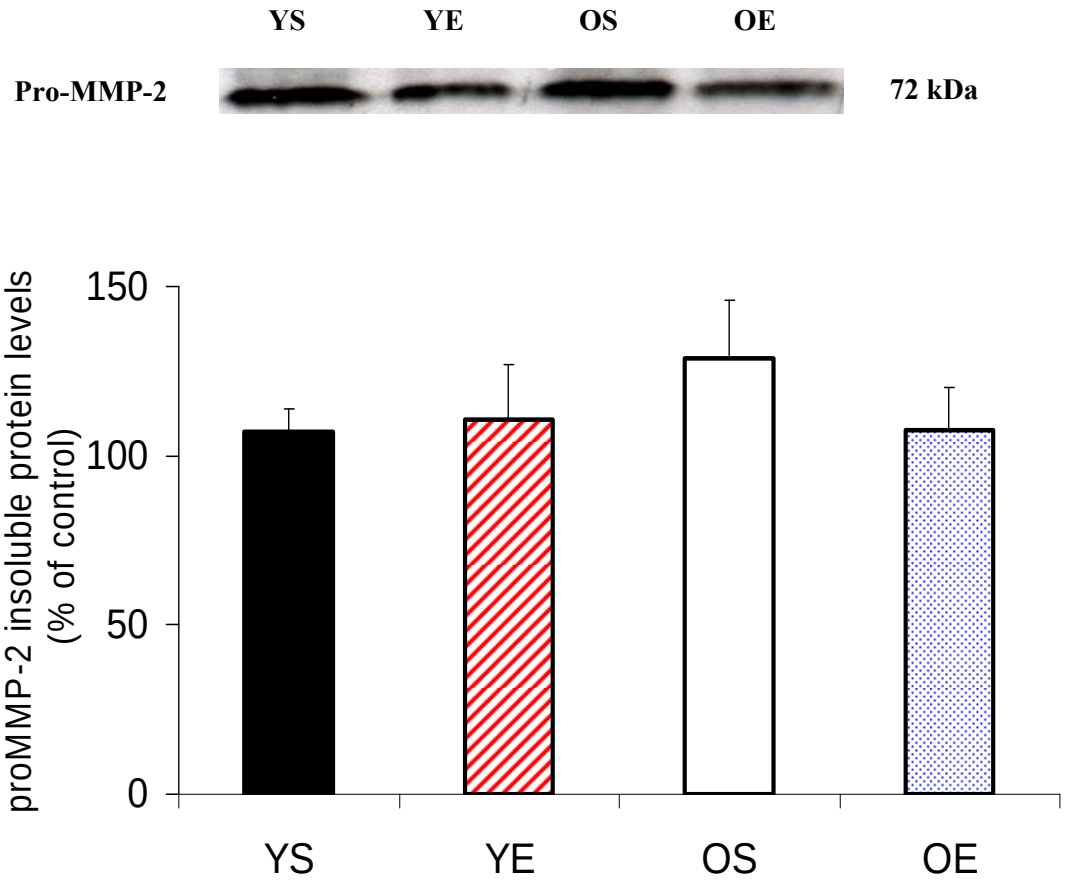

Fig. 10. Effect of aging and exercise training on pro-MMP-2 protein levels in the insoluble fraction. Values are mean ± SEM.

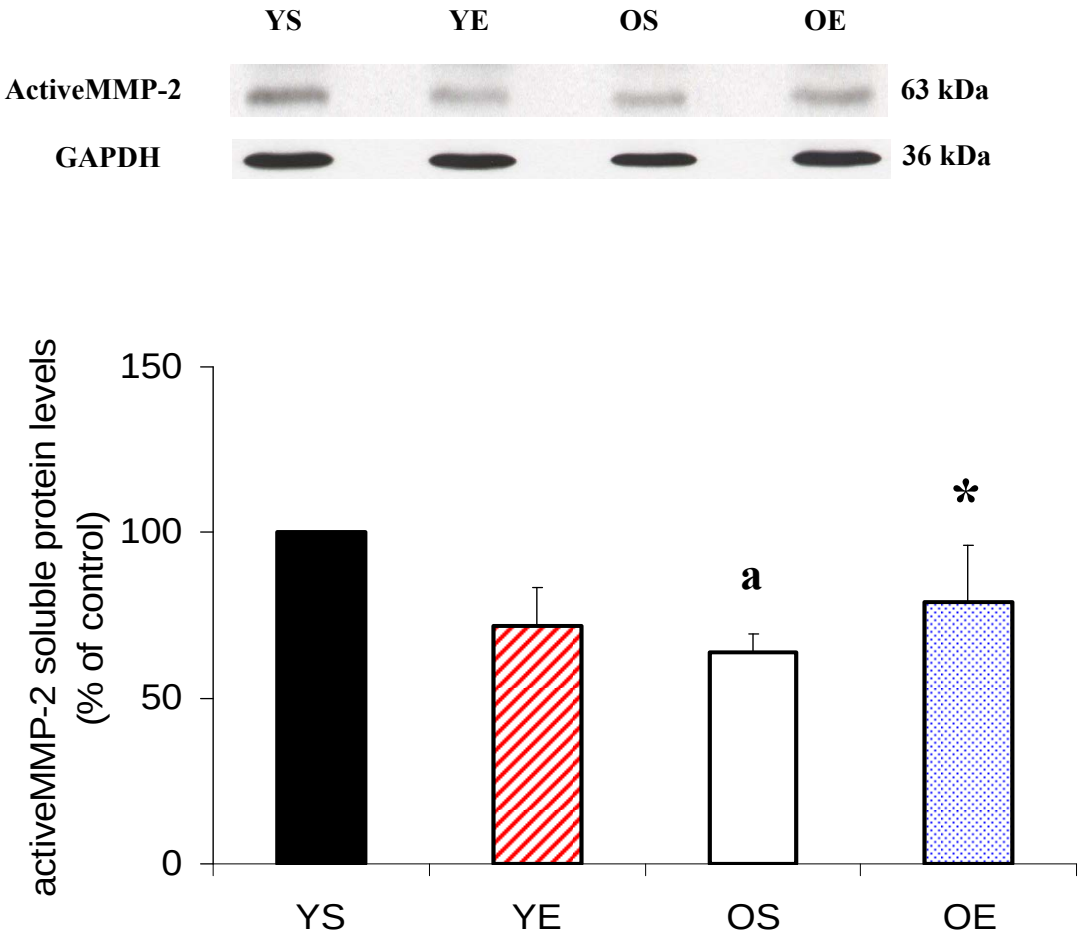

Fig. 11. Effect of aging and exercise training on active MMP-2 protein levels in the soluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

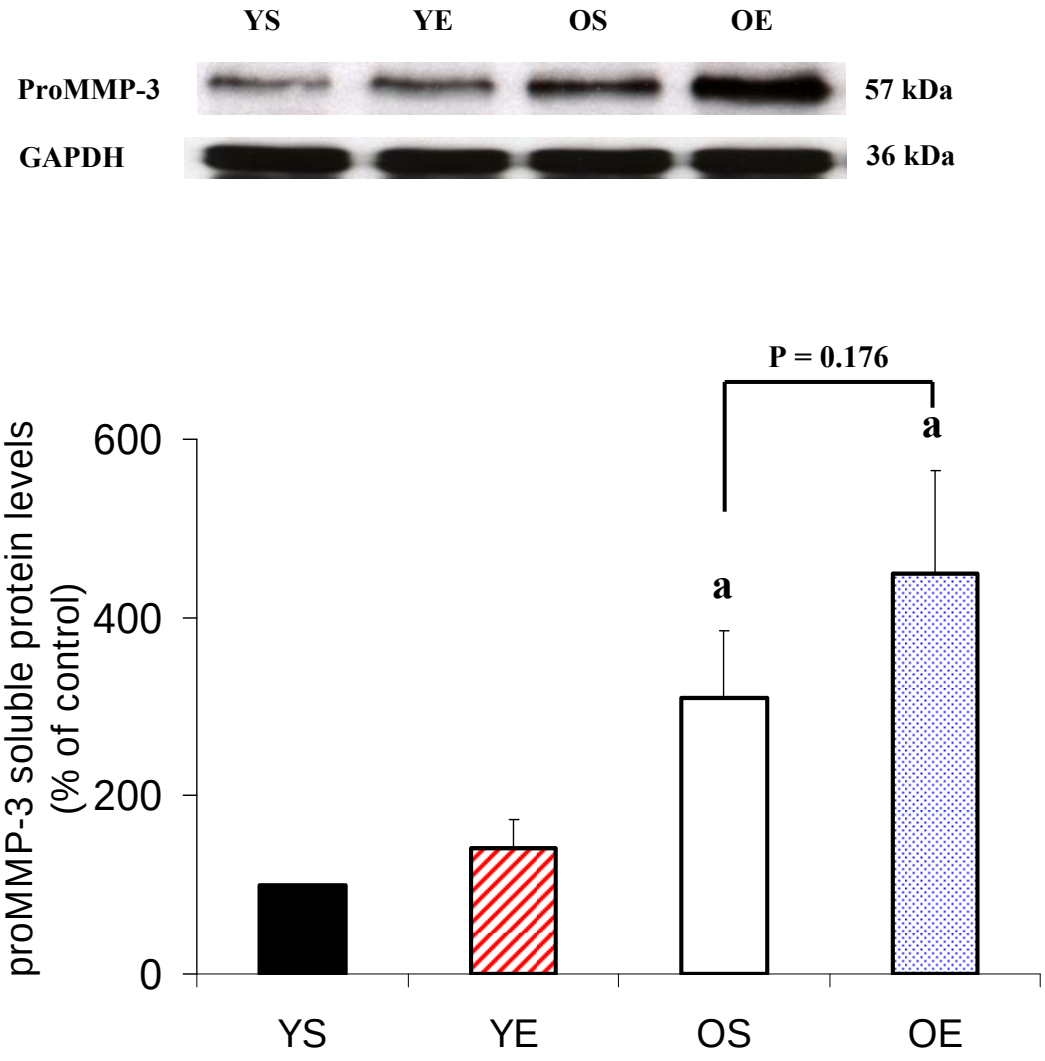

Fig. 12. Effect of aging and exercise training on pro-MMP-3 protein levels in the soluble fraction. Values are mean ± SEM. [a]Indicates old groups (OS, OE) are significantly different from young sedentary control (YS) ($P < 0.05$).

