SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of the cardioprotective effect of recreational exercise in oestrogen-deficient animal models

by Dr. Zoltán Karácsonyi

Supervisors: Dr. Anikó Pósa, Dr. Béla Juhász

UNIVERSITY OF DEBRECEN

LAKI KÁLMÁN DOCTORAL SCHOOL

DEBRECEN, 2023

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	Sándor Somodi, MD, PhD

The Examination takes place at the Library of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
10:00, 10th April, 2024

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
13:00, 10th April, 2024

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Introduction

Menopause is a natural process marked by the gradual reduction in female sex hormone levels, primarily endogenous oestrogen. This hormonal shift brings about notable changes in the functioning of various body tissues and organs. Notably, oestrogen deficiency during this period has profound effects on the cardiovascular (CV) system, carbohydrate, protein, and lipid metabolism, as well as bone health. In addition to these, many women experience vascular symptoms during menopause, which are associated with an increased risk of CV issues.

Despite a decrease in the mortality rate from cardiovascular diseases, these conditions remain a leading cause of death among older women. While men typically experience CV diseases earlier than women by about 7-10 years, the pace of progression significantly accelerates after menopause. Although the incidence of these conditions is lower in women before menopause compared to men, it levels off in postmenopause and even reverses in advanced age, resulting in higher rates of CV morbidity and mortality among women.

Oestrogen plays a pivotal role in safeguarding cardiovascular health by providing protection to blood vessels, influencing serum lipid profiles, and mitigating inflammation, among other key mechanisms. The reduction in ovarian hormone levels during and after menopause is a substantial contributing factor to the development of CV diseases in women. Research has indicated that both oestrogen deficiency and obesity are risk factors that promote structural and tissue changes in the CV system, as well as alterations in heart function.

As women transition into menopause and oestrogen levels decrease, the protective effects that oestrogen provides diminish, rendering them more susceptible to CV diseases. Concurrently, the prevalence of obesity, often linked to hormonal changes and an unhealthy lifestyle, further elevates the risk of CV issues by promoting inflammation and fostering the development of insulin resistance and other pathological processes within the CV system.

Ultimately, oestrogen deficiency significantly increases the risk of CV diseases, particularly atherosclerosis, ischemic diseases, and hypertension in postmenopausal women. These hormonal changes also impact metabolic functions, compounding the risk of CV problems.

The decline in oestrogen production can result in endothelial dysfunction and an increase in body mass index, both of which are factors contributing to hypertension. Hypertension, or arterial hypertension (AHT), is a well-known significant factor in cardiovascular (CV) mortality. Research has shown that various risk factors, such as psychological stress, exposure to environmental pollutants, unhealthy lifestyle choices, and physical inactivity, are associated with the development of multiple health conditions. These include vasculomotor dysfunction, neuropsychological issues, endocrine and metabolic disturbances, and cardiovascular problems. These factors not only diminish the quality of life but also have a long-term impact on prognosis.

Hypertension is the leading preventable risk factor in the development of CV diseases, significantly affecting global morbidity and mortality. Nevertheless, as women enter postmenopause, they typically undergo unfavourable changes in arterial wall stiffness, muscle strength, and overall health status. These changes are closely tied to hormonal shifts, particularly the decline in oestrogen levels. This hormonal change weakens the protective effects on the cardiovascular system, resulting in an increased prevalence of hypertension among postmenopausal women. This gradual increase in hypertension prevalence contributes to higher morbidity and mortality from CV diseases.

Consequently, postmenopausal women face a substantially higher risk of ischemic heart disease compared to men of the same age. The clinical course of ischemic heart disease in postmenopausal women exhibits distinct characteristics, with microcirculatory disorders being more prevalent. These often manifest as positive findings in electrocardiographic exercise tests, despite minimal hemodynamic changes in the epicardial arteries. Alongside microvascular dysfunction, the increased risk is frequently associated with issues like abnormal coronary reactivity and heart failure with preserved ejection fraction (HFpEF).

Oestrogen depletion also leads to adverse changes in early diastolic relaxation and late diastolic compliance, contributing to left ventricular diastolic dysfunction, fibrosis, and concentric left ventricular hypertrophy. These cardiac remodelling processes are typically associated with the menopausal transition. Left ventricular diastolic dysfunction and changes in cardiac morphology can result from various factors, including increased collagen concentration, fibrosis, and reduced cardiac function. These changes are influenced by the autonomic nervous system and the renin-angiotensin system. Moreover, the climacteric phase negatively affects the hemodynamic profile, characterized by unfavourable alterations in blood flow dynamics. Furthermore, the balance of the autonomic nervous system is disrupted, leading to a decrease in parasympathetic activity and/or an increase in sympathetic activity. These changes in autonomic function have long-term repercussions, potentially impacting inflammatory and oxidative stress markers.

The aging process itself has been shown to induce autonomic, biochemical, and functional changes, which can be exacerbated by menopause. This underscores the significance of early interventions during menopause to mitigate the adverse effects of aging in women. Addressing or ameliorating autonomic dysfunction associated with the aging process can result in a more favourable inflammatory and oxidative stress profile, preserve proper heart function, and ultimately enhance the quality of life and life expectancy of postmenopausal women, especially those who have undergone oophorectomy.

The role of physical activity in the prevention and treatment of cardiovascular diseases

Exercise plays a crucial role in the prevention and treatment of cardiovascular diseases (CVD). It is a well-recognized non-pharmacological intervention with numerous benefits for cardiovascular health. Engaging in regular exercise leads to significant cardiovascular adaptations, including the reduction of blood pressure, promotion of beneficial myocardial remodelling known as physiological hypertrophy, and mitigation of myocardial fibrosis. Furthermore, exercise-induced cardio-protection is closely associated with the activation of antioxidant defence mechanisms and the reduction of metabolic risk factors.

In women, the aging of the vascular system accelerates after menopause, resulting in increased arterial stiffness. This age-related stiffening of the arteries is partly due to changes in the extracellular matrix (ECM) within arterial walls. These changes involve an increase in collagen at the expense of elastic fibres (fibrosis) and the elevated presence of cross-linking molecules, such as advanced glycation end products (AGE).

Perimenopausal women often experience a persistent rise in arterial blood pressure. However, there is limited information available regarding changes in their quality of life, and tailored recreational physical activity programs designed to improve the quality of life during menopause have not received adequate attention. Moderate aerobic physical activity is known to significantly contribute to the prevention of arterial hypertension. Prospective studies have shown that physically inactive middle-aged individuals have a 30% higher risk of developing cardiovascular disease. Regular, moderate physical activity fosters adaptive responses, enhances resistance to environmental stressors, increases tissue metabolism, improves coping with hypoxia, and enhances heart function efficiency.

To enhance the quality of life for perimenopausal women, it is essential to develop comprehensive wellness programs. Key components of such programs include physiotherapy (physical exercise), cognitive training, and massage. Additionally, initiatives aimed at expanding social relationships can be valuable supplements. The North American Menopause Society (NAMS) recommends that women experiencing mild vasculomotor symptoms consider lifestyle changes and other non-pharmacological interventions as part of their treatment plan.

Physical activity has proven to be an effective non-pharmacological treatment option for blood pressure management. Aerobic exercises are widely recommended for their positive effects on cardiovascular and metabolic parameters. Various studies have indicated that the combination of specific risk factors, such as hypertension, menopause, and excessive fructose intake, can exacerbate metabolic, cardiovascular, autonomic, inflammatory, and oxidative stress parameters. Both aerobic and combined exercises, which incorporate aerobic and resistance components, can alleviate these dysfunctions. While aerobic and resistance exercises may not significantly alter resting blood pressure in this combination of risk factors, they do positively affect the autonomic regulation of circulation, leading to improvements in vascular sympathetic modulation and baroreflex sensitivity.

