Antiapoptotic effect of exercise training on ovariectomized rat hearts

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Huang CY, Lin YY, Hsu CC, Shyu WC, Ting H, Yang AL, Ho TJ, Lee SD. Antiapoptotic effect of exercise training on ovariectomized rat hearts. J Appl Physiol 121: 457–465, 2016. First published June 23, 2016; doi:10.1152/japplphysiol.01042.2015.—The purpose of this study was to evaluate the effects of exercise training on cardiac Fas receptor-dependent and mitochondria-dependent apoptotic pathways in ovariectomized rats. Histopathological analysis, TUNEL assay, and Western blotting were performed on the excised hearts from three groups of Sprague-Dawley rats, which were divided into a sham-operated group, a bilaterally ovariectomized group (OVX), and a bilaterally ovariectomized group that underwent treadmill running exercise for 60 min/day, 5 sessions/wk, for 10 wk (OVX-EX). The abnormal myocardial architecture, cardiac trichome-stained fibrosis and cardiac TUNEL-positive apoptotic cells in ovariectomized rats improved after exercise training. The protein levels of tumor necrosis factor-α, tumor necrosis factor receptor 1, Fas ligand, Fas receptors, Fas-associated death domain, activated caspase-8 and activated caspase-3 (Fas receptor-dependent apoptotic pathways), as well as t-Bid, Bad, Bak, Bax, cytosolic cytochrome c, activated caspase-9, and activated caspase-3 (Fas receptor-dependent apoptotic pathways) were decreased in OVX-EX compared with the OVX group. Exercise training suppressed cellular apoptosis in menopausal or bilaterally oophorectomized women.

**NEW & NOTEWORTHY**

Cardiac widely dispersed apoptosis was found after ovariectomy. Exercise training on treadmill could prevent ovariectomy-induced cardiac widely dispersed apoptosis in Fas receptor-dependent apoptotic pathway (TNF-α, TNFR1, Fas-L, Fas, activated caspase-8, and activated caspase-3) and mitochondria-dependent apoptotic pathway (t-Bid, Bad, Bak, Bax, cyto-

solic cytochrome c, activated caspase-9, and activated caspase-3). Exercise training has antiapoptotic effects on ovariectomized rat hearts via both Fas receptor-dependent and mitochondria-dependent apoptotic pathways.

**EVIDENCE SHOWS THAT MENOPAUSE or early ovariectomy (oophorectomy) is associated with an increased risk of ischemic heart disease (26, 42). Deficiency of estrogen worsens cardiovascular dysfunction and increases the possibility of heart failure (3, 28, 38). In Europe, about 55% of female deaths are caused by cardiovascular diseases, such as myocardial infarction, heart failure, and sudden cardiac death (44). A report published in 2006 from a study of 38,283 women showed that each 5-yr increment in age after menopause is associated with a 44% increase of the risk of heart failure and with a 52% risk of all-cause mortality (36).**