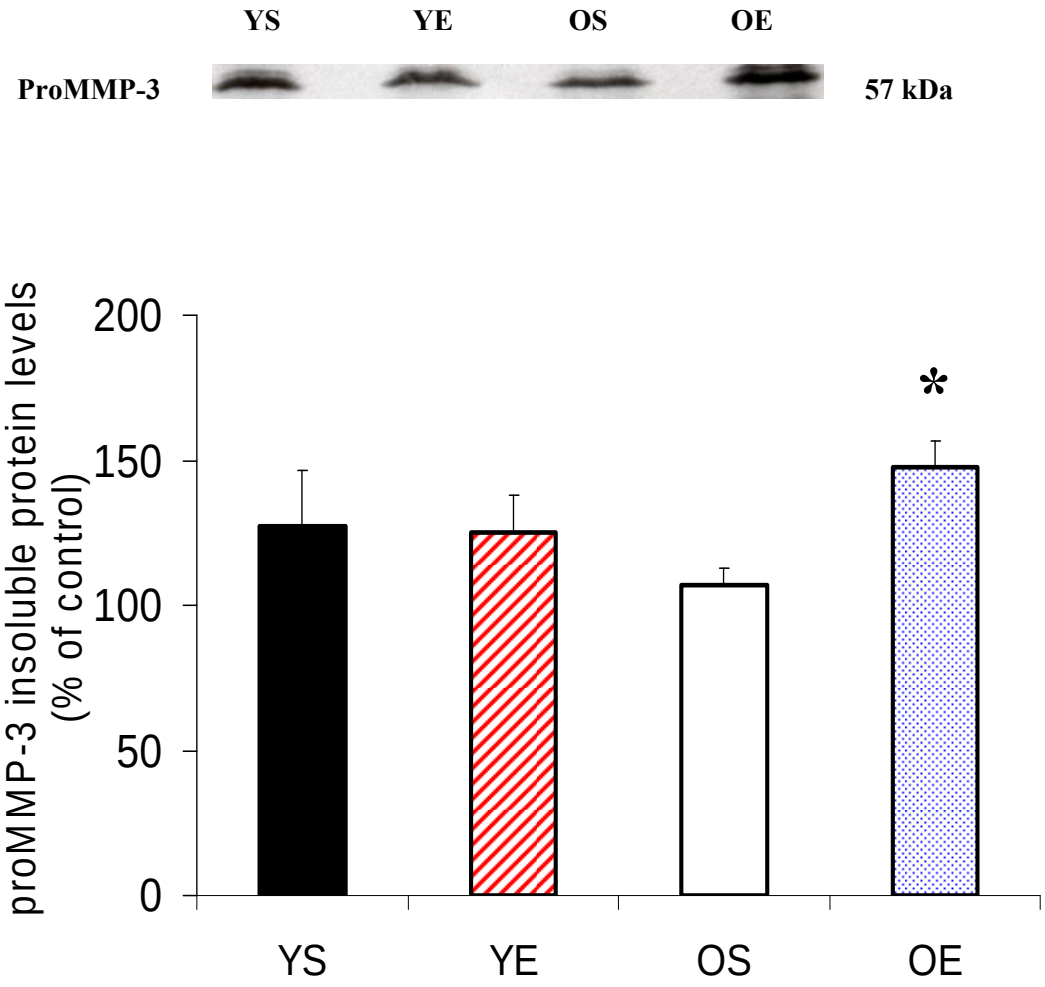

Fig. 13. Effect of aging and exercise training on pro-MMP-3 protein levels in the insoluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$).

However, exercise training had no effect on pro-MMP-3 insoluble protein levels of left ventricles in the young age group (Fig. 13).

Active MMP-3 protein levels

The proteins of active MMP-3 in both soluble fraction and insoluble fraction of left ventricle tissues were not detectable in either young or old groups.

Pro-MMP-9 protein levels

There were no aging and exercise training effects on pro-MMP-9 protein levels in the soluble fraction of left ventricle tissues (Fig. 14). Consistently, we also did not find significant differences in pro-MMP-9 protein levels with aging and exercise training in the insoluble fraction of left ventricles (Fig. 15).

Active MMP-9 protein levels

The proteins of active MMP-9 in both soluble fraction and insoluble fraction of left ventricle tissues were also undetectable in either young or old groups.

Pro-MMP-14 protein levels

We found that protein levels of pro-MMP-14 in the soluble fraction of left ventricle of old sedentary controls were significantly lower (-23.8%) compared to the young sedentary controls (Fig. 16). Exercise training resulted in a significant increase (+19.7%) in pro-MMP-14 soluble protein levels in the old trained group compared to old sedentary controls (Fig. 16).

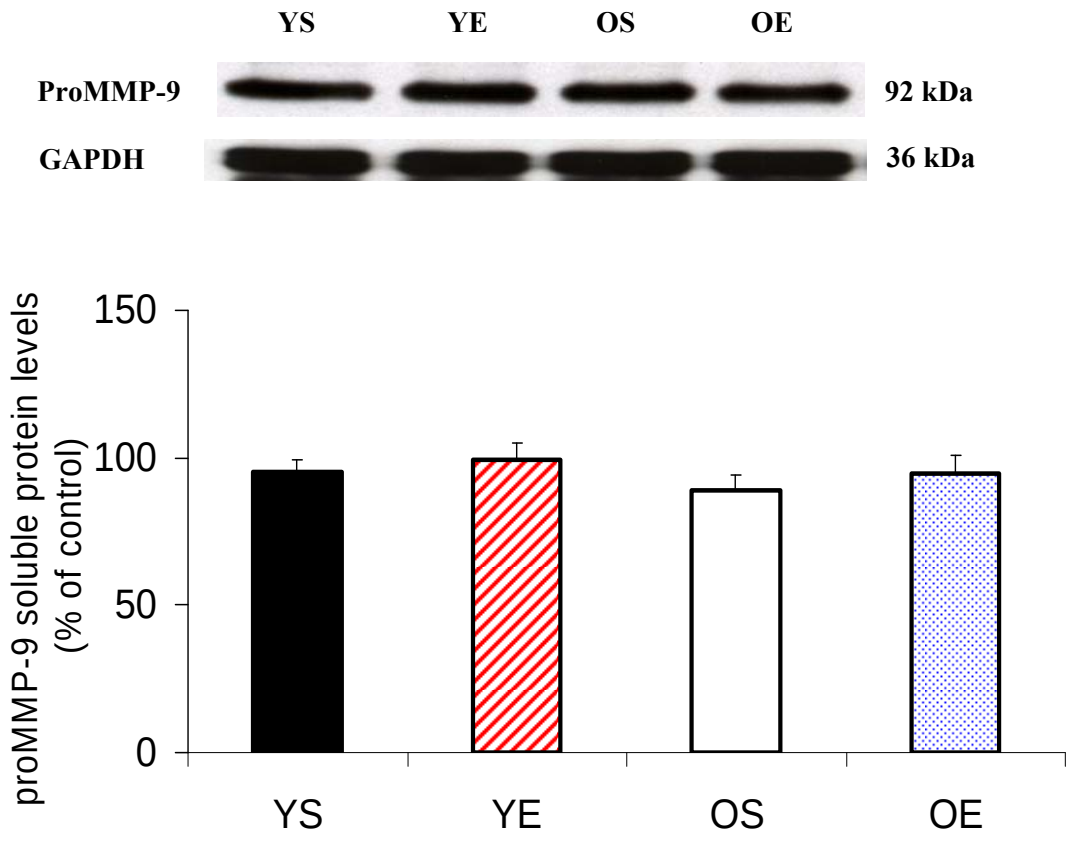

Fig. 14. Effect of aging and exercise training on pro-MMP-9 protein levels in the soluble fraction. Values are mean ± SEM.