Aerobic exercise can significantly reduce blood pressure in postmenopausal women, whether they have hypertension or not. It also enhances endothelial function, nitric oxide (NO) levels, arterial compliance, and reduces insulin resistance. Research has demonstrated that resistance training has favourable effects on blood pressure in both hypertensive and non-hypertensive postmenopausal women. Resistance training involves muscle contractions against external resistance to increase muscle strength, mass, and bone density. Postmenopausal women commonly experience reduced muscle strength and mass, which are associated with increased arterial stiffness. Increased arterial stiffness contributes to the development of hypertension. Most

studies show that postmenopausal women who engage in regular endurance exercise exhibit lower arterial stiffness compared to sedentary individuals. Furthermore, some research has highlighted the potential role of flexibility in promoting cardio-metabolic health in women and the elderly. Therefore, including resistance training in the routine of postmenopausal women is advisable.

Research has yielded mixed results regarding the impact of exercise on blood pressure in menopausal and postmenopausal women. Some studies have shown significant reductions in both systolic and diastolic blood pressure, while others have suggested that aerobic exercise alone may not substantially lower blood pressure. However, a combination of aerobic and resistance training has demonstrated more substantial reductions. Several previous investigations have indicated that engaging in aerobic exercise three times a week can lead to significant blood pressure reduction in postmenopausal women. Interestingly, combined aerobic and resistance exercises, even when done less frequently than three times a week, have also proven effective in lowering blood pressure. These two forms of exercise complement each other in mitigating menopause-induced hypertension by addressing key mechanisms involved in the development of cardiovascular disease. Given that aerobic and resistance exercises impact blood pressure through different mechanisms, combining them may offer enhanced benefits. Some researchers have recommended using both aerobic and resistance exercises together, as resistance exercises not only enhance muscle strength and mass but also improve the efficiency of aerobic exercise, further contributing to the reduction in blood pressure.

In the larger context, hormonal changes associated with aging in women create a chronic, low-grade inflammatory state that contributes to the development of systemic arterial hypertension (SAH) and type 2 diabetes mellitus. A decrease in the serum levels of female sex hormones, particularly 17β-estradiol, affects various metabolic pathways and inflammatory mechanisms, leading to changes in adipose tissue distribution throughout the body.

Abdominal obesity represents a significant risk factor for metabolic disorders and cardiovascular-related mortality, particularly in postmenopausal women. Thus, it is crucial for them to explore effective strategies to combat obesity. Physical activity also plays a role in reducing the circulating levels of specific proinflammatory cytokines, which contributes to its overall impact on weight management and metabolic health.

Physical activity has been described as an effective countermeasure against structural and functional cardiac changes associated with cardiovascular disease by preventing the shift from physiological cardiac hypertrophy to pathological hypertrophy. Exercise not only reduces body mass index but also improves insulin sensitivity, glucose uptake, and lipid profiles. Older women who engage in exercise, even if they have diabetes mellitus and hypertension, exhibit overall improvements in various risk factors. These include more favourable body proportions in terms of fat distribution, blood pressure, lipid profiles, and inflammatory status, taking into account both pro- and anti-inflammatory cytokine serum concentrations.

Lipid profiles are significantly affected by aging, especially in women with type 2 diabetes mellitus. Typically, triglyceride and LDL serum concentrations increase, while HDL levels decrease, contributing to an increased cardiovascular risk (referred to as an atherogenic lipid profile). Physical activity has been associated with improved lipid profiles in older postmenopausal women with diabetes and hypertension. This improvement is observed not only in individual parameters but also in the ratios between HDL and specific risk factors, such as LDL and VLDL.

Resistance training (RT) has been found to lead to a significant acute energy expenditure. It is well-established that RT enhances insulin sensitivity, thus positively impacting glucose metabolism. However, in sedentary women, the beneficial effects of acute exercise, especially intense RT causing muscle damage, might be diminished. Nunes et al. reported that low-volume (less intense) RT is more effective at reducing HbA1c% than high-volume RT. Notably, the HbA1c%-reducing effects of high-volume RT appear to be more reliable in postmenopausal women. The results suggest that low-volume RT primarily improves HbA1c, muscle strength, and body fat percentage, while high-volume RT is more effective at reducing waist circumference, waist-to-hip ratio, total cholesterol (TC), LDL-cholesterol (LDL-C), and also inhibiting the increase of interleukin 6 (IL-6) in postmenopausal women. Age-related muscle loss and weakness are exacerbated by a decline in the hormone oestrogen, which is a common consequence of menopause. Studies indicate that the decrease in muscle strength observed in women over 50 is linked to oestrogen deficiency resulting from menopause. Previous research has demonstrated that RT can be safely and effectively integrated into the therapy programs of older women. Many individuals incorporate RT into their healthy lifestyle to enhance muscle strength and endurance. RT performed three times a week can help reduce the metabolic syndrome by lowering fasting glucose, improving body fat percentage, and increasing muscle strength. For postmenopausal women, a short-term hypertrophy RT program can be performed up to twice weekly, either as a starting point for a progressive RT program or in combination with other treatments.

Recent literature underscores the significance of exercise volume and physical fitness levels in cardiovascular disease prognosis. However, most studies have primarily focused on the relationship between cardiorespiratory fitness, muscle strength, and cardiovascular disease. The influence of other aspects of physical fitness, such as flexibility and mobility, on cardiovascular disease risk remains a topic of discussion. Key findings from one study indicate that less sedentary work, cardiorespiratory fitness (CRF), greater upper body flexibility, and enhanced lower body muscle strength are associated with a more favourable cardiovascular health profile, especially in postmenopausal women. Several studies have stressed the protective role of various physical fitness components in women's cardiovascular health. For instance, CRF has demonstrated positive effects on body fat percentage, blood pressure, autonomic nervous system activity, glucose and lipid profiles, susceptibility to inflammation, and oxidative stress. Regarding flexibility, a recent comprehensive study suggested that stretching skeletal muscles can reduce sympathetic nervous system activity and arterial stiffness. While a significant portion of research has concentrated on cardiorespiratory fitness and muscle strength, it is becoming increasingly evident that factors like sedentary behaviour, flexibility, and mobility also significantly contribute to cardiovascular health, especially in postmenopausal women. These findings emphasize the importance of conducting a comprehensive evaluation of physical fitness to assess cardiovascular disease risk.

Numerous studies have investigated the impact of various exercise regimens in combination with specific dietary intake or vitamin supplements on the health parameters of healthy postmenopausal women. These parameters include body composition, lipid and glucose profiles, antioxidant status, prothrombotic burden, and cardiovascular (CV) parameters. This research is of particular significance since epidemiological studies have revealed that the risk of CV diseases is higher after menopause compared to premenopausal women. Both aging and menopause can lead to elevated circulating levels of catecholamines, particularly adrenaline and norepinephrine. This is believed to result in a reduction in autonomic nervous system activity, as elderly and postmenopausal populations have demonstrated increased sympathetic nervous system activity at rest, which

decreases in response to exercise. Impaired autonomic nervous system activity may contribute to the development of CV diseases, as higher catecholamine levels have been observed in this disease group, including older hypertensive individuals, postmenopausal and hypertensive women. Increased pulse wave velocity, an indicator of arterial stiffness, is a significant risk factor for atherosclerosis, high blood pressure, and CV disease, often observed in postmenopausal women. Muscle strength is thought to be protective against arterial stiffness and hypertension, implying that improvements in muscle strength may yield CV benefits and overall health. As a result, addressing high blood catecholamine levels, arterial stiffness, reduced muscle strength, and muscle mass in postmenopausal women with hypertension is crucial. Taekwondo can be a valuable form of exercise for this population due to its ability to be performed at different intensities, encompass both aerobic and resistance training components, and enhance muscle strength, motor coordination, and cardiovascular health. Literature suggests that Taekwondo training may serve as an effective therapeutic approach to enhance blood catecholamine levels, reduce arterial stiffness, lower blood pressure, and enhance muscle strength in postmenopausal women with stage 2 hypertension. These findings support the use and recommendation of this type of exercise for preventing and treating CV disease risk factors in this population.