Cellular apoptosis in cardiomyocytes is one of the critical pathological mechanisms in the development of heart failure, and is recognized as a predictor of adverse outcomes in patients with cardiac diseases or heart failure (31, 32). The “extrinsic” Fas receptor-dependent (type I) apoptotic pathway and “intrinsic” mitochondria-dependent (type II) apoptotic pathway are two major pathways that directly trigger cardiac apoptosis (7). Tumor necrosis factor-α (TNF-α) or Fas ligand binding the TNF receptor 1 or Fas receptor starts with the formation of a death-inducing signaling complex through recruitment of Fas-associated death domain (FADD) and caspase-8, which then leads to caspase-3 cleavage and executes the cell death program (7, 41). Additionally, a downstream protein of the Fas-dependent apoptotic pathway, caspase-8, can cleave Bcl-2 homology domain 3 (BH3)-interfering domain death agonist (Bid) to truncates Bid (t-Bid), thus translocating to the mitochondria, causing the release of cytochrome c, leading to the activation of caspase-9, which can then activate caspase-3 (1, 7). The mitochondria are the main sites of action for members of the apoptosis-regulating protein family exemplified by the Bcl-2 family, such as Bad, Bak, and Bax (15, 45). Bcl-2-associated death promoter (Bad), BCL-2 antagonist or killer (Bak), and Bcl-2-associated X protein (Bax) are proapoptotic proteins that can enhance cytochrome c release from the mitochondria (7, 15, 45). When cytochrome c is released from mitochondria into cytosol, it is responsible for activating...
caspase-9, which further activates caspase-3 to execute the apoptotic program (7, 15). In addition, our previous study showed that cardiac Fas-dependent apoptosis and mitochondria-dependent apoptosis were significantly increased after ovariectomy (22). Continuing 17-β-estradiol treatment can prevent ovariectomy-induced cardiac Fas-dependent and mitochondrial apoptotic pathways in the heart (25) and also prevent cardiomyocyte apoptosis in animal models of myocardial infarction (34). However, long-term estrogen therapy is associated with an increased risk of breast cancer or severe side effects in some women (16, 27). Alternative therapeutic approaches need be discovered for menopausal women.

Exercise training has been used as an important therapeutic approach in managing cardiovascular diseases (11, 21, 30), and regular exercise has been reported to have benefits for the cardiovascular system of postmenopausal women (48). A previous study has reported that exercise training exerts beneficial effects by diminishing adverse cardiac remodeling in ovariectomized hypertensive rats (29). Recently, we have found that exercise training can significantly improve cardiac apoptosis in hypertension (18), genetic obesity (23), and diabetes (10). However, the effect of exercise training on cardiac apoptosis in postmenopausal or early oophorectomized women is unclear. The current study was undertaken to determine whether exercise training can prevent cardiac apoptosis in ovariectomized rats. We hypothesized that exercise training would prevent cardiac Fas receptor-dependent and mitochondria-dependent apoptotic pathways in ovariectomized rats.

MATERIALS AND METHODS

Animal model. Forty-two female Sprague-Dawley rats (14 wk old) were purchased from National Laboratory Animal Center, Taiwan. Ambient temperature was maintained at 25°C and the animals were kept on an artificial 12-h light-dark cycle. The light period began at 7:00 AM. Rats were provided with standard laboratory chow (Lab Diet 5001, PMI Nutrition International, Brentwood, MO) and water ad libitum. All protocol designs and animal care were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee of the China Medical University Animal Center, Taichung, Taiwan.

Ovariectomy and sham operation. The ovariectomy was performed following the protocol from our previous study (22). At 14 wk old, all rats were randomly assigned to either the sham-operated group (Sham, n = 14) or bilateral ovariectomized group (OVX, n = 28). All rats were operated on with survival surgical procedures with aseptic technique. The OVX rats were anesthetized with ketamine/xylazine (100:10 mg/kg, ip), and the bilateral ovaries were removed. The sham-operated rats underwent the same surgical procedure except for the removal of the ovaries. After surgery, each rat was injected with penicillin G procaine (0.2 ml, 20,000 IU, im). Two weeks after the surgery, the 28 OVX rats were randomly divided into sedentary (OVX, n = 14) and exercise (OVX-EX, n = 14) groups. Exercise training. The OVX-EX group was trained on a motor-driven leveled treadmill (Model T408E, Diagnostic & Research Instruments, Taoyuan, Taiwan) at a speed of 12 m/min for 20 min on the first day. The running time was extended by 10 min/day until a running time of 60 min/day was reached and reached in the familiarization period of 5 days. During the training period, the training speed of 15 m/min gradually increased by 3 m/min every 2 wk until 24 m/min was achieved. These animals were trained for 60 min/day, 5 days/wk, for 10 wk. The sedentary groups were placed on the treadmill without any exercise training for the same environmental stimulation. At the end of the experimental period, rats were anesthetized with 2% isoflurane delivered in oxygen (95% O2 and 5% CO2), and all efforts were made to minimize suffering.