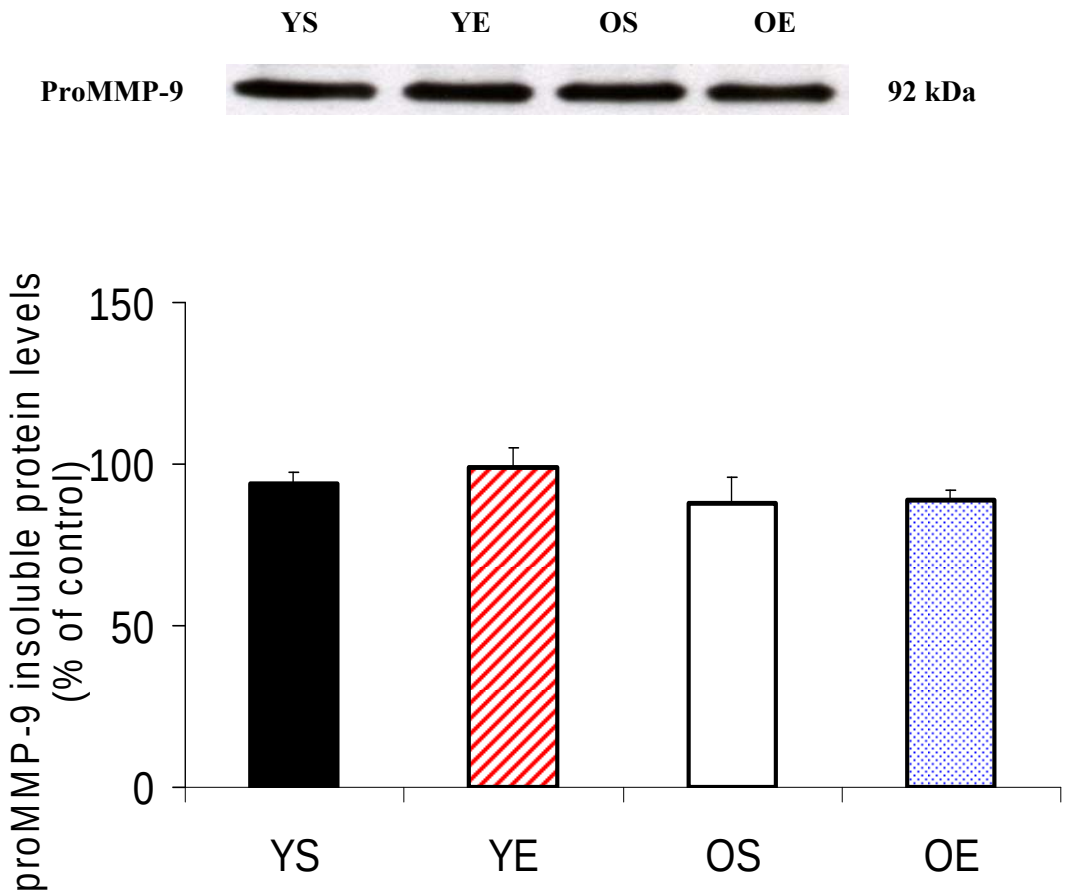

Fig. 15. Effect of aging and exercise training on pro-MMP-9 protein levels in the insoluble fraction. Values are mean ± SEM.

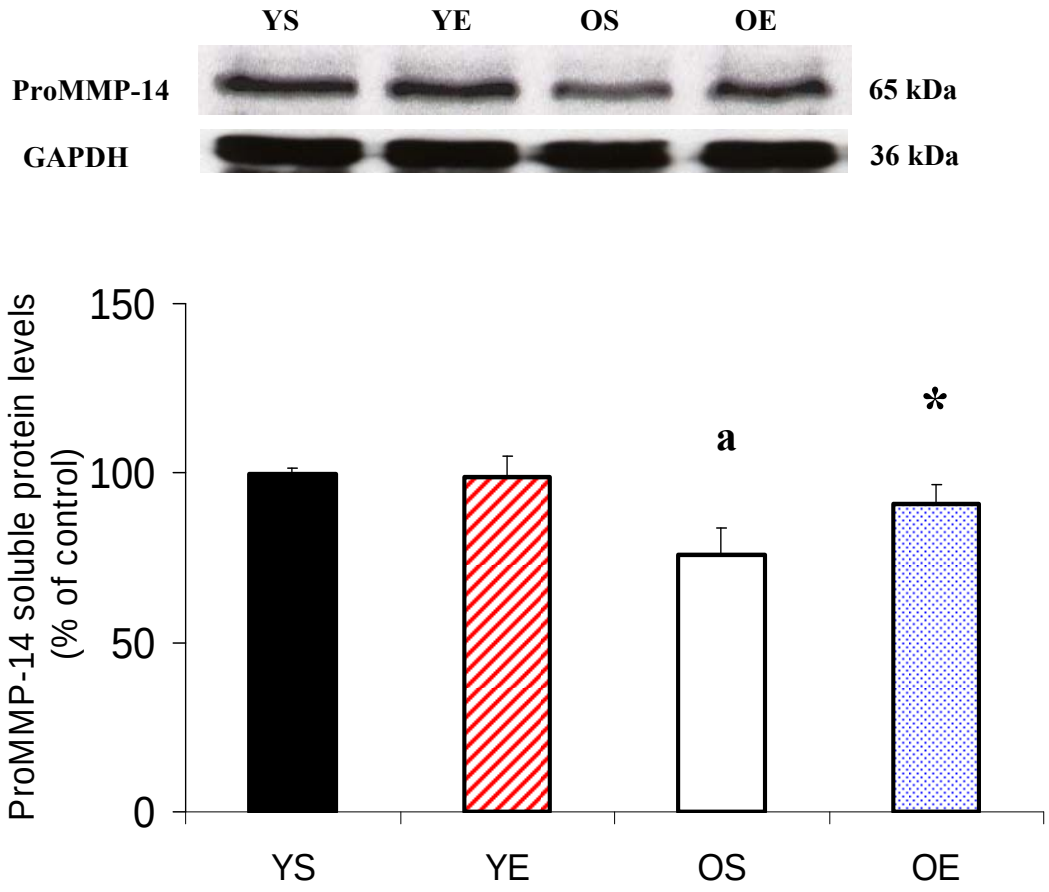

Fig. 16. Effect of aging and exercise training on pro-MMP-14 protein levels in the soluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

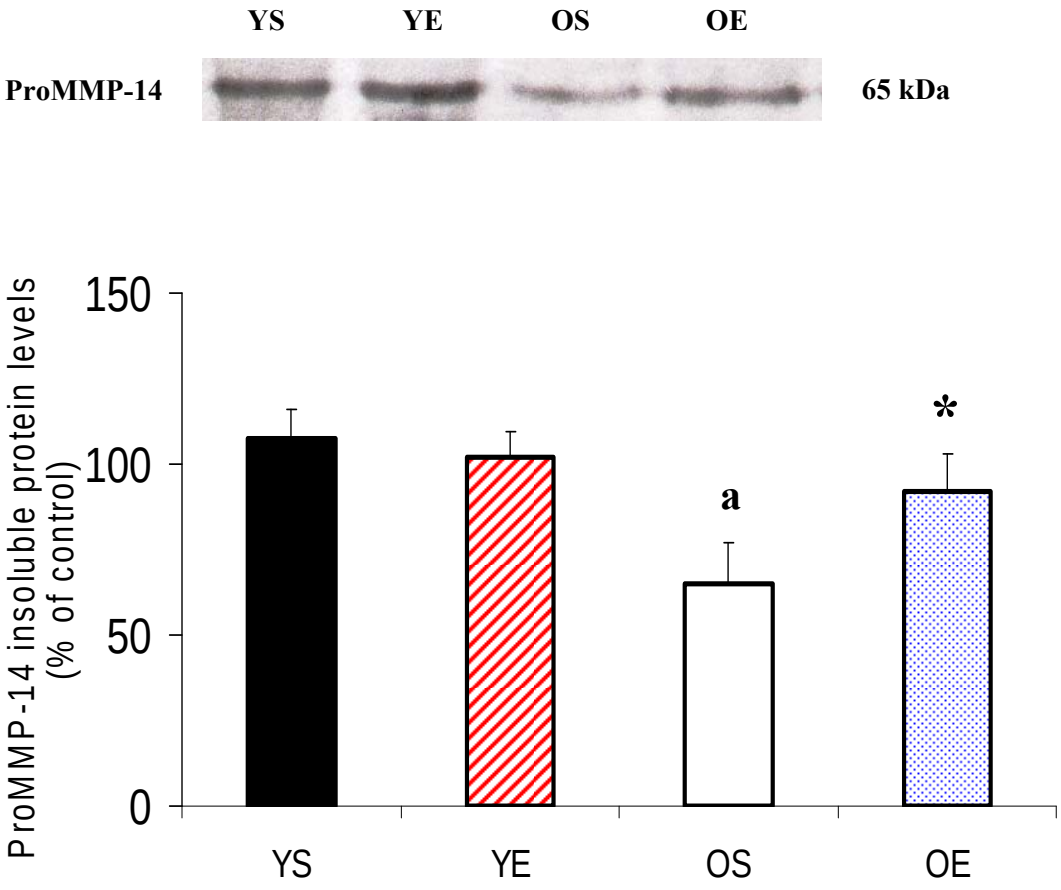

Fig. 17. Effect of aging and exercise training on pro-MMP-14 protein levels in the insoluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

However, there was no significant difference in pro-MMP-14 soluble protein levels between young sedentary controls and young trained rats (Fig. 16).

We also found similar results of pro-MMP-14 protein levels in the insoluble fraction of left ventricles (Fig. 17). Pro-MMP-14 insoluble protein levels in the left ventricle of old sedentary controls were significantly lower (-39.9%) than young sedentary controls. Exercise training significantly increased (+41.9%) pro-MMP-14 insoluble protein levels in old exercise trained group compared to old sedentary controls (Fig. 17). But, exercise training had no effect on pro-MMP-14 insoluble protein levels of left ventricles in the young age group (Fig. 17).

TIMP-1 protein levels

We found that protein levels of tissue inhibitor of metalloproteinase-1 (TIMP-1) in the soluble fraction of left ventricles in the old rats were markedly higher (+123.0%) than young sedentary rats (Fig. 18). In contrast, exercise training significantly decreased TIMP-1 soluble protein levels by 50.8% in old trained rats compared to old sedentary rats (Fig. 18). However, there was no significant difference in TIMP-1 soluble protein levels between young sedentary rats and young trained rats (Fig. 18).

The proteins of TIMP-1 in the insoluble fraction of left ventricle tissues were not detectable in both young and old rats.

TIMP-2 protein levels

There were no aging and exercise training effects on TIMP-2 protein levels in the soluble fraction of left ventricle tissues (Fig. 19). We just found reduced trend (P=0.263) in old trained groups compared to old sedentary controls (Fig. 19). In addition, the proteins of TIMP-2 in the insoluble fraction of left ventricles were undetectable in both young and old groups.

TIMP-3 protein levels

We did not find the effects of aging and exercise training on TIMP-3 protein levels in the soluble fraction of left ventricles in both young and old groups (Fig. 20). Consistently, there were also no aging and exercise training effects on TIMP-3 protein levels in the insoluble fraction of left ventricles in both young and old groups (Fig. 21).