Although the positive effects of physical activity and structured exercise are well-established, the World Health Organization (WHO) recommendations for physical activity continue to be insufficiently followed, especially among women. Physical inactivity stands as a major risk factor for global mortality and CV disease, and this is closely linked to severe menopausal symptoms and obesity in postmenopausal women. A sedentary lifestyle has also been associated with metabolic disorders, CV disease, cancer, psychological distress, and higher mortality rates. Hence, reducing sedentary behaviour offers an alternative strategy for decreasing the risk of CV disease and CV-related mortality in older adults.

In addition to engaging in physical activity, adherence to exercise programs is a critical factor that can significantly influence health outcomes, especially in the older adult population. Exercise adherence is typically considered successful if participants complete a prescribed exercise routine at least two-thirds of the time. Structured exercise, which is a planned and organized form of physical activity, has numerous positive effects on the cardio-metabolic profile throughout one’s life and plays a crucial role in countering the adverse changes associated with the menopausal transition. Physical activity has become an essential complement to traditional pharmacological therapies, with public health programs increasingly incorporating regular physical activity as a valuable tool in the treatment of chronic diseases.

Currently, the American College of Sports Medicine and the American Heart Association recommend 150 minutes of physical activity per week for adults and seniors. Moreover, when accounting for the amount of weekly exercise, postmenopausal women who engage in 150 minutes or more of exercise per week experience significant reductions in both systolic and diastolic blood pressure compared to those who exercise for less than 150 minutes per week. This aligns with the minimum recommended weekly physical activity time outlined by the World Health Organization.

The quantity of exercise also appears to be a critical determinant of the rate of fat loss. It is proposed that the reduction in body fat among postmenopausal women is directly linked to enhanced muscle aerobic function, increased mitochondrial content, and elevated energy expenditure.

In general, regular exercise, whether it involves aerobic or resistance training, can contribute to mitigating vascular aging in women. It is essential to note, however, that these effects may not manifest uniformly across all biomarkers of vascular aging.

All of this underscores the profound therapeutic potential intrinsic to physical exercise. It stands as the cornerstone of both the prevention and treatment of cardiovascular diseases, offering a multitude of benefits that encompass structural, functional, and metabolic enhancements to the cardiovascular system. These benefits can extend to situations where hormonal imbalances come into play.

Aims

Our primary goal in this study was to investigate the postconditioning-like effects of voluntary exercise on postmenopausal cardiovascular outcomes following a myocardial infarction (MI), with a focus on its influence on myocardial extracellular matrix (ECM) and collagen homeostasis.

Expanding on our previous research, another objective of this study was to assess the enduring impacts of isoproterenol (ISO)-induced cardiac damage on the antioxidant and inflammatory profile within various oestrogen-deficient rat models, including those in the fertile state. We hypothesized that voluntary physical exercise could offer an effective therapeutic approach for mitigating ISO-induced cardiac damage.

Furthermore, we sought to explore the potential of longer-term voluntary exercise as a strategy to modify cardiac remodelling in the context of oestrogen deficiency. The myocardial ECM plays a pivotal role in heart development, maintaining homeostasis, and undergoing remodelling processes. In this context, our second objective was to investigate potential protective effects against the dysregulation and detrimental impacts on MMP-2 and collagen content, particularly as they relate to the detection of necrotic ratios following ischemia/reperfusion injury.

The acceleration of vascular aging and the decline in ovarian hormone levels during menopause have profound implications for the proactive implementation of cardiovascular prevention strategies aimed at perimenopausal women. As women's life expectancy continues to increase, a substantial portion of their lives is spent in the postmenopausal phase. Consequently, there is a clinical imperative to employ evidence-based therapeutic approaches for preventing cardiovascular disease in women.

Nonetheless, a vital area for future research involves determining the optimal type, quantity, and intensity of physical activity that can effectively enhance vascular health in women. Current recommendations may need adjustment to account for women’s hormonal status, ensuring that exercise’s full range of benefits is realized.

To formulate effective treatment and intervention strategies, it is critical to gain a comprehensive understanding of the mechanical deficiencies associated with vascular aging and the diminished sensitivity of the vasculature in older women. This knowledge will serve as the foundation for future research, enabling the development of gender-specific therapeutic approaches to preserve vascular function as women age.

Materials and Methods

Ethical Approvals

All animal experiments adhered to international regulations regarding the protection and use of laboratory animals and followed the local ethical guidelines of the University of Szeged, as per the approvals I.74-40/2017 MÁB and 16/2007 DE MÁB.

Role of Exercise-Induced Cardiac Remodelling in Ovariectomized Female Rats

Experimental Animals:

Female Wistar rats, aged 9 weeks and weighing 180-200 g, were procured from Toxi-Coop Zrt., Hungary, for this study. The rats were housed in standard-sized cages with three animals per cage. The animal housing facilities, both at the Department of Physiology, Organization and Neuroscience of the University of Szeged and the Institute of Pharmacology and Pharmacotherapy of the University of Debrecen, maintained a consistent temperature range of 20-23°C and followed a 12-hour light and dark cycle throughout the one-week acclimatization period and the entire experimental duration.

Surgical Procedures:

Following the acclimatization period, surgical interventions were performed on the 10-week-old rats. Ovariectomy (OVX) and sham operation (SO) were conducted under anaesthesia induced by thiopental (5 mg/100 g i.p.). The OVX procedure involved a bilateral dorsolateral incision and removal of the ovaries. In contrast, the SO group underwent sham surgery, where the ovaries were not removed but were instead exteriorized to induce the same stress effect. A four-week recovery period followed, during which serum oestrogen levels were assessed using ELISA (Quantikine rat oestrogen ELISA kit, R&D Systems Inc.) to monitor the onset of OVX-induced menopause.

Experimental Protocol:

The OVX and SO groups were further subdivided into eight groups based on diet (regular rodent food - control group, and high triglyceride content food - HT group) and physical activity (running and non-running groups). Throughout the 12-week experimental phase, the CTRL animals were provided standard rodent feed, while the HT group received rodent feed with a high triglyceride content (a blend of 40% triglyceride and 60% standard feed). These groups were then categorized into running and non-running subgroups. Animals in the running group were individually housed in cages equipped with running wheels, offering 24-hour access to the wheel over the 12-week period to minimize the stress caused by forced exercise. The non-running animals (3 animals/cage) remained in standard cages. At the endpoint of the experimental period, the animals were humanely euthanized with a combination of 100/5 mg/kg ketamine/xylazine during the pre-oestrogen phase of the menstrual cycle. Giemsa staining was employed to verify the oestrogenic phase in sham-operated animals. This involved obtaining vaginal secretion from the animals with an ear cleaner, smearing it on a slide, and fixing the smear with a 1:9 mixture of 96% alcohol and ether. Giemsa solution (methylene blue eosin) was then used for staining. The smears were examined under a microscope after the filtered Giemsa solution was left on the smear for 3-5 minutes, washed with hot water, and rinsed with distilled water. Following gentle euthanasia, the hearts were either subjected to ex vivo ischemia/reperfusion damage assessment using a Langendorff perfusion

system (10 animals/group) or immediately frozen and stored at -80°C (10 animals/group) for subsequent biochemical and molecular biological tests.

MMP-2 Activity measurement:

To determine MMP-2 activity in heart muscle tissues, 50 µg of protein samples were subjected to electrophoresis on an 8% polyacrylamide gel copolymerized with 20 mg/ml of gelatine (Sigma). Following electrophoresis, the gels underwent washing in a 2.5% Triton X-100 solution, followed by a 20-hour incubation in an activity buffer at 37°C. Subsequently, MMP-2 activity was visualized through Coomassie Brilliant Blue staining at 0.05%. The identification of the 2 isoforms of the enzyme was aided by a protein ladder (Spectra Multicolor Broad Range Protein Ladder, Thermo Scientific). Quantification of band optical activity was performed by scanning the zymograms digitally using Quantity One software (Bio-Rad, Hercules, CA, USA).