Hematoxylin and eosin staining. The hearts of six rats from each group (from a total of 14 rats per group) were soaked in 4% formalin, dehydrated through graded alcohols, and embedded in paraffin wax. Paraffin sections 0.2-μm thick were cut from the paraffin-embedded tissue blocks. The tissue sections were deparaffinized by immersing in xylene for 5 min three times and rehydrated through graded alcohols (100, 90, 85, and 75%) for 5 min. Slides were then washed in running tap water for 5 min. All slides were dyed with hematoxylin (Merck, Darmstadt, Germany) for 5 min and then washed in running tap water for 5 min. This was followed by submersion in eosin (Merck) for 5 min followed by washing in running tap water for 5 min. Each slide was dehydrated through graded alcohols (75, 85, 90, and 100%). Finally, they were soaked in xylene twice. Photomicrographs were obtained with a phase-contrast microscope (×200, Olympus BX43, Tokyo, Japan).

Masson’s trichrome staining. The hearts of six rats from each group (from a total of 14 rats per group) were soaked in 4% formalin, dehydrated through graded alcohols, and embedded in paraffin wax. Paraffin sections 0.2-μm thick were cut from the paraffin-embedded tissue blocks. The tissue sections were deparaffinized by immersing in xylene for 5 min three times and rehydrated through graded alcohols (100, 90, 85, and 75%) for 5 min. Next, all slides were dyed with Masson’s trichrome kit (Scytek Laboratories, Logan, UT). In brief, The sections were soaked in a warmed Bouin’s solution at 60°C for 45 min, then washed in running tap water until the yellow color in the samples disappeared. All slides were immersed in Weigert’s Haematoxylin for 5 min, followed by washing in running tap water for 2 min. The slides were stained with Acid Fuchsin for 15 min and rinsed in distilled water. Next, slides were treated with phosphomolybdic acid solution for 10 min and then immediately stained with methyl blue solution for 10 min. Slides were rinsed with distilled water and treated with 1% acetic acid solution for 3 min. Each slide was dehydrated through two changes of alcohol (95, 95, 100, and 100%). Finally, they were soaked in xylene twice. Photomicrographs were obtained with a phase-contrast microscope (×200, Olympus BX43). The quantification of fibrotic areas (stained blue) and myocardial areas (stained red) were performed with Image J analysis software. The fibrosis percentage of the left ventricular area was obtained by calculating the ratio of fibrotic area to myocardial area.

DAPI and TUNEL staining. For terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) assay, the sections were incubated with proteinase K for 30 min, washed in phosphate-buffered saline followed by 3% hydrogen peroxide for 30 min. After washing, the sections were treated with 10% normal goat serum in PBS for 1 h and then incubated with 1:200 DAPI (20 min) followed by 1:5000 TUNEL (2 h) mixture on the same day. The slides were washed in PBS, then mounted in antifade mounting medium and stored at 4°C. The slides were observed with a fluorescence microscope (Olympus BX43, Tokyo, Japan). Table 1. Cardiac characteristics of Sham, OVX, and OVX-EX groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>OVX</th>
<th>OVX-EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>284±12</td>
<td>389±9***</td>
<td>342±13**##</td>
</tr>
<tr>
<td>Heart weight index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHW, g</td>
<td>0.83±0.07</td>
<td>0.99±0.05**</td>
<td>0.93±0.06**</td>
</tr>
<tr>
<td>LVW, g</td>
<td>0.60±0.05</td>
<td>0.73±0.03**</td>
<td>0.68±0.03**#</td>
</tr>
<tr>
<td>LVW/WHW, g</td>
<td>0.74±0.13</td>
<td>0.74±0.07</td>
<td>0.73±0.07</td>
</tr>
<tr>
<td>WHW (g)/BW (g) × 10^3</td>
<td>2.90±0.29</td>
<td>2.55±0.14**</td>
<td>2.70±0.17</td>
</tr>
<tr>
<td>LVW (g)/BW (g) × 10^3</td>
<td>2.11±0.17</td>
<td>1.87±0.09**</td>
<td>1.98±0.13</td>
</tr>
<tr>
<td>WHW (g)/TL (mm) × 10^3</td>
<td>23.12±1.87</td>
<td>26.92±1.15**</td>
<td>25.58±1.85*</td>
</tr>
<tr>
<td>LVW (g)/TL (mm) × 10^3</td>
<td>16.88±1.59</td>
<td>19.79±1.06**</td>
<td>18.66±0.77**</td>
</tr>
</tbody>
</table>