TIMP-4 protein levels

In addition to TIMP-3 protein levels, there were no aging and exercise training effects on TIMP-4 protein levels in the soluble fraction of left ventricle tissues (Fig. 22). However, the protein levels of TIMP-4 in the insoluble fraction of old sedentary controls were significantly lower (-56.3%) compared to the young sedentary controls (Fig. 23). Exercise training had no significant effects on TIMP-4 protein levels in the old trained group compared to old sedentary controls (Fig. 23). In addition, there was no significant difference in TIMP-4 protein levels between young sedentary controls and young trained group (Fig. 23).

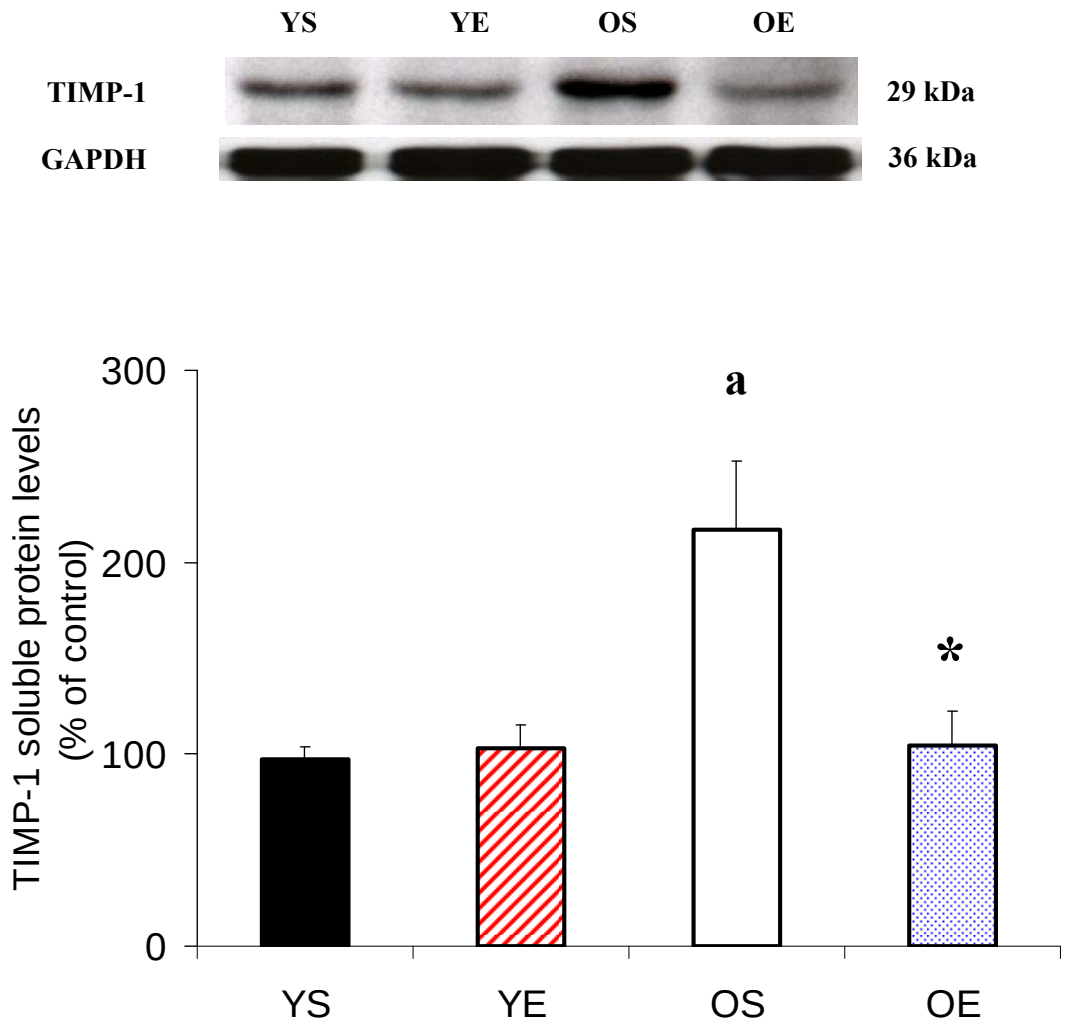

Fig. 18. Effect of aging and exercise training on TIMP-1 protein levels in the soluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

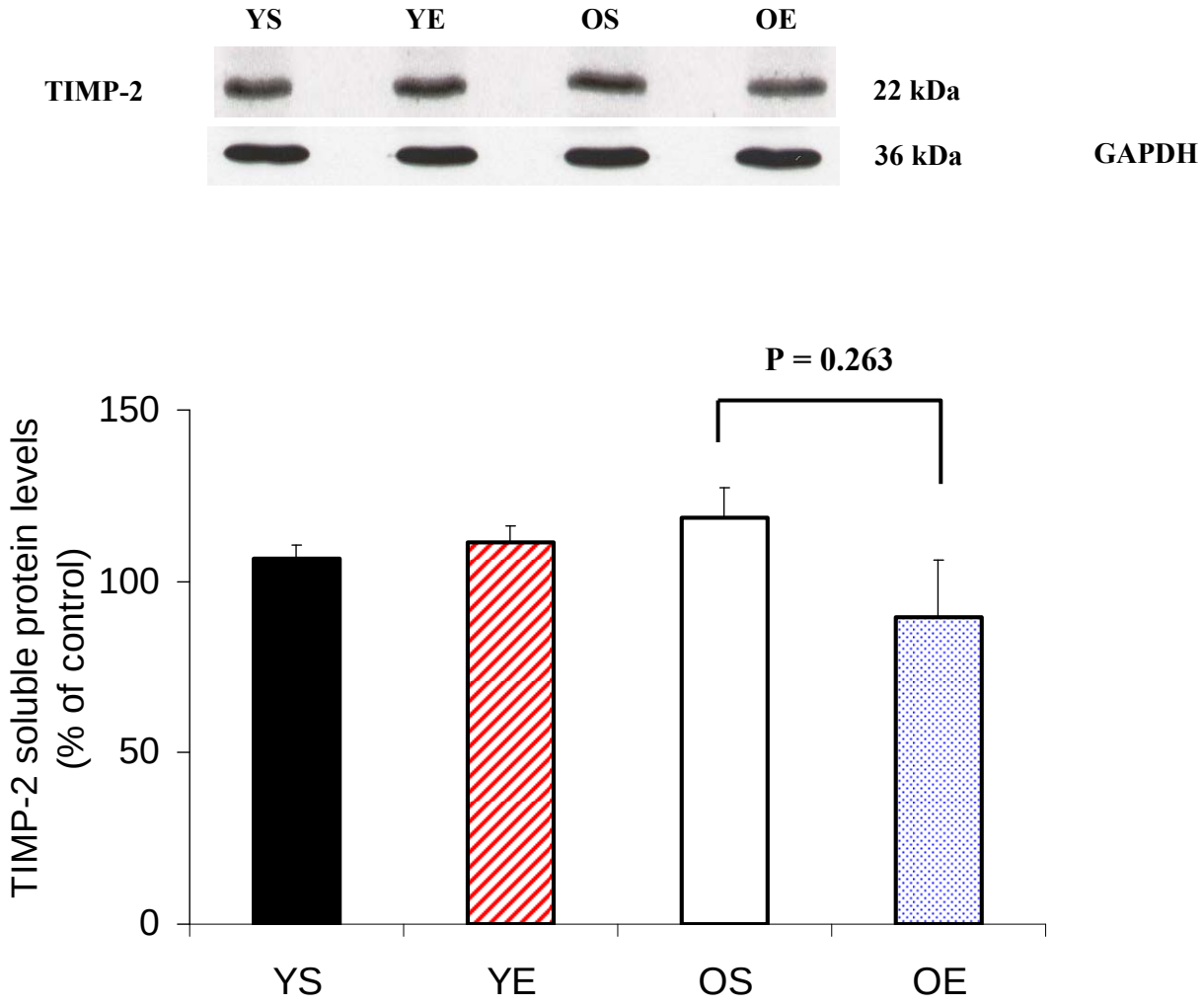

Fig. 19. Effect of aging and exercise training on TIMP-2 protein levels in the soluble fraction. Values are mean ± SEM.

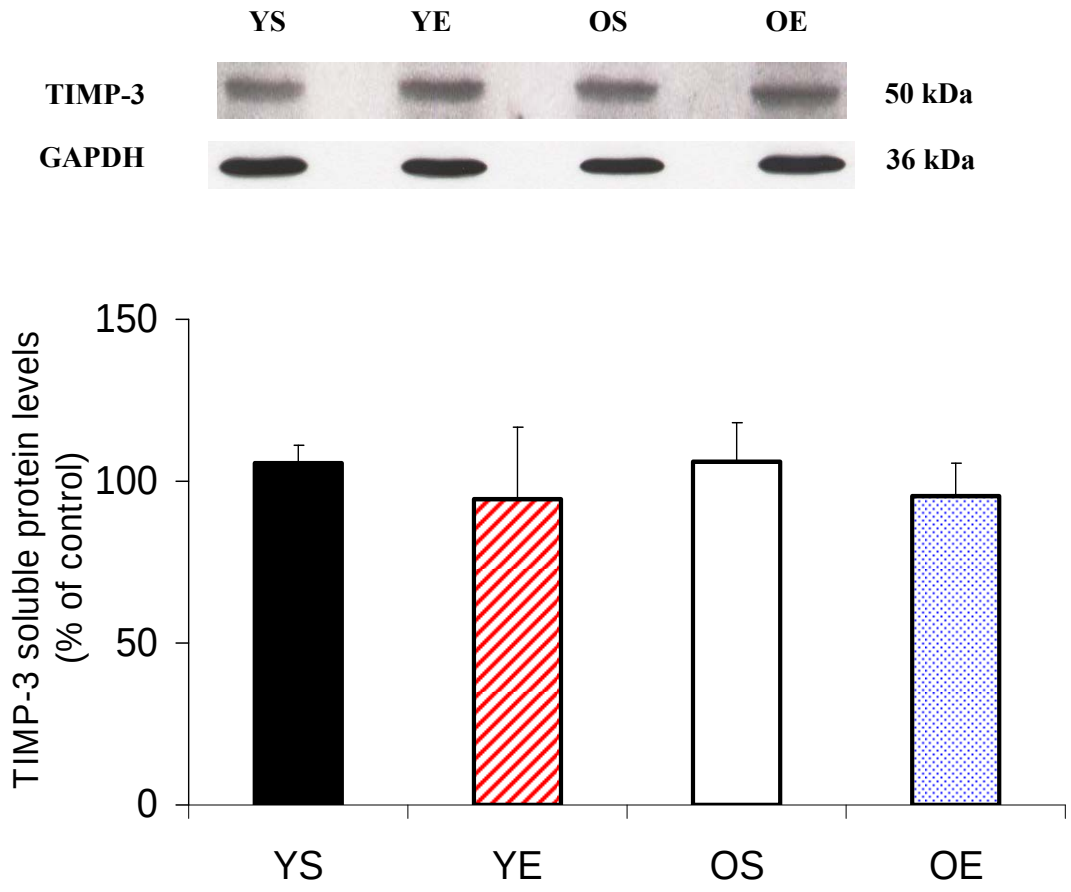

Fig. 20. Effect of aging and exercise training on TIMP-3 protein levels in the soluble fraction. Values are mean ± SEM.