Total Glutathione Level Measurement:

Myocardial tissue samples weighing 70 mg were homogenized through centrifugation (30 minutes at 15,000 g, 4°C) in 280 ml of homogenizing buffer "A" (comprising 0.25 M sucrose, 20 mM Tris, and 1 mM dithiothreitol (DTT)). Following this, 150 µl aliquots of the supernatant were mixed with 600 µl of buffer "B" (containing 0.1 M CaCl2, 0.25 M sucrose, 20 mM Tris, and 1 mM DTT). These mixtures were then incubated at 0°C for 30 minutes and centrifuged again (for 60 minutes at 21,450g, 4°C). The resulting cytosolic fraction was used for the subsequent enzyme assays. The determination of the total glutathione (GSH+GSSG) level involved the use of commercially available kits (GenAsia, Shanghai, China). The measurement was conducted at a wavelength of 450 nm using a microplate reader (Benchmark Microplate reader; Bio-Rad), and the results were expressed as nmol/mg protein.

Analysis of 3-Nitrityrosine (3-NT), Tissue Inhibitor 2 (TIMP-2), and Type I Collagen Concentrations in Cardiomyocytes:

After 12 weeks of experimentation, the hearts of the animals were collected and frozen. The tissue samples were prepared for enzyme assays in a phosphate buffer (pH=7.4) with the aid of a tissue homogenizer (Ultra-Turrax T8; 2 x 30 sec) and subsequent centrifugation (20 minutes, 2000 rpm, 4°C). Commercially available kits (GenAsia, Shanghai, China) were used for the enzyme tests. The absorbance was measured at 450 nm using a microplate reader (Benchmark Microplate reader; Bio-Rad). To determine protein concentration, a diluted homogenate (20 µl) was mixed with dH2O (980 µl) and Bradford reagent (200 µl) and incubated for 10 minutes at room temperature. The optical density of the sample was then measured at a wavelength of 595 nm. The concentration of 3-NT was expressed as pmol/mg protein, type I collagen as pg/mg protein, and TIMP-2 as ng/mg protein.

Ischemia/Reperfusion Experimental Protocol:

The hearts of the animals were dissected under general anaesthesia (30 mg/kg thiopental) and placed in ice-cold Krebs-Henseleit solution (composition: 11.2 mM glucose, 1.24 mM KH2PO4, 20.1 mM NaHCO3, 119 mM NaCl, 4.7 mM KCl, 1, 25 mM CaCl2, and 1.24 mM MgSO4). The hearts were subsequently inserted into a Langendorff perfusion system, where retrograde perfusion of the heart was carried out via the aorta at a constant pressure of 75 mmHg, using Krebs-Henseleit buffer saturated with 5% CO2 and 95% O2 at 37°C. Ischemia was induced by ligating the anterior descending branch of the left coronary artery (LAD) for 30 minutes, followed by reperfusion for 120 minutes. At the endpoint of the experiment, the LAD was closed again, and perfusion was

terminated. The ischemic area was determined by staining the heart with a 1% Evans blue solution injected through the aorta, followed by overnight freezing at -20°C.

Measurement of Myocardial Infarction-Induced Necrotic Area:

The frozen heart tissue samples were sliced into 2 mm thick sections, which were then immersed in a 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution at 37°C for 10 minutes. After TTC staining, the slices were treated with 10% formalin for 10 minutes, followed by phosphate buffer (pH=7.4). Images were captured from both sides of the slices using a digital camera, and the extent of the infarcted area was calculated, expressed as a percentage of the at-risk area.

Statistical Analysis:

The results are expressed in mean ± S.E.M (standard error of mean). Statistical differences between the groups were determined using an analysis of variance (ANOVA) test, with significance defined as $p \leq 0.05$.

Postconditioning-like effect of exercise in pharmacologically induced menopause in a rat model

Experimental Animals:

Female Wistar rats (Toxi-Coop Zrt., Hungary) weighing between 180-200 g were utilized for this study. The rats were housed in controlled conditions with a constant temperature of 20-23°C and a 12-12-hour dark/light lighting cycle. They were kept in standard-sized cages, with three animals per cage, and provided with phyto-oestrogen-free food and unrestricted access to tap water.

Test Protocol:

Following a one-week acclimatization period, a total of 100 animals were divided into two main groups: control (CTRL) and pharmacologically induced ovariectomy (POVX) groups (Figure 5). The oestrogen-deficient state was induced in the POVX group by administering 750 µg/kg of triptorelin (Decapeptyl depot, Ferring Germany) intramuscularly every 4 weeks. In contrast, animals in the control group received an equivalent volume of physiological saline solution intramuscularly every 4 weeks. On the 56th day of treatment, the oestrogen levels of the POVX group animals were assessed using a quantitative enzyme-linked immunosorbent assay (Rat E2 ELISA kit, SunRed Biological Technology Co., Shanghai, China).

Both the control and POVX groups were further subdivided into two groups each: CTRL, ISO-CTRL, POVX, and ISO-POVX. In the ISO-CTRL and POVX-CTRL groups, myocardial infarction was induced by a single subcutaneous injection of 0.1 mg/kg isoproterenol (ISO) (dissolved in 1 ml of physiological saline solution) (Sigma Chemicals Co.). Animals in the CTRL and POVX groups received a 1 ml subcutaneous injection of physiological saline without the active ingredient. To confirm the occurrence of myocardial infarction, serum lactate dehydrogenase (LDH) and myoglobin concentrations were measured 20 hours after the isoproterenol injection. Additionally, myocardial damage was confirmed through a 1% TTC staining process. For this, the frozen heart tissue samples were sliced into 2 mm thick sections transversely and immersed in 1% TTC solution at 37°C for 10 minutes.

In the fourth week after isoproterenol or physiological saline treatment, the animals were further divided into two subgroups: running and non-running (sedentary). The running group animals were placed in cages with running wheels. To alleviate stress and maintain socialization, two animals were housed together in each cage. A

voluntary treadmill use model was chosen for the training protocol to eliminate the stress associated with mandatory (forced) exercise. The animals had unrestricted access to the running wheels 24 hours a day, with an average daily distance of 4.0 km ± 10% per animal. The training protocol continued for 6 weeks. Non-running animals were housed in standard cages (three animals per cage) throughout the training period.

At the endpoint of the experiment, after euthanizing the animals using a combination of 100/5 mg/kg body weight of ketamine/xylazine, two samples were obtained from the heart muscle tissue: one sample was subjected to TTC staining, and the other was homogenized and stored at -80°C for subsequent biochemical tests.

Determination of Laboratory Parameters of Myocardial Infarction:

Determination of LDH and Myoglobin Concentration:

To measure LDH concentration, blood samples were obtained from the vena safaena 20 hours after isoproterenol treatment and then centrifuged (20 minutes, 2000 rpm, 4°C). LDH and myoglobin concentrations were determined in the obtained serum using commercially available kits (GenAsia Biotech Co., Ltd, Shanghai, China) and a Benchmark Microplate device (Bio-Rad) at a wavelength of 450 nm. LDH concentration was expressed in U/l, while myoglobin concentration was expressed in ng/ml.

Determination of GOT, GPT, and ALP Concentrations:

GOT, GPT, and ALP levels were assessed using a Biolis 24i Premium system analyser (Siemens) from blood samples collected 20 hours after isoproterenol treatment. The absorbance for GOT and GPT was measured at 340 nm, and for ALP at 405 nm. Concentrations were expressed in U/l.

Assessing the Antioxidant Status of Cardiomyocytes:

HO-1 Activity:

For determining HO-1 activity, 70 mg of myocardial tissue was isolated from rats and homogenized in 280 ml of ice-cold buffer (10.0 mM HEPES, 32.0 mM sucrose, 1.0 mM DTT, 0.10 mM EDTA, 10.0 µg/ml trypsin inhibitor, 10.0 µg/ml leupeptin, and 2.0 µg/ml aprotinin), with a pH of 7.4. This homogenate was obtained by centrifugation (20 minutes, 15,000 g, 4°C). A supernatant was collected, and a reagent mixture was prepared. The reagent mixture, with a total volume of 1.5 ml, contained glucose-6-phosphate, glucose-6-phosphate dehydrogenase, hemin, biliverdin reductase (rat liver cytosol), $MgCl2 \cdot 6H2O$, $KH2PO4$, and the supernatant. The chemical reaction was initiated by adding reduced β-NADPH, and after 60 minutes of incubation at 37°C, it was stopped by cooling on ice. Bilirubin concentration was measured at 465 and 530 nm, and the difference between the results at these two wavelengths was calculated. HO activity was expressed in nmol concentration of bilirubin produced per hour per mg of protein.