Values are means ± SD. Three groups: sham-operated rats (Sham), ovariectomized rats (OVX), and ovariectomized rats with exercise training (OVX-EX). WHW, whole heart weight; LVW, left ventricular weight; TL, tibia length. *P < 0.05, **P < 0.01, significant differences from the Sham group. ##P < 0.05, ###P < 0.01, significant differences between OVX-EX and OVX group.
buffered saline (PBS) two times for 5 min, followed by incubation with permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate, freshly prepared) for 8 min, and then washed in PBS two times for 5 min. The sections were soaked in a blocking buffer [Tris-HCl, 0.1 M, pH 7.5, containing 3% bovine serum albumin and 20% normal bovine serum] for 60 min, and then washed two times in PBS for 5 min. The slides were incubated with terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP conjugated from the In Situ Cell Death Detection Kit, Fluorescein (Roche Applied Science) for 60 min, and then washed two times in PBS for 5 min. The slides were mounted with DAPI-fluoromount G (Southern Biotech, Birmingham, AL). TUNEL-positive nuclei (fragmented DNA) were fluoresced by bright green light at 450-500 nm. The nucleus position was fluoresced by blue light at 340–380 nm. Photomicrographs were obtained with a phase-contrast microscope (×400, Olympus BX43). The numbers of TUNEL-positive and DAPI-stained nuclei were determined, and the percentage of TUNEL-apoptosis was calculated as the ratio of TUNEL-positive relative to DAPI-stained nuclei from the left ventricular area.

Tissue extraction. The hearts of eight rats from each group (from a total of 14 rats per group) were extracted by homogenizing the left ventricle samples in a lysis buffer (20 mM Tris, 2 mM EDTA, 10% glycerol, 50 mM 2-mercaptoethanol, protease inhibitor, phosphatase inhibitor, pH 7.4) at a ratio of 100 mg tissue/1 ml buffer. The homogenates were placed on ice and then centrifuged at 7,000 g for 40 min. The supernatant was collected and stored at −80°C for further investigation.

Western immunoblotting. Protein concentration of cardiac tissue extracts was determined by the Lowry protein assay. Protein samples (40 μg/lane) were separated on a 10 or 12% SDS polyacrylamide gel by electrophoresis (SDS-PAGE) with a constant voltage of 75 V. Electrophoresed proteins were transferred to polyvinylidene difluoride (PVDF) membrane (0.45-μm pore size, Millipore, Bedford, MA) with a transfer apparatus (Bio-Rad). PVDF membranes were incubated in 5% nonfat dried milk in TBST buffer (25 mM Tris-HCl, 150 mM NaCl, pH 7.6, and 0.1% Tween-20). Primary antibodies including TNF-α, TNF receptor 1 (TNFR1), Fas ligand, Fas receptors, FADD, t-Bid, Bad, Bak, Bax, cytochrome c, caspase-8, caspase-9, caspase-3...
and α-tubulin (Santa Cruz Biotechnology, Santa Cruz, CA) were
diluted to 1:500 in antibody binding buffer overnight at 4°C. The
immunoblots were washed three times in TBST buffer for 10 min
three times, followed by incubation with a HRP-conjugated second
antibody solution (1:5,000 dilution, Santa Cruz) for 1 h at room
temperature. The immunoblots were then washed three times in
TBST buffer, for 10 min each time. The immunoblotted proteins
were visualized with an enhanced chemiluminescence Western blotting
luminal reagent (Millipore) and quantified with a Fujifilm LAS-3000
chemiluminescence detection system (Fuji, Tokyo, Japan).