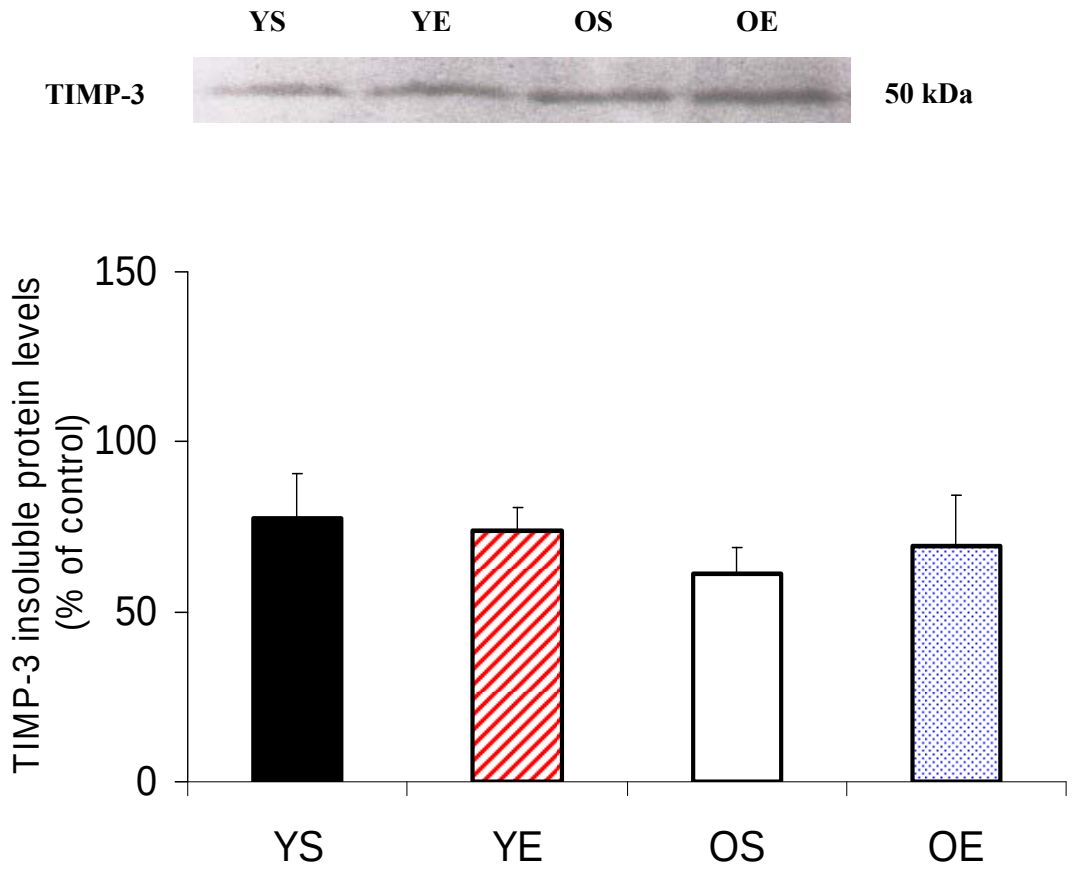

Fig. 21. Effect of aging and exercise training on TIMP-3 protein levels in the insoluble fraction. Values are mean ± SEM.

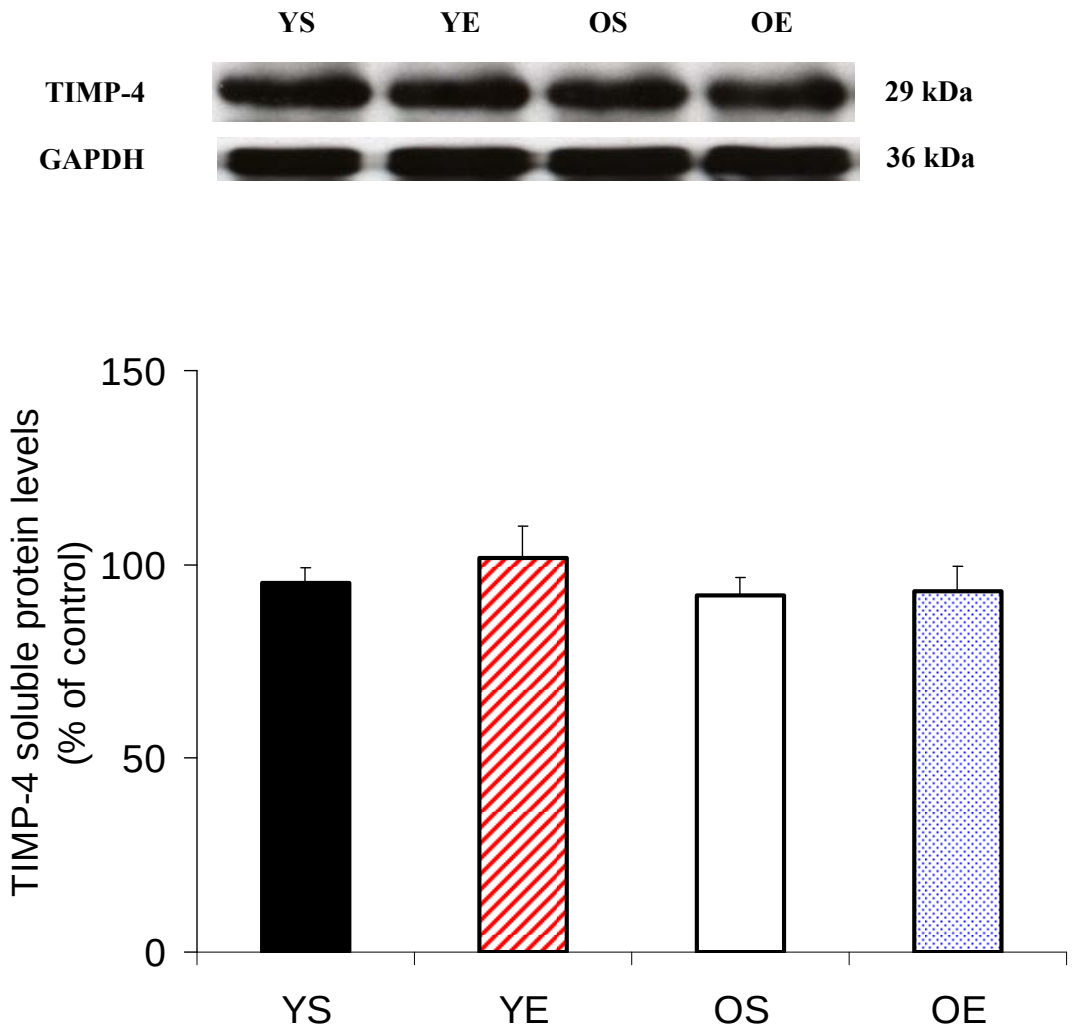

Fig. 22. Effect of aging and exercise training on TIMP-4 protein levels in the soluble fraction. Values are mean ± SEM.