HO-1 Concentration:

The semi-quantitative determination of HO-1 concentration was carried out through Western blot analysis. Heart muscle tissue was homogenized in RIPA buffer by sonication, followed by centrifugation and boiling. Equal amounts of proteins (80 µg) were separated by gel electrophoresis and transferred to a nitrocellulose membrane. The membranes were then blocked, incubated with anti-HO-1 rabbit polyclonal primary antibody, and washed. After incubating with a secondary antibody, detection was performed using Quantity One software (Bio-Rad). β-actin was utilized as a control protein, and a Uvi Chemi Pro scanner was employed for development.

Determination of Total Glutathione Concentration:

To assess the total glutathione concentration, 70 mg of isolated rat heart muscle tissue was homogenized in a 4x amount of "A" buffer, comprising a solution of 0.25 M sucrose, 1 mM DTT, and 20 mM Tris. The homogenate was then subjected to centrifugation for 30 minutes at 15,000 g and 4°C. Subsequently, 600 µl of buffer B, composed of 0.1 M CaCl2, 0.25 M sucrose, 20 mM Tris, and 1 mM DTT, was added to the 150 µl supernatant. The mixture was incubated at 0°C for 30 minutes, followed by another centrifugation step lasting 60 minutes at 21,450 g and 4°C. A dilution buffer containing 125 mM Na-phosphate and 6.0 mM EDTA was used for GSH, *GSH reductase, DTNB, and β-NADPH stock solutions.*

In each well of the plate, 40 µl of background/standard/sample, 20 µl of DTNB stock solution, and 140 µl of β-NADPH were pipetted, and the plate was incubated for 5 minutes at 25°C. The reaction was initiated by adding 10 µl of GSH reductase, and 10 minutes later, the absorbance was measured at 405 nm. During this spectrophotometric test, GSH was intermittently oxidized by DTNB and reduced by NADPH in the presence of GSH reductase. The total GSH concentration was quantified as nmol/mg protein.

Myocardial GSH and TNF-α Levels:

Myocardial tissue was homogenized in phosphate buffer with a pH of 7.4 using an Ultra-Turrax T8 for two cycles of 30 seconds each, and then centrifuged for 20 minutes at 2000 rpm and 4°C. GSH and TNF-α concentrations were determined using a commercially available kit from GenAsia, with readings taken at 450 nm using a Benchmark Microplate reader (Bio-Rad). GSH levels were expressed in mg/l, and TNF-α levels in pg/mg.

Myeloperoxidase (MPO) Activity:

To measure MPO activity, myocardial tissue was homogenized with an ice-cold PBS and 0.5% hexadecyltrimethylammonium bromide solution, maintaining a pH of 6.0. After undergoing freezing and thawing three times, the samples were then centrifuged for 15 minutes at 15,000 g and 4°C. To initiate the reaction, 12 µl of the supernatant was added to a solution containing 280 µl PBS (pH=6.0) and 0.167 mg/ml O-dianisidine dihydrochloride. The reaction was adjusted with 10 µl of 0.03% hydrogen peroxide. After 90 seconds of shaking, cardiac MPO activity was measured at a wavelength of 490 nm and expressed in µU/mg of protein.

Determination of Total Protein Level:

The Bradford method was used to determine the total protein concentration. Specifically, 20 µl aliquots of the diluted protein samples were mixed with 200 µl of Bradford reagent from Bio-Rad Laboratories. After 10 minutes of stirring incubation, protein concentrations were measured using the spectrophotometric method at 595 nm, expressed as mg/ml.

Statistical Methods:

The results are presented as mean ± SE (standard error). Normality of the data was assessed with the Shapiro-Wilk test. Differences between groups were determined using a one-way analysis of variance (ANOVA) supplemented with a Tukey post-test. Significance was considered when $p \leq 0.05$. Additionally, interactions between paired combinations of the three parameters (ISO treatment, oestrogen status, and exercise) were examined using two-way ANOVA and Tukey's post-test.

Results:

Role of Exercise-Induced Cardiac Remodelling in Ovariectomized Female Rats

Effect of oestrogen deficiency, diet and exercise on cardiac MMP-2 activity

To investigate the influence of oestrogen deficiency, dietary factors, and exercise on cardiac fibrosis, we assessed the activity of the 64kDa and 72kDa isotypes of MMP-2 at the study's endpoint. The groups SO-HT-F, OVX-CTRL-NF, and OVX-HT-NF exhibited significantly lower activity of the 64kDa MMP-2 ($p < 0.05$) in comparison to the SO-CTRL-NF group. Physical exercise significantly increased the protein's activity ($p < 0.05$) in animals from the SO-HT-F, OVX-CTR-F, and OVX-HT-F groups, compared to their non-running counterparts. Notably, in the running groups, the high-fat diet significantly reduced the values in both the sham-operated and ovariectomized animals ($p < 0.05$).

Regarding the 72kDa isoform, significantly lower activity was observed in ovariectomized non-running animals fed a high-fat diet. Physical exercise significantly improved these values, both in sham-operated and OVX animals, compared to the control group and their non-running counterparts ($p < 0.05$). Nevertheless, the highest values were recorded in SO-CTRL-F animals.

Changes in 3-Nitrotyrosine (3-NT) Levels:

The levels of 3-nitrotyrosine in the myocardium were significantly lower ($p < 0.05$) in the ovariectomized groups compared to the control group. The high-fat diet also led to a decrease in the 3-NT level in sham-operated animals, though it did not reach statistical significance. Physical exercise significantly increased the 3-NT level ($p < 0.05$) in animals fed a high-fat diet (both SO and OVX groups) compared to their non-running counterparts. In contrast, in the OVX-CTRL-F group, a significant decrease ($p <0.05$) was observed.

Changes in Glutathione (GSH) Levels:

At the endpoint of the protocol, GSH levels measured via spectrophotometry in the non-exercising groups (SO-HT-NF, OVX-CTRL-NF, and OVX-HT-NF) were significantly reduced ($p <0.05$) in comparison to the control group. Exercise significantly increased GSH levels ($p <0.05$) compared to non-running animals.

Changes in Tissue Inhibitor of Metalloproteinases-2 (TIMP-2) Levels:

Measuring cardiac TIMP-2 concentration is pivotal for understanding the role of the MMP-TIMP system in myocardial extracellular matrix regulation. The results showed that both a high-fat diet and oestrogen deficiency led to a significant decrease ($p <0.05$) in TIMP-2 concentration in the myocardium. However, exercise induced a significant increase ($p <0.05$) in TIMP-2 levels in each group compared to sedentary animals.

Changes in Type I Collagen Levels:

In line with our hypothesis, regular exercise appeared to modify the accumulation of fibrotic tissue in the myocardium. Thus, we quantified type I collagen concentration using the ELISA method. As anticipated, oestrogen deficiency and a high-fat diet induced collagen accumulation, which was significantly reduced by physical exercise in animals of the OVX-HT-F group.

Changes in the Necrotic Area Caused by Myocardial Infarction:

The high-fat diet, particularly in combination with oestrogen depletion, significantly increased the necrotic area caused by myocardial infarction ($p < 0.05$) compared to the control group. However, these effects were significantly mitigated by regular exercise in all running groups.

Postconditioning-like effect of exercise in pharmacologically induced menopause in a rat model

Changes in Serum Estradiol Levels:

After two triptorelin injections, serum estradiol levels significantly decreased in the POVX animals, with CTRL animals having a level of 290.92 ± 3.10 ng/l and POVX animals having a level of 160.90 ± 2.98 ng/l ($p < 0.0001$).