Statistical analysis. Data for weight index, echocardiography in-
dex, and protein levels were compared between the Sham, OVX, and
OVX-EX groups by one-way ANOVA with preplanned contrast
comparison with the control group. Sham and OVX served as the
negative control and the positive control, respectively. P < 0.05 was
considered to be significant.

RESULTS

Cardiac characteristics. The body weight (BW), whole heart weight
(WHW), left ventricular weight (LVW), whole heart weight/tibia
length (WHW/TL), and left ventricular weight/tibia length (LVW/TL)
in the OVX group was higher than in the Sham group. After exercise
training, BW and LVW were reduced in the OVX-EX compared with
the OVX (Table 1). There was no significant difference in LVW/WHW
among the three groups (Table 1).

Cardiac histopathological changes. To investigate whether
there were changes in cardiac architecture and fibrosis after
exercise training, we performed histopathological analysis
of left ventricular slices with hematoxylin and eosin staining
and Masson’s trichrome staining in hearts from the Sham,
OVX, and OVX-EX groups. Compared with the Sham
group, the ventricular myocardium in the OVX group
showed abnormal myocardial architecture, increased inter-
stitial space, and cardiac fibrosis (Fig. 1, A, B, and D). These
myocardial architectural abnormalities and cardiac fibrosis
in the OVX-EX group were less than those in the OVX
group (Fig. 1, A, B, and D).

TUNEL-positive apoptotic cells of left ventricle. To clarify
the apoptotic activity in cardiac tissues after exercise training,
TUNEL assay and DAPI staining were measured in the left
ventricular slices from the Sham, OVX, and OVX-EX groups.
We observed that the left ventricles of the OVX groups had a
greater number of TUNEL-positive cardiac cells than those in
the Sham group (Fig. 1, C and D). The number of TUNEL-
positive cardiac cells in the OVX-EX group was less than in
the OVX group (Fig. 1, C and D).

Upstream components of cardiac Fas receptor-dependent
apoptotic pathways. To investigate the upstream components
of cardiac Fas receptor-dependent apoptotic signaling path-
ways in ovariectomized models with exercise training, the
protein levels of TNF-α, TNF receptor 1 (TNFRI), Fas ligand
(Fas-L), Fas receptors (Fas), and FADD in the left ventricles
excised from the Sham, OVX, and OVX-EX groups were
measured by Western blotting. Compared with the Sham
group, the protein levels of TNF-α, TNFRI, Fas-L, Fas, and
FADD were significantly increased in the OVX (Fig. 2),
whereas they were significantly decreased in the OVX-EX
group compared with the OVX group (Fig. 2).

Fig. 2. A: the representative protein products of
tumor necrosis factor-α (TNF-α), TNF receptor 1 (TNFRI), Fas ligand
(Fas-L), Fas receptors (Fas), and Fas-associated death do-
main (FADD) extracted from the left ventricles of excised hearts of sham-operated rats
(Sham), ovariectomized rats (OVX), and ovariectomized rats with exercise training
(OVX-EX), as measured by Western blotting analysis. B: the bars represent the relative
protein quantification of TNFα, TNFRI, Fas-L, Fas, and FADD on the basis of α-tubulin
and indicate mean values ± SD (n = 8 in each group). *P < 0.01 denotes significant
difference from the Sham group, *P < 0.05 and **P < 0.01 denote significant difference
between the OVX group and OVX-EX group, respectively.
Upstream components of cardiac mitochondria-dependent apoptotic pathways. To further understand the upstream of cardiac mitochondria-dependent apoptotic signaling pathways in ovariectomized models with exercise training, we measured the protein levels of t-Bid, Bad, Bak, Bax, and cytosolic cytochrome c in the left ventricles excised from the Sham, OVX, and OVX-EX groups by Western blotting. Compared with the Sham group, the proapoptotic protein levels of t-Bid, Bad, Bak, Bax, and cytosolic cytochrome c were significantly increased in the OVX (Fig. 3). The protein levels of t-Bid, Bad, Bak, Bax, and cytosolic cytochrome c were significantly decreased in the OVX-EX group compared with the OVX group (Fig. 3).