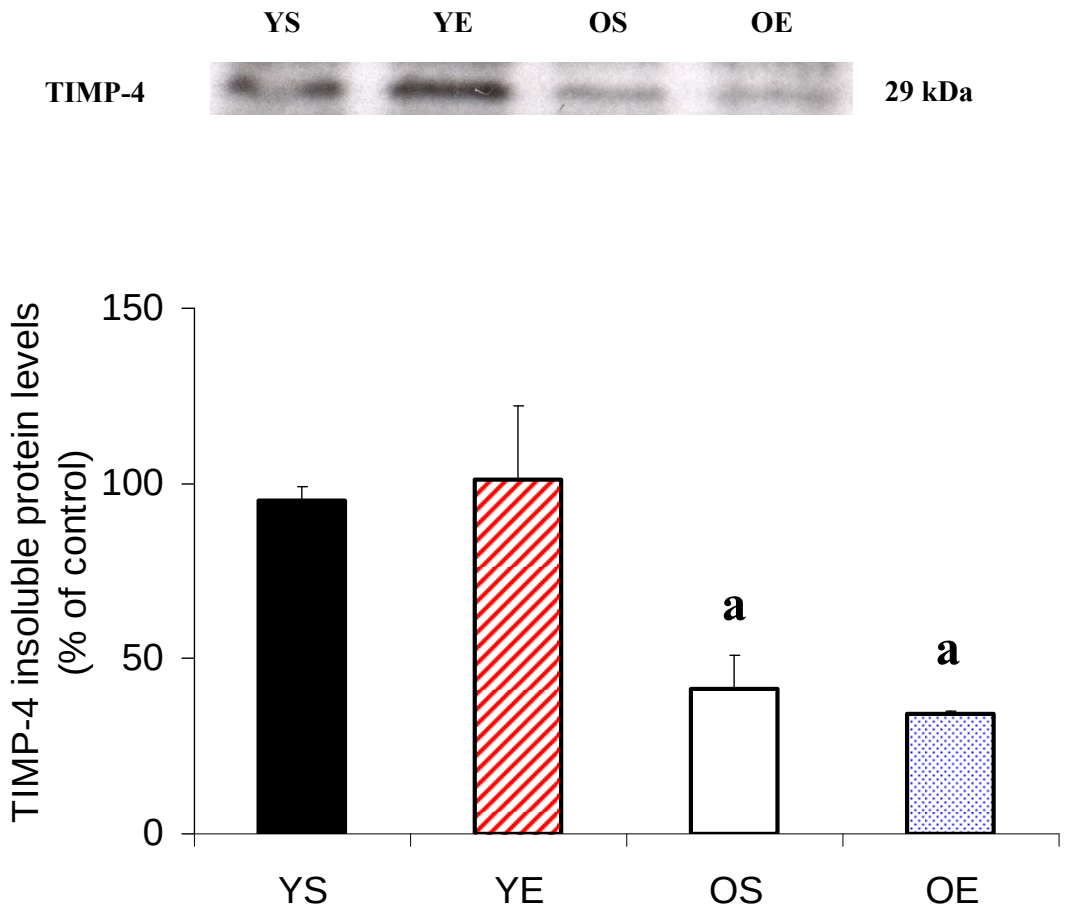

Fig. 23. Effect of aging and exercise training on TIMP-4 protein levels in the insoluble fraction. Values are mean ± SEM. [a]Indicates old groups (OS, OE) are significantly different from young sedentary control (YS) ($P < 0.05$).

TNF-α levels

Left ventricle muscle levels of the inflammatory cytokine tumor necrosis factor-α (TNF-α) were not changed with aging (Fig. 24). However, 12 weeks of exercise training significantly reduced TNF-α levels by 19.4% compared with old sedentary control group (Fig. 24). No significant differences between young sedentary control and young exercise group were seen for TNF-α levels.

TGF-β1 protein levels

The protein levels of transforming growth factor-β1 (TGF-β1) in the soluble fraction of the left ventricle in old sedentary controls were dramatically higher (+93.1%) than young sedentary controls (Fig. 25). Conversely, we found a significant decrease (-23.5 %) in TGF-β1 protein levels with exercise training in the soluble fraction of left ventricle from old groups (Fig. 25). But, there was no significant training effect on TGF-β1 protein levels in young groups (Fig. 25). The TGF-β1 proteins in the insoluble fraction of left ventricles were undetectable in both young and old groups.

Myofibroblast (α-smooth muscle actin)

No changes in the protein levels of α-smooth muscle actin (α-SMA, myofibroblast marker) with aging and exercise training occurred in the soluble fraction of left ventricles (Fig. 26). The α-SMA proteins in the insoluble fraction of left ventricles were undetectable in both young and old groups.

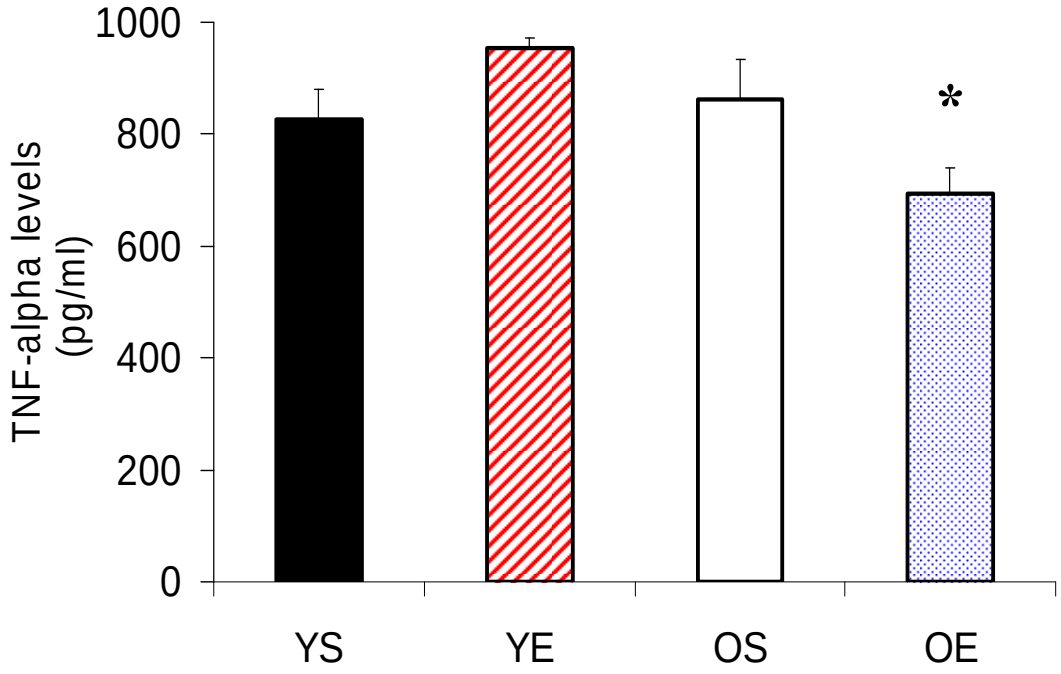

Fig. 24. Effect of aging and exercise training on TNF-α levels in the soluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$).

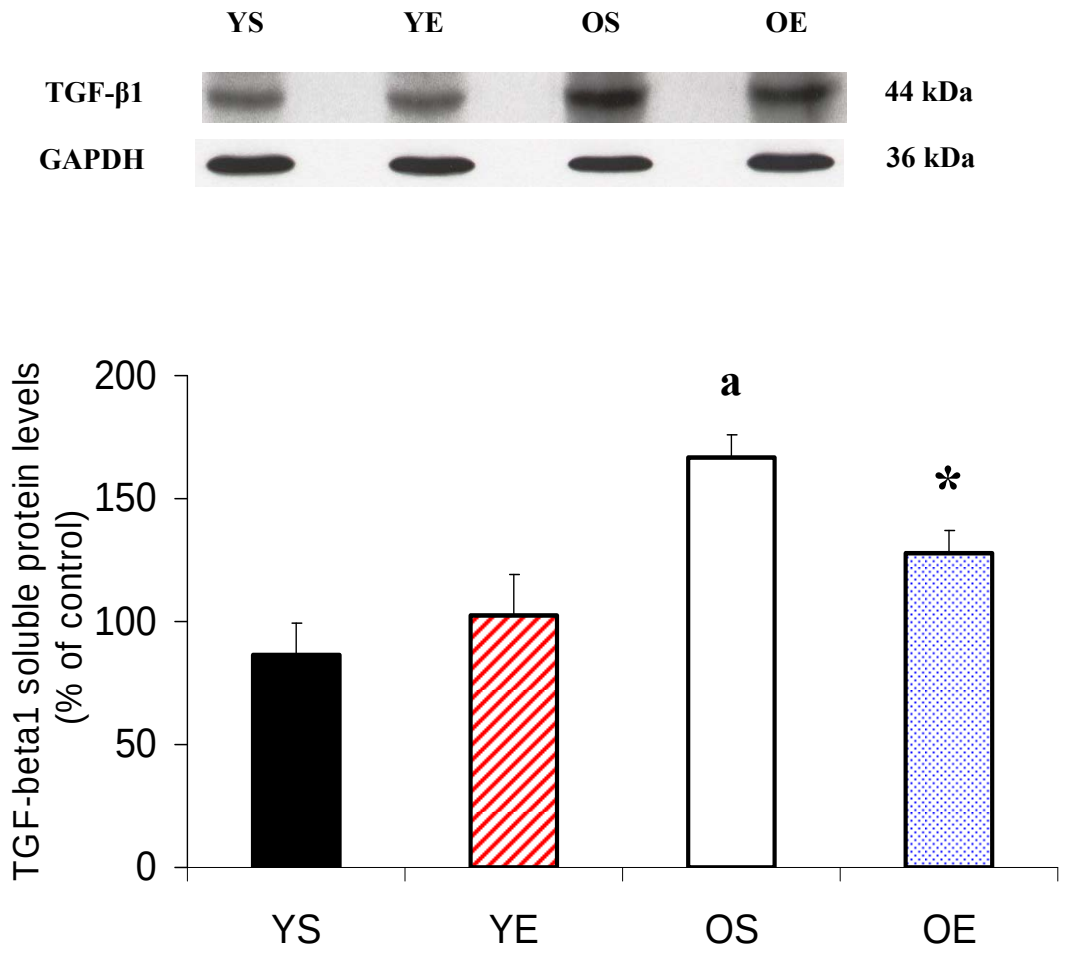

Fig. 25. Effect of aging and exercise training on TGF-β1 protein levels in the soluble fraction. Values are mean ± SEM. *Significantly different from control within same age group ($P < 0.05$). [a]Indicates old sedentary control (OS) is significantly different from young sedentary control (YS) ($P < 0.05$).