Effect of ISO Treatment on Myocardial Infarction Markers:

Serum lactate dehydrogenase (LDH) activity significantly increased 20 hours after ISO treatment in both the control and POVX groups. The myoglobin concentration also increased in both ISO-treated groups after ISO treatment, with a significant increase in the ISO-POVX group compared to the ISO-CTRL group.

While 0.1 mg/kg ISO treatment significantly increased glutamate-oxaloacetate aspartate aminotransferase (GOT) concentrations in both the CTRL and POVX groups, neither POVX nor ISO altered serum glutamate-pyruvate alanine aminotransferase (GPT) levels. Serum alkaline phosphatase (ALP) was significantly increased in the POVX groups compared to the CTRL groups, and ISO treatment further increased ALP concentrations in the POVX groups in contrast to the CTRL-ISO group.

Changes in HO-1 Concentration and Activity:

The impact of exercise on antioxidant homeostasis was assessed by determining the activity and concentration of heme oxygenase-1 (HO-1) in cardiomyocytes at the end of the 6-week treatment period. HO-1 activity was decreased in the ISO-CTRL group compared to the negative control. In the ISO-POVX group, however, the decrease was already significant compared to the CTRL group. As a result of 6 weeks of exercise, an increase in HO-1 activity was observed in all groups compared to the non-running groups, with significantly higher values found in the CTRL, ISO-CTRL, and ISO-POVX groups.

The expression of HO-1 significantly decreased as a result of pharmacologically induced ovariectomy in the non-running animals compared to their own control group, but exercise significantly improved the expression in the same group compared to the non-running animals.

Changes in GSH+GSSG and GSH Concentration:

In comparison to the control animals, GSH and glutathione disulphide activity (GSH + GSSG) in the myocardium were significantly reduced in the animals of the POVX and POVX-ISO groups. Physical exercise resulted in a significant improvement in the GSH levels of both CTRL and POVX rats; however, these levels were reduced by oestrogen deficiency and ISO treatment. A significant association ($p = 0.0176$) was found between exercise and oestrogen status.

The concentration of GSH in the cardiomyocytes decreased as a result of a single dose of ISO in all ISO-treated groups. Still, a significant decrease was observed only in the ISO-CTRL and running-ISO-POVX groups compared to the non-ISO-treated groups. The GSH concentration was also significantly lower in oestrogen-

deficient animals compared to the animals in the absolute CTRL group. Six weeks of training improved the GSH changes induced by oestrogen deficiency in both the POVX and ISO-POVX groups. The results demonstrate a significant interaction between exercise, oestrogen concentration, and GSH levels ($p = 0.0164$).

Changes in Cardiac TNF-α Concentration:

As anticipated, the concentration of tumour necrosis factor-alpha (TNF-α) significantly increased in oestrogen-deficient animals, which was significantly reduced by 6 weeks of training ($p = 0.011$).

Changes in Cardiac Myeloperoxidase (MPO) Activity:

At the end of the experiment, myocardial MPO activity was determined, showing significantly high values in the POVX and ISO-POVX groups compared to the control animals. However, 6 weeks of training restored MPO pathological activity in oestrogen-deficient animals compared to their non-running peers. The results indicate that exercise and oestrogen status significantly affect cardiac MPO activity ($p = 0.0077$) and demonstrate the effectiveness of exercise in reducing inflammatory processes (decrease in MPO activity).

Discussion

Role of Exercise-Induced Cardiac Remodelling in Ovariectomized Female Rats

Cardiovascular diseases (CVDs) are a leading cause of global mortality, with approximately 17.9 million individuals succumbing to various CVDs annually, according to the World Health Organization's 2016 statistics. Multiple risk factors contribute to the development of CVDs, including inflammation, dyslipidaemias, oestrogen deficiency, alterations in antioxidant status, and the detrimental consequences of a sedentary lifestyle. These factors can lead to cardiac hypertrophy and heart failure, resulting in characteristic changes such as heart muscle remodelling, fibrosis, collagen infiltration, apoptosis, and cardiomyocytolysis due to insufficient oxygen supply, all of which worsen the prognosis for these pathologies.

Physical activity has emerged as a safe, non-pharmacological therapeutic approach for both preventing and treating CVDs. It offers several health-preserving benefits, including a reduced risk of age-related cardiomyocyte apoptosis and heart failure, ultimately enhancing cardiac pumping function.

Recent research is increasingly focused on demonstrating how tightly regulated extracellular matrix (ECM) homeostasis significantly influences the myocardial function. Cardiac fibrosis involves a considerable build-up of ECM proteins, primarily collagen, which contributes significantly to cardiomyocyte remodelling, leading to extreme muscle stiffness, hypertrophy, and, in severe cases, acute myocardial infarction. Interstitial collagen deposits can accumulate due to various factors, including aging processes, myocardial ischemia, inflammation, diabetes, and hormonal imbalances. Sex hormones and their receptors play pivotal roles in regulating ECM proteins. Furthermore, the interactions observed between matrix metalloproteinase-2 (MMP-2), peroxynitrite ($ONOO^-$), and glutathione open new avenues for exploring cardiac pathophysiology in oestrogen-deficient states.

Thus, our investigation delved into the pathomechanism of collagen accumulation and fibrosis resulting from oestrogen depletion, which may be linked to MMP-2 downregulation and cardiac hypertrophy. Our findings reveal that oestrogen deficiency and a high triglyceride diet significantly increase cardiac collagen levels while decreasing 3-nitrotyrosine (3-NT) and glutathione (GSH) concentrations. Nonetheless, our study demonstrates that 12 weeks of moderate exercise can reduce myocardial fibrosis in ovariectomized animals, likely through the regulation of GSH/3-NT and MMP-2. Our previous research also highlighted that ovariectomy-induced hypertension is involved in driving these changes. The antioxidant impact of both endogenous and exogenous oestrogen plays a decisive role in inducing vasoprotective effects, supported by the observation that oxidative stress processes escalate in postmenopausal women and animals. Studies by Pedram et al. indicate that oestrogen helps prevent cardiac remodelling by regulating myofibroblast, collagen, and fibronectin production. Conversely, oestrogen deprivation leads to left ventricular hypertrophy, collagen deposition, increased vasoconstriction, and heightened sensitivity to substances such as angiotensin II. Our results, consistent with existing literature, demonstrate that ovariectomy results in myocardial damage characterized by the accumulation of type I collagen. Furthermore, fibrosis is a complex process influenced by multiple factors, including a lack of exercise, sedentary lifestyles, declining oestrogen levels, oxidative stress, and aging. These elements collectively contribute to the excessive build-up of the collagen matrix and the progression of cardiac dysfunction. However, the mechanisms underlying the reduction of exercise-induced myocardial fibrosis are not yet fully understood. Our current research has sought to examine the effects of voluntary exercise and diet on MMP-2 regulation. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) govern the ECM profile in both normal and

pathological conditions, with the balance between MMPs and TIMPs playing a critical role in cardiac remodelling. Previous studies by Felix et al. showed that the lack of ovarian hormones has a detrimental effect on heart morphology, while low-intensity aerobic exercise can inhibit fibrosis proliferation. Nonetheless, this research did not investigate the regulatory mechanisms of MMP-2. Another study by Kwak et al. explored changes in collagen profiles resulting from exercise and highlighted the protective effect of exercise against the age-related downregulation of active MMPs.

Our research has revealed that 12 weeks of voluntary exercise effectively inhibited MMP-2 activity, offering protection against collagen accumulation and fibrosis. MMP-2 plays a pivotal role in ECM protein degradation, influencing cardiac remodelling. We also found that exercise led to reduced levels of type I collagen and an improved MMP/TIMP ratio, effectively shielding the heart from damage.