Downstream components of cardiac Fas-dependent and mitochondria-dependent apoptotic pathways. To identify the downstream components of cardiac Fas and mitochondria-dependent apoptotic pathways, the protein levels of activated caspase-8, activated caspase-9, and activated caspase-3 were measured in the excised hearts of Sham, OVX, and OVX-EX groups by Western blotting. The protein levels of activated caspase-8, activated caspase-9, and activated caspase-3 were significantly increased in the OVX group compared with those in the Sham group (Fig. 4), whereas they were significantly decreased in the OVX-EX group compared with the OVX group (Fig. 4).

DISCUSSION

Our main findings can be summarized as follows. 1) Abnormal myocardial architecture enlarged interstitial space and increased cardiac fibrosis, and more cardiac TUNEL-positive apoptotic cells were observed in ovariectomy, but they became less severe by exercise training. 2) The cardiac Fas receptor-dependent apoptotic pathways in ovariectomy were less activated after exercise training, with evidence of attenuates in tumor necrosis factor-α (TNF-α), TNF receptor 1 (TNFR1), Fas-L, Fas receptors (Fas), FADD, activated caspase-8, and activated caspase-3, when compared with the sedentary ovariectomy. 3) The cardiac mitochondria-depen-
Physical activity is a common lifestyle modification which is always recommended for postmenopausal women and in patients with chronic heart diseases (48). Regular cardiorespiratory exercise is able to decrease the risk of cardiovascular disease (17, 43). A previous study has reported that exercise training exerts beneficial effects by diminishing adverse cardiac remodeling and reducing interstitial myocardial fibrosis, improving myocardial vascularization and sustaining the number of cardiomyocytes in ovariectomized hypertensive rats (29). We have shown that estrogen deprivation by ovariectomy can be prevented through the exercise intervention (Fig. 5).

The estrogen deficiency after ovariectomy may cause cardiovascular remodeling and dysfunction (6, 22, 47). Brower et al. (9) observed that left ventricular hypertrophy and left ventricular dilatation were increased after ovariectomy. Left ventricular hypertrophy is recognized as the most important predictor of cardiovascular morbidity and mortality, and an important risk factor for heart failure (20). In the present study we observed that, after ovariectomy, an estrogen deficiency increased LVW, enlarged interstitial spaces, and increased cardiac fibrosis.

Physical activity is a common lifestyle modification which is always recommended for postmenopausal women and in patients with chronic heart diseases (48). Regular cardiorespiratory exercise is able to decrease the risk of cardiovascular disease (17, 43). A previous study has reported that exercise training exerts beneficial effects by diminishing adverse cardiac remodeling and reducing interstitial myocardial fibrosis, improving myocardial vascularization and sustaining the number of cardiomyocytes in ovariectomized hypertensive rats (29). We have shown that estrogen deprivation by ovariectomy displays cardiomyopathic changes, such as increased LVW, abnormal myocardial architecture, enlarged interstitial space, and cardiac fibrosis, all of which appear to be improved after exercise training by running on a treadmill for 10 wk.