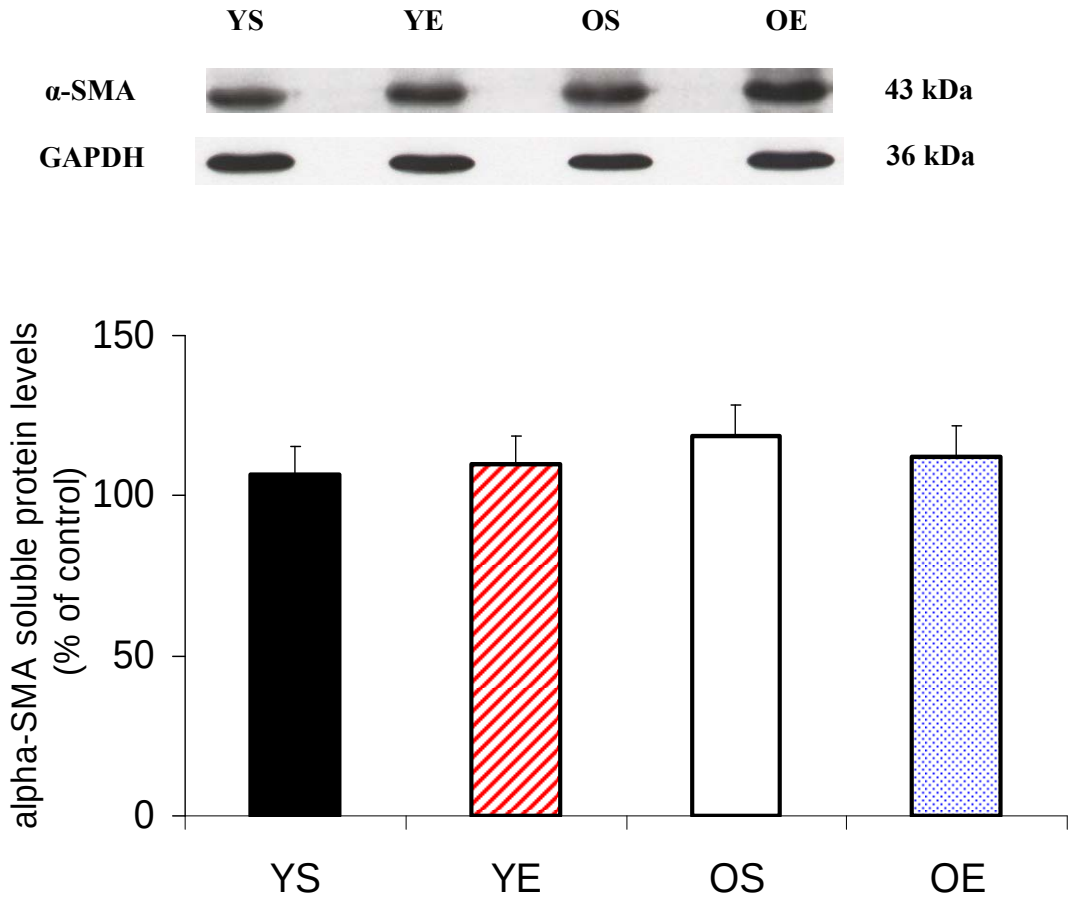

Fig. 26. Effect of aging and exercise training on α-SMA (myofibroblast marker) protein levels in the soluble fraction. Values are mean ± SEM.

CHAPTER IV

DISCUSSION

The major and novel findings of the current study include the following observations. Regular endurance exercise training ameliorated the age-induced increase in extramyocyte space of the left ventricle. We demonstrated exercise training might protect against age-induced fibrosis by increasing MMP-2, MMP-14 in the soluble fraction and MMP-1, MMP-3, MMP-14 in the insoluble fraction of old rat heart. Conversely, exercise training might reduce the fibrosis by decreasing TIMP-1 in the soluble fraction of old rat heart. Endurance exercise training reduced upstream pro-fibrotic mediators including TNF-α and TGF-β1 in the aging rat heart, while no changing α-smooth muscle actin as a myofibroblast marker. To our knowledge, these are the first data to indicate that exercise training has a protective effect against age-induced extracellular collagen matrix remodeling in the aging heart, associated with MMPs, TIMP-1, TNF-α, and TGF-β1 expression that was more similar to young hearts.

Left ventricle morphology

Long-term endurance exercise training reduced age-induced accumulation of extramyocyte space (Fig. 3) and collagen contents (Fig. 4) in the heart. Increased extramyocyte space with aging may be caused by increased extracellular matrix including collagens, increased fat, and increased fluid volume in the heart. Our findings are consistent with previous studies (19, 35, 54, 76, 85, 124, 125, 135). Taken together,

the increased collagens might be an integral part of extracellular matrix remodeling that takes place in the left ventricle consequent to the natural aging process leading to an increase in myocardial passive stiffness and impaired contractile function.

Our findings provide a theoretical paradigm by which endurance exercise can ameliorate progressive impairment of function in the aging heart. Preservation of myocyte number and reduced fibrosis may increase the capacity to pump blood while reducing internal work (57). Endurance training has also been documented to protect against reduced left ventricle filling during diastole (43). Changes in early ventricular filling are a marker of altered material properties of the left ventricle (43). Increased filling and enhanced systolic function that result from exercise training in the aging human heart suggest improved Ca^{2+} handling and protection against i) reduced elasticity, ii) increased stiffness, and iii) elevated internal work. Such observations in human patients are consistent with amelioration of fibrosis and ventricular remodeling in the left ventricles of exercise-trained aging rats as indicated in Fig. 2 and Fig. 4. Centurione et al. (25) postulated that reduction in myocyte number and increased fibrosis contributed to impaired diastolic filling rate. Indeed, amelioration of age-related changes in myocyte number, size, and connective tissue by exercise training could help to explain exercise-induced protection of diastolic and ventricular function in the aging heart noted in clinical studies (43, 68).

The mechanical relationship between the cardiac myocytes and extracellular collagen matrix is complex and interactive. Collagens are a regulated family of proteins that provide the structure and optimize the function of the heart (8). A healthy

arrangement of collagens provides a framework for myocyte sheath sliding, transmittance of force from myocyte to the ventricular chamber, prevents excessive stretch and damage, and preserves heart function (37), which is essential for efficient cardiac function. However, excessive accumulation of collagen matrix called fibrosis with aging results in elevated myocardial stiffness and cardiac dysfunction (8, 30). Aging increases the rate of ventricular collagen turnover and deposition by fibroblasts with aging (8, 124, 125). Fibrosis with aging is characterized by increased collagen content (35, 54), decreased collagen solubility, and increased collagen cross-linking (124, 125). This increase in collagen deposition during aging may be thought to result from a combination of cellular events including increased collagen synthesis and decreased collagen degradation. The collagen might become more resistant to collagenase degradation with aging (59).

Aging not only clearly increased the amount of connective tissue but also altered the geometry (Fig. 4), being ingredients for a dysfunctional mechanical environment. This occurred primarily in the left ventricle toward the endocardial surface in the current study, which could most affect diastolic filling and wall motion (62). Remarkably, amelioration of age-induced remodeling by 12 weeks of endurance exercise training was not simply a reduction in the area of the left ventricle occupied by collagen connective tissue, but importantly a more normal, linear geometric pattern (Fig. 2. Fig. 4). Sheaths of myocardial cells with exercise training in old heart appeared largely preserved, dissimilar to the discontinuous and more random pattern of connective tissue exhibited in the left ventricles of old, sedentary animals. Although untested in the current study,

better retention of normal geometry by regular exercise could ameliorate internal ventricular work of the aging heart. In addition, Thomas et al (124, 125) observed that ten weeks of treadmill exercise training modulated age-induced upregulation of collagen cross-linking (HP) in the left ventricle of rats.

Effects of aging and exercise training on MMPs and TIMPs

Cardiac ECM plays an important role in cardiac structure and function. In particular, accumulation of cardiac collagens with aging could result in diminished systolic performance, decreased compliance, and diastolic dysfunction (37). The collagen ECM depends on a balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), which determines cardiac remodeling (1, 59). MMPs are an endogenous family of enzymes that degrade ECM proteins, which are responsible for ECM remodeling in a number of physiological and pathological process (1, 127).

Most of MMPs are inactive, secreted enzymes that are stimulated extracelluarly after activation. However, some membrane-type MMPs are anchored to the cell membrane (60). It has been shown that important MMPs highly related with myocardial remodeling are collagenases (e.g., MMP-1 and MMP-13), gelatinases (e.g., MMP-2 and MMP-9), stromelysin (e.g., MMP-3), and the membrane-type MMP (e.g., MMP-14) (63). In contrast, MMPs are regulated by endogenous inhibitors, TIMPs (59). TIMPs are specific MMP inhibitors in the ECM (78). The role of TIMPs is to prevent excessive ECM degradation by MMPs.

Effect of aging

We found differential responses of MMPs to aging in the left ventricles. Contrary to the hypothesis that aging would result in the elevation of MMPs in the left ventricles, aging decreased protein levels of pro-MMP-2 (Fig. 9), active MMP-2 (Fig. 11), pro-MMP-14 (Fig. 16) in the soluble fraction and active MMP-1 (Fig. 8), pro-MMP-14 (Fig. 17) in the insoluble fraction of left ventricles, while no changes with aging in pro/active MMP-1 (Fig. 5, Fig. 7), pro-MMP-9 (Fig. 14) in the soluble fraction and pro-MMP-1 (Fig. 6), pro-MMP-2 (Fig. 10), pro-MMP-3 (Fig. 13), pro-MMP-9 (Fig. 15) in the insoluble fraction. But, overall effects of aging on MMPs in the current study were reduced protein levels of MMPs in the left ventricle, indicating that the depression of the degradation by MMPs is in part responsible for age-associated fibrosis.

There were controversial results regarding effect of aging on MMPs in the heart. Lindsey et al. (76) indicated that the levels of MMP-3, MMP-8, MMP-9, MMP-12, and MMP-14 increased in the insoluble fraction of old mouse heart, compared to young group. However, Robert et al. (98) showed that there were 40-45% decreases of both MMP-2 and pro-MMP-1 activity and mRNA in 24-month-old rat heart compared to young groups. The current data appear to rectify some of the contradictory findings of previous investigations. However, discrepancies between current study and previous data from Lindsey et al. (76) may be due to differences in dissection, protein extraction, species, and aging of animals. For example, we used Fischer 344×Brown Norway F1 hybrid rats (34 mon for old rats) as opposed to the CB6F1 mice (23 mon for old mice).