In our study, we observed a significant reduction in infarcted areas following 12 weeks of physical exercise, alleviating the extent of necrotic areas associated with oestrogen deficiency, high-fat diets, and obesity in an ischemia/reperfusion model. Many studies support the notion that both climacteric and postmenopausal women face an increased risk of cardiovascular events, including myocardial infarction. This heightened risk is primarily linked to increased oxidative damage and reduced nitric oxide (NO) bioavailability. Regular physical exercise is considered a non-pharmacological therapeutic approach for the prevention of cardiovascular diseases in both women and men. Improved myocardial capillary function, intracellular redox balance, and endothelial dysfunction due to enhanced NO production through physical activity can minimize infarct size. Almeida et al. reported that physical exercise reduced the protein expression of a significant oxidative stress pathway while increasing the activity of the antioxidant enzyme catalase. This dual effect enhanced cardiac function and remodelling in ovariectomized, post-infarction rats.

Clinical studies have also demonstrated a close association between cardiac fibrosis and obesity, contributing to the development of cardiac dysfunction in obese women. Research conducted by Kosmala et al. revealed that left ventricular function abnormalities are linked to changes in the MMP/TIMP system, which helps reduce ECM protein degradation due to MMP-2 downregulation.

In summary, our current investigation underscores the potential of voluntary exercise to protect against myocardial fibrosis, most likely through the regulation of MMP-2 and its interactions with GSH and 3-NT. Regular physical activity offers promising prospects for preventing and mitigating cardiac damage, particularly in the context of oestrogen deficiency and high-fat diets. These findings contribute to our understanding of how exercise can be a crucial element in cardiovascular health promotion.

In our experimental protocol, exercise has shown a significant ability to enhance MMP-2 activity and reduce collagen accumulation within the myocardium, both in sham-operated and ovariectomized animals. This underscores the critical role that exercise can play as a prophylactic strategy in preventing and treating heart diseases. The mechanisms underlying collagen turnover and cardioprotection induced by exercise are closely related to the MMP/TIMP profile and are a result of the activation of the MMP pathway.

MMP-2 activation can occur through two distinct pathways: proteolytic and non-proteolytic. Proteolytic activation involves the removal of the auto-inhibitory propeptide from the 72 kDa zymogen, leading to the production of the active 64 kDa MMP-2. Alternatively, peroxynitrite can be involved in post-translational modifications under the influence of cellular glutathione. The exact role of peroxynitrite in either activating or inhibiting MMP-2 remains a topic of ongoing debate. Some studies, like Rajagopalan et al., have suggested that

peroxynitrite increases the activity of unpurified MMP-2 in smooth muscle cells. Post-translational modifications resulting from oxidative stress can trigger the activation of MMP-2. Intracellular glutathione and peroxynitrite can lead to the production of S-glutathione and conformational changes, ultimately promoting the production of the active MMP-2 form. Glutathione (GSH) is a critical non-enzymatic antioxidant within cells, and a decrease in its concentration is a significant marker of oxidative damage. In our experiments, both oestrogen deficiency and a high-fat diet led to a substantial reduction in GSH levels. Our findings align with other research, highlighting that 12 weeks of exercise can effectively restore GSH concentrations. GSH plays a pivotal role in maintaining cardiac function by preserving redox homeostasis. Studies conducted by Frasier et al. have demonstrated that physical activity helps maintain cardiac GSH stores and reduces cardiac damage induced by ischemia.

Nevertheless, it is important to note that the functional properties of MMPs are complex and not uniform, especially in the context of cardiovascular pathology. Activation and regulation of MMP-2 remain subjects of ongoing research, and other studies have yet to investigate the role of lifestyle factors such as oestrogen deficiency, exercise, and diet in this context.

In conclusion, the data suggest that a 12-week exercise regimen significantly enhances cardiac 3-nitrotyrosine (3-NT) and GSH concentrations. These, in conjunction with the activation of the 72 kDa MMP-2, play a pivotal role in preventing cardiac fibrosis. These findings are consistent with our previous research, highlighting that exercise-induced MMP-2 activation and the maintenance of the MMP/TIMP balance contribute to cardioprotection, making exercise a promising therapeutic option for addressing cardiac remodelling.

Postconditioning-like effect of exercise in pharmacologically induced menopause in a rat model

Cardiovascular diseases (CVD) stand as a prominent cause of morbidity and mortality among postmenopausal women. The deficiency of oestrogen imparts adverse effects on both metabolic and cardiovascular functions in this population. Menopause is often linked to disruptions in body fat distribution, disorders in lipid and glucose metabolism, hypertension, and inflammation, all of which significantly elevate the risk of CVD, particularly myocardial infarction (MI). Several studies have established that the absence of oestrogen has deleterious effects on the cardiovascular system and inflammatory status. It diminishes the biological utilization of nitric oxide (NO), heightens aortic reactivity, increases inflammatory responses, and raises oxidative stress, cumulatively diminishing life expectancy in postmenopausal women post-MI.

While many preclinical and clinical studies have affirmed the effectiveness of exercise in preventing MI, only a few have explored the effects of exercise in the post-infarction phase. The objective of our study is to investigate the impact of voluntary exercise on the balance between oxidants and antioxidants and alterations in inflammatory status following MI. We have adopted a non-invasive animal model, wherein oestrogen deficiency is induced through the administration of triptorelin, and MI is induced by a single dose of isoproterenol (ISO). Unlike the “coronary ligation” procedure, which is associated with higher morbidity and mortality, the ISO-induced MI rat model offers more reliable cardiovascular effects with significantly lower mortality rates. We conducted a dose-finding study, using five different doses (100; 10; 1; 0.1 and 0.01 mg/kg) of ISO. Based on our mortality data, the dose of 0.1 mg/kg was determined to be optimal for inducing MI lesions in oestrogen-deficient female animals. In the surviving rat model employed by our research team, subcutaneous ISO treatment significantly elevated serum markers of myocardial necrosis, such as lactate dehydrogenase (LDH), myoglobin,

glutamate oxaloacetate transaminase (GOT), and alkaline phosphatase (ALP), which aligns with findings from previous studies. Furthermore, the extent of myocardial damage was assessed through TTC staining in myocardial samples of both ISO-treated and post-ovariectomy (POVX) animals.

A single 0.1 mg/kg ISO treatment induced 15.83% necrosis in the ISO-CTRL group and 23.54% in the ISO-POVX group. Presently, there is growing interest in exploring the consequences of acute ISO treatment and the examination of various potential prophylactic agents in ISO-induced MI. To the best of our knowledge, our study, which aims to investigate the long-term effects of ISO treatment and the role of exercise in a non-invasive MI rat model, is unique. It is well-established that ISO treatment triggers inflammatory processes and activates the generation of free radicals, prompting questions regarding the timing and intensity of physical activity to significantly ameliorate myocardial damage caused by MI. Garza et al. summarized existing literature that underscores the importance of the timing of exercise post-MI, revealing that exercise initiated immediately after MI can extend myocardial necrosis. Early exercise (within the first week after MI) may exacerbate left ventricular remodelling, while exercise initiated at a later stage (around three weeks post-MI) does not worsen the prognosis of the disease.

In our current research protocol, rats commenced voluntary running wheel training three weeks after ISO treatment. Our results unequivocally demonstrate that the cardioprotective effects of physical exercise result from increased activity and expression of the heme oxygenase (HO) enzyme in both the control (CTRL) and ovariectomy-induced oestrogen deficiency (POVX) groups. HO and its metabolic by-products possess both short-term and long-term cardioprotective attributes. HO catalyses the conversion of heme into biologically active metabolites, including carbon monoxide (CO), iron, and biliverdin, which is subsequently converted into bilirubin by cytosolic biliverdin reductase. These cytoprotective end products serve to reduce apoptosis and inflammation, regulate vasomotor tone, and exhibit antioxidant and immunomodulatory properties.

Notably, oestrogen deficiency and/or early ISO treatment significantly diminish the activity and expression of the HO enzyme. However, six weeks of voluntary training successfully restored the oxidant/antioxidant balance. In a study by Ren et al., moderate aerobic training in spontaneously hypertensive rats significantly enhanced HO activity and expression in aorta and myocardial tissue samples. Consequently, exercise-induced upregulation of HO contributes to increased endogenous CO production and participates in regulating cyclic guanosine monophosphate (cGMP) levels to maintain vascular tone. Activation of the HO enzyme, whether by pharmacological agents or exercise, improves post-infarction cardiac functions, reducing apoptosis, inflammatory cell infiltration, and oxidative damage. A study by Wang et al. demonstrated the effects of HO overexpression in a mouse model, revealing improvements in post-infarction survival, reduced left ventricular dilatation, apoptosis, fibrosis, and oxidative damage.