Apoptosis in terminally differentiated cardiomyocytes is a critical pathological mechanism that causes heart failure (31). Therefore, we set out to investigate whether an understanding of the process of apoptosis could allow for the development of novel strategies to reverse or attenuate heart failure. Exercise training has been shown to protect more activated cardiac Fas to mitochondria-dependent apoptotic pathways in hypertension (18), genetic obesity (23), and diabetes (10). In addition, our previous studies have indicated that estrogen deficiency by ovariectomy could promote a cardiac apoptosis-related death process (22, 25). In the present study, we further set out to investigate if exercise training can prevent cardiac apoptosis in ovariectomized rats. We observed that exercise training for 10 wk appeared to suppress the cardiac Fas-dependent and mitochondria-dependent apoptotic pathway in ovariectomized rats. Therefore, our findings strongly suggest that exercise training did prevent or attenuate ovariectomy-induced cardiac Fas-dependent and mitochondria-dependent apoptosis. The current study is the first to report that exercise training prevents ovariectomy-induced cardiac Fas to mitochondria-dependent apoptotic pathways in ovariectomized rats’ hearts.

TNF-α is a proapoptotic molecule and pro-inflammatory factor (4). TNF-α binding to the receptor TNFR1 can activate caspase-8 and initiate apoptosis (7). Moreover, TNF-α stimulating an inflammatory cascade through activation of nuclear factor κB (NF-κB), and its chronic stimulation through the enhancement of oxidative stress promotes the development of heart failure (13). As we observed, the suppression of cardiac...
apoptosis after exercise training may be a mechanism of the action of TNF-α, providing further evidence for the anti-inflammatory effects of exercise.

Exercise training has been demonstrated to modulate a variety of risk factors for cardiovascular disease as well as enhancing myocardial endothelial nitric oxide synthase (eNOS) function (14), augmenting vascular function (24), and suppressing systemic inflammation (35). Long-term training increases antioxidant capacity in superoxide dismutase activity and may be associated with reduced apoptosis in the heart of middle-aged rats (2). In addition, an 8-wk aerobic physical activity program was found to enhance insulin sensitivity and improve serum glucose, LDL cholesterol, and oxidative stress markers in postmenopausal women (19). Further studies are required to evaluate these points.

A previous study has indicated that women who became overweight or obese after menopause had an increase in many health risks (46). In our current study, weight gain can be observed after ovariectomy and weight can be partially maintained by running on a treadmill. We might further hypothesize that exercise training is a therapeutic agent for preventing obesity associated problems in menopausal obese women.

There are some limitations in the current experimental design. Bilateral ovariectomized animal models not only impact the female hormonal system but also impact female systemic physiology, such as weight gain and increased inflammation (37). In addition, exercise training has multiple beneficial effects on the whole body system such as the cardiovascular, neurological, musculoskeletal, and hormonal systems (12, 30, 33). Therefore, we need to make a cautious note that any detrimental effect of ovariectomy or any preventive effect of exercise training on hearts cannot be isolated to one specific factor, such as BW changes, estrogen receptor mechanism, dyslipidemia, lipid accumulation, insulin resistance, oxidative stress, inflammation, or unclear interacting factors.

The ovariectomized animal model presents many of the same pathophysiological deficits as noted in menopausal women (5, 8), such as decreased levels of progesterone and estrogen (39) and increased risk of cardiovascular diseases (40). Because cardiac tissues are difficult to sample from the hearts of menopausal women, the current ovariectomized animal model under exercise training provides a reasonable explanation for clinical exercise training preventing heart failure or apoptosis-related cardiac diseases in menopausal women. However, the cardiac function of the human heart after exercise training in menopausal women will be required to support our hypothesis. In sum, menopausal women should be highly aware of the progressive development in cardiac abnormality and should devote themselves to exercise training and lifestyle modification.

Hypothesized and clinical application. Our current findings indicate that exercise training may be an important lifestyle modification to prevent cardiac Fas receptor-dependent and mitochondria-dependent apoptotic pathways after menopause. Exercise training might provide one possible mechanism to
interrupt cardiac apoptosis and prevent the development of heart failure in postmenopausal women. We might further hypothesize that exercise training may be one of several important therapeutic approaches to prevent cardiac apoptosis in menopausal women. Of course, further therapeutic or clinical studies are required to clarify any possible therapeutic application.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