Interestingly, in contrast to other MMPs, aging led to a significant elevation of pro-MMP-3 protein levels in the soluble fraction of left ventricle (Fig. 12). Although age-induced increase in pro-MMP-3 levels was not directly associated with cardiac fibrosis, this finding may suggest that aging is presumably associated with increased ECM degradative capacity as well.

In addition to MMPs, we also found differential responses of TIMPs to aging in the left ventricles. Aging increased the protein levels of TIMP-1 (Fig. 18) in the soluble fraction and decreased TIMP-4 protein levels (Fig. 23) in the insoluble fraction of left ventricles. Increased TIMP-1 in the soluble fraction with aging might be associated with age-induced cardiac fibrosis, whereas decreased TIMP-4 in the insoluble fraction with aging would support the additional degradation of ECM proteins. Taken together, the regulation of MMPs and TIMPs is implicated in ECM remodeling with aging. The overall decrease of MMPs in both fractions and increased TIMP-1 in soluble fraction potentially might promote the accumulation of ECM proteins including collagens with advancing age.

Proteins found in the soluble fraction include cytoplasmic proteins and soluble ECM proteins, while proteins found in the insoluble fraction contain membrane proteins and insoluble ECM proteins (63). The fact that TIMP-1 and TIMP-2 known soluble proteins were only detected in the soluble fraction in the current study verifies this idea.

Effect of exercise training

We found that exercise training increased protein levels of active MMP-2 (Fig. 11), pro-MMP-14 (Fig. 16) in the soluble fraction and active MMP-1 (Fig. 8), pro-MMP-3 (Fig. 13), pro-MMP-14 (Fig. 17) in the insoluble fraction in the aging left ventricles. Overall effects of exercise training on MMPs in the current study were to elevate protein levels of MMPs in the aging left ventricles, suggesting that the degradation by MMPs is in part responsible for exercise training-induced protection against cardiac fibrosis. These data are consistent with the hypothesis that exercise training would attenuate age-induced alterations in MMPs in the aging heart.

The exercise training effects on MMP protein levels take place in the only old rat groups except soluble pro-MMP2, which was reduced by exercise training in the young rat groups. Although the efficacy of exercise training regimen is effective in the young group including increased heart-to-body weight ratio and citrate synthase activity of soleus skeletal muscle with the exercise training (Table 1), the exercise training effects on MMP protein levels in the young groups were minimal. These results were confirmed by other studies (38, 68).

In addition, protection of left ventricle morphology and MMP changes in the old rat hearts by exercise training did not appear to be related to changes in oxidative capacity in skeletal muscle. While citrate synthase activity was significantly reduced with age, exercise training surprisingly had little positive benefit on citrate synthase activity in the old skeletal muscle rats (Table 1). The lack of an exercise effect for citrate synthase in soleus muscle of old groups may be indicative of relatively low exercise

intensity in the current study. However, based on the training experience in the current study, the exercise performance of old rats groups was almost maximum because they are too heavy and fat compared to young groups. We can also predict that the skeletal muscle of old rats might be degradative even though they exercised.

It is also extremely interesting that exercise training resulted in a marked reduction of only TIMP-1 soluble protein levels in the aging heart (Fig. 18). The attenuation of TIMP-1 in the soluble fraction by exercise training potentially may protect against fibrosis in the aging heart. These results from current study provide the foundation for future intervention studies that TIMP-1 inhibition with exercise training might ameliorate age-induced fibrosis much more in the heart. Taken together, exercise training protects against age-related fibrosis or connective tissue accumulation by the upregulation of pro- or active MMP-1, -2, -3, -14 in the soluble or insoluble fractions and by the downregulation of TIMP-1 in the soluble fraction.

Effects of aging and exercise training on potential upstream mediators

Tumor necrosis factor-alpha (TNF-α)

TNF-α has a variety of biological functions in response to the changes in environmental stress in the heart, suggesting that TNF-α might be involved in alterations in cardiac myocytes and extracellular matrix (81). In particular, it seems like that TNF-α may lead to an imbalance in myocardial MMP/TIMP ratio as a potential upstream mediator of MMPs and TIMPs, resulting in altered myocardial ECM structure and LV dysfunction (59, 118). We found that TNF-α activity levels did not change with age in

the left ventricle (Fig. 24), which is consistent with previous studies (103, 105), even though there are conflicting evidences that TNF-α is associated with aging (21, 34, 104). In contrast, exercise training decreased the levels of TNF-α activity in the aging left ventricles in the current study (Fig. 24) as other studies (7, 10, 71, 72), indicating exercise training-induced suppression of TNF-α.

The downregulation of TNF-α activity with exercise training in old rats might be associated with elevated levels of insoluble active MMP-1, soluble active MMP-2, insoluble pro-MMP-3, and soluble/insoluble pro-MMP-14, and reduced levels of soluble TIMP-1 with exercise training in the old rats in the current study. We postulate that TNF-α may presumably stimulate extracellular collagen matrix synthesis by downregulating MMPs and upregulating TIMP-1. These findings are consistent with previous data. For example, Sivasubramanian et al. (116) reported that there were significant decrease in total MMP activity and increase in TIMP-1 levels in the cardiac overexpression of TNF-α in transgenic mice, suggesting a possible mechanism for the increase in myocardial fibrosis.

In contrast, there are controversial reports that TNF-α could increase the extracellular collagen matrix degradation by upregulating MMPs and downregulating TIMPs (59, 74, 118). For example, Li et al. (74) reported that cardiac overexpression of TNF-α in transgenic mice resulted in the elevation in MMP-2 and MMP-9 activity as well as marked diastolic dysfunction. In the cardiac fibroblasts, TNF-α reduces collagen synthesis, increases MMP expression, and decreases TIMP expression (75, 117). Taken together, although the mechanisms are not fully understood, the imbalance in

MMP/TIMP ratio in the heart would be ameliorated by exercise training-induced downregulation of TNF-α in the aging rat hearts.

Transforming growth factor-beta1 (TGF-β1)

We found that aging increased TGF-β1 protein levels in the left ventricle (Fig. 25). These findings are consistent with the hypothesis that aging results in the upregulation of TGF-β1 leading to the fibrosis in the heart. TGF-β consists of three isoforms, TGF-β1, TGF-β2, and TGF-β3 that are structurally and functionally closely related to one another (2). The TGF-β, an anti-inflammatory cytokine, is a potent stimulator of collagen synthesis (112, 118). It mediates collagen synthesis via reduced MMPs or enhanced TIMPs, thus favoring an accumulation of ECM (12, 118).

TGF-β may play an important role in stimulating abnormal accumulation of ECM proteins including collagens in the cardiovascular diseases. Rosenkranz et al. (100) showed that TGF-β overexpression in the transgenic mice heart resulted in cardiac hypertrophy and fibrosis. Similarly, Brooks & Conrad (15) found that TGF-β1 deficient old mice heart exhibited a decrease in myocardial fibrosis and reduced myocardial stiffness, indicating the role of TGF-β to contribute to ECM component synthesis in the heart.

In contrast, exercise training resulted in a marked reduction of TGF-β1 protein levels in the left ventricle from old rats (Fig. 25).To our knowledge the current study is the first to demonstrate that TGF-β1 protein levels are reduced in the left ventricle as a result of prolonged exercise training. The responses of TGF-β1 with exercise training in

old rat hearts might be associated with the changes in MMPs and TIMP-1 levels, indicating that TGF-β is an upstream regulator of MMPs and TIMPs leading to cardiac fibrosis. Taken together, exercise training ameliorates age-induced cardiac fibrosis by downregulating an upstream regulator, TGF-β1.

Alpha-smooth muscle actin (myofibroblast marker)

Myofibroblast is a differentiated cell type from fibroblast characterized by increased ECM protein synthesis especially collagen type I, providing an essential role for ECM remodeling during normal and pathological wound healing (30, 96, 126, 129). Myofibroblast expression may be not detectable in the normal healthy adult hearts, while myofibroblasts are often associated with injured heart such as myocardial infarction for wound healing (8, 94, 133). In particular, the expression of α-smooth muscle actin (α-SMA) is considered to be the most reliable marker of differentiated myofibroblasts (28, 96, 113).

We found that there were no significant effects of aging and exercise training on alpha-smooth muscle actin (α-SMA) as a myofibroblast marker in the current study (Fig. 26). Contrary to our expectation, α-SMA did not follow a pattern reflective of changes in TGF-β1 with aging and exercise training. These data are inconsistent with the hypothesis that alterations in myofibroblasts with aging and exercise training are exclusively downstream of the TGF-β (28, 36). Taken together, the current study indicated that the α-SMA levels are delinked with TGF-β1 with aging and exercise training in the left ventricles.

However, current data on α-SMA with exercise training were confirmed by a previous study (57). The results indicated that there was no change of mRNA levels of α-smooth muscle actin in the young (6-8 wk) rat heart after13 week treadmill exercise training.

CHAPTER V

SUMMARY AND CONCLUSIONS

This dissertation provides a comprehensive analysis of the effects of exercise training on extracellular matrix and remodeling in the aging rat left ventricles. The purpose of this study was to (a) to determine whether aging affects extracellular matrix remodeling, and (b) to identify the effects of exercise training on age-induced changes in extracellular matrix remodeling in the heart. The results of the present study demonstrated that cardiac fibrosis with aging was associated with decreased soluble MMP-2, MMP-14 and insoluble MMP-1, MMP-3, MMP-14, and increased soluble TIMP-1 and TGF-β1. However, 12 weeks of endurance exercise training might reverse the age-induced changes in extracellular matrix remodeling-related factors in the heart. These novel findings indicate protective and reversible effects of exercise training against fibrosis and extracellular matrix remodeling in the aging heart.

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2005 Student Research Manuscript Award, first place at Texas Regional Chapter of American College of Sports Medicine

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PUBLICATIONS

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