Elevated oxidative stress damage often results from an imbalance between reactive oxygen radicals and the activity of the antioxidant system. During our experiments, we observed that ISO administration had a long-term impact on the heart's glutathione (GSH) concentration, reducing GSH levels in both control and POVX groups. Similarly to the HO enzyme, GSH values improved following exercise. By regulating the redox status of proteins, GSH safeguards the cardiac muscle against the deleterious effects of oxidative stress. Both the GSH and HO pathways provide significant antioxidant effects, thereby offering physiological cytoprotection. Bilirubin, the end product of heme metabolism, offers broad protection against lipid peroxidation, while GSH primarily inhibits the oxidation of water-soluble proteins. The enhanced antioxidant capacity attributed to

physical activity can be linked to GSH's ability to scavenge free radicals within the heart muscle. Several studies support the role of the HO-1 enzyme in maintaining oxidant/antioxidant homeostasis. The nuclear factor E2-related factor 2 (Nrf2) protein serves as the key regulator in the antioxidant response by transcriptionally regulating HO-1 enzyme activity. Nrf2 plays a pivotal role in regulating cysteine uptake, thereby protecting against oxidative stress by maintaining intracellular GSH and cysteine levels. Yu et al. also reported the importance of the Nrf2/HO-1 pathway in cardiovascular pathology. Activation of the Nrf2/HO-1 signalling pathway has been shown to reduce necrosis, inflammation, and oxidative processes. Further exploration of this regulatory mechanism may offer more comprehensive evidence for future research on physical exercise and its antioxidant effects.

In addition to its antioxidant effects, increased HO-1 activity induced by exercise also serves as a safeguard against inflammatory processes. By elevating biliverdin and endogenous CO production, it promotes the upregulation of HO-1 activity. CO inhibits the release of proinflammatory mediators, such as tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), while increasing the expression of anti-inflammatory mediators, such as interleukin-10 (IL-10). Several studies have demonstrated a link between physical inactivity and a heightened inflammatory state, further increasing the risk of developing age-related diseases. In our research, oestrogen deficiency induced by POVX elicited the most pronounced response, as evidenced by increased levels of TNF-α and myeloperoxidase (MPO) activity. MPO catalyses the oxidative modification of lipoproteins and depletes endothelial nitric oxide (NO), thus impairing the cardiovascular system, particularly vasodilator function. MPO serves as an early biomarker of inflammation and plays a substantial role in the initiation and progression of cardiovascular diseases. In our previous studies, we demonstrated that treatment with tin protoporphyrin IX (SnPP), one of the most potent competitive inhibitors of HO, significantly increased cardiac MPO activity in both healthy and oestrogen-deficient rats. Moreover, the HO knock-out mouse model has also underscored the role of HO-1 in inflammation. Kapturczak and others noted that HO-1-deficient animals experience an elevation in the production of proinflammatory cytokines and develop a progressive inflammatory process. Consequently, six weeks of running wheel training, presumably due to its anti-inflammatory effects, reduced MPO activity and TNF-α levels in the heart, mitigating the myocardium's inflammatory state.

In conclusion, we can assert that six weeks of voluntary running wheel training ameliorated cardiac antioxidant and inflammatory status in our applied non-invasive MI animal model. The presumed mechanisms behind the cardioprotective effects of physical exercise are closely linked to increased HO enzyme activity and expression, as well as elevated GSH levels, collectively reducing the inflammatory response within the heart muscle. As such, voluntary physical exercise may serve as a potential therapeutic approach in preventing and treating cardiovascular complications in women who have experienced menopausal myocardial infarction.

Summary

In the first study we explored the impact of exercise on the myocardium in a rat model with ovariectomy-induced oestrogen deficiency. The study focused on the role of extracellular matrix in heart function. Cardiac fibrosis, characterized by excessive collagen build-up in the heart, can lead to detrimental cardiac remodelling and dysfunction, and oestrogen deficiency was identified as a contributing factor to this condition. The results showed that 12 weeks of moderate exercise effectively reduced myocardial fibrosis in ovariectomized rats. Physical activity appeared to regulate glutathione, peroxynitrite, and matrix metalloproteinase-2, leading to increased MMP-2 activity, which helped degrade ECM proteins and prevent collagen accumulation. Exercise also reduced infarcted tissue, especially in cases of oestrogen deficiency, high-fat diets, and obesity. This reduction was associated with improved capillary function, intracellular redox balance, and endothelial function. Consequently, exercise's benefits included modulating the MMP-2 pathway, maintaining ECM protein balance, and protecting against heart-related complications.

In the second study, we examined the effects of voluntary exercise as a post-treatment approach in a rat model with oestrogen deficiency induced by triptorelin administration and MI induced by ISO administration. While previous research highlighted exercise's preventive effects on MI, this study focused on its post-infarction benefits. ISO treatment induced inflammation and free radical formation, raising questions about the timing and intensity of exercise post-infarction. The study found that initiating exercise early might worsen left ventricular remodelling, but starting it later did not exacerbate the condition. Exercise also demonstrated anti-inflammatory effects by reducing myocardial MPO activity and TNF-α levels. In summary, voluntary exercise had cardioprotective effects in a pharmacologically induced menopausal rat model with MI. The mechanisms included increased HO enzyme activity, elevated GSH levels, and reduced inflammation in the myocardium.

In summary, exercise is a cornerstone in reducing CV disease risk among postmenopausal women. It offers a wide range of advantages, spanning structural and functional enhancements in the CV system, hormonal regulation, inflammation management, and metabolic improvements. Tailoring exercise to accommodate hormonal changes is vital for preserving vascular function and overall health in postmenopausal women.

Acknowledgment

I extend my heartfelt gratitude to Prof. Dr. Zoltán Szilvássy, the head of the Institute of Pharmacology and Pharmacotherapy (University of Debrecen) and the rector of our university, for providing the necessary resources and support for conducting this research within the framework of the UD Laki Kálmán Doctoral School.

I am deeply appreciative of my supervisors, Associate Professor Dr. Anikó Pósa and Professor Dr. Béla Juhász, for accepting me as their Ph.D. student. Their expert guidance, valuable insights, and unwavering support have been instrumental throughout my research journey.

I would like to express my gratitude to the late Professor Lajos Zoltán Csernátony, who served as the former Director of the Orthopaedic and Traumatology Clinic at the University of Debrecen. He not only provided me with the opportunity to practice my profession in the institution he led but also supported my scientific work.

My sincere thanks go out to both my former and current colleagues, whose camaraderie and dedication have fostered an excellent working environment that significantly contributed to the completion of this dissertation.

I am immensely thankful to my dear family and friends for their unending support, patience, and love.

Lastly, I wish to convey my appreciation to all the staff at the Department of Physiology, Anatomy, and Neuroscience, University of Szeged as well as the Department of Pharmacology and Pharmacotherapy at the University of Debrecen for their valuable assistance.

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Registry number: DEENK/473/2023.PL
Subject: PhD Publication List

Candidate: Zoltán Karácsonyi
Doctoral School: Doctoral School of Molecular Medicine

List of publications related to the dissertation

1. Szabó, R., Börzsei, D., **Karácsonyi, Z.**, Gesztelyi, R., Nemes, K., Magyariné Berkó, A., Veszelka, M., Török, S., Kupai, K., Varga, C., Juhász, B., Pósa, A.: Postconditioning like effect of exercise- New paradigm in experimental menopause.
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DOI: http://dx.doi.org/10.1152/ajpheart.00485.2018
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Total IF of journals (all publications): 18,172
Total IF of journals (publications related to the dissertation): 8,732

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

24 October, 2023

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