Summary of Ph.D thesis

The cardioprotective and anti-inflammatory effects of recreational physical exercise in rats

Médea Hegyköziné Veszelka

Supervisor:

Anikó Pósa Ph.D.

associate professor

Ph.D. School in Biology

University of Szeged

Faculty of Science and Informatics

Department of Physiology, Anatomy and Neuroscience

2018

Abbreviations

AVP: arginine vasopressin

CF: coronary flow

CO: carbon monoxide

CTRL: phytoestrogen free chow

CVD: cardiovascular disease

ECG: electrocardiogram

HO-1, HO-2, HO-3: heme oxygenase-1, heme oxygenase-2,heme oxygenase-3

HT: high-triglyceride diet

IL-6: interleukin-6

I / R: ischemia /reperfusion

LAD: left anterior descending artery

MAPK: mitogen activated protein kinase

MMP-2: matrix metalloproteinase-2

MPO: myeloperoxidase

OVX: ovariectomy surgery

R: running

SO: sham operation

TNF-α: tumor necrosis factor-alpha

1.INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death in the world. Chronic inflammation and oxidative stress are associated with the development of various cardiovascular diseases including hypertension and heart failure. The risk of these diseases is significantly reduced by appropriate lifestyle modifications, such as increased physical activity. The exact mechanisms by which exercise influence the development and progression of CVD are unclear. Estrogen deficiency in women is one of the main causes of age-associated diseases in the cardiovascular system. Cardiovascular morbidity and mortality are far less in premenopausal women compared to age-matched men because the estrogen seems to have a protective effect. Though, estrogen deficiency in itself increases overweight and obesity in postmenopausal women, many genetic and environmental effects (e.g. lifestyle, nutrition, and smoking) can further determine the pathophysiology of body fat accumulation. Postmenopausal women spend the third of their lifetime in estrogen-depleted state, therefore the management of obesity and obesity-related comorbidities has important health significance in the 21th century. While estrogen plays a fundamental role in antioxidant and anti-inflammatory mechanisms and positively regulates lipid and glucose metabolisms, postmenopausal women more likely tend to suffer from obesity, inflammation and oxidative stress. Moderate-intensity exercise causes low-degree oxidative stress which can induce the antioxidant defence systems,

including the heme oxygenase enzyme (HO). HO is a rate-limiting enzyme responsible for the catabolism of heme into carbon monoxide (CO), ferrous iron, and biliverdin, which is converted to bilirubin. CO and biliverdin metabolites have major functions in the cardiovascular system. CO is a vasodilator and antioxidant molecule and in addition, its antiapoptotic and anti-inflammatory actions are also important.

Body fat accumulation causes low-grade chronic inflammation by enhancing the production of pro-inflammatory citokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).

This obesity-related inflammatory state can be associated with disruption of the oxidant/antioxidant homeostasis which cause an enhancement of oxidative stress.

2.AIMS

2.1. In our first experiment, we examined the effects of physical exercise on the cardiovascular system in male rats.

We wanted to investigate the effects of a 6 week-voluntary exercise on the levels of serum and coronary effluent matrix metalloproteinase-2 (MMP-2) activity.

- How does the physical exercise influence basal blood pressure, the cardiac infarct size, ST depression, blood pressure as a response to AVP and aorta contraction?
- What are the differences between running and non-running groups regarding to MMP-2 activity?

2.2. In our second experiment we planned to examine the effects of high triglyceride diet (HT) and physical exercise (R) in ovariectomized rats (OVX).

There were two aspects of our investigation:

- Does 12-week exercise affect HO activity in aorta and heart, estrogen deficiency and HT diet?
- How does 12-week exercise affect the inflammatory parameters (IL-6, TNF-α, MPO activity) on HT diet and in the lack of estrogen?

3.METHODS

3.1.The effects of physical exercise on the cardiovascular system in male rats

3.1.1. Animals and Experimental Design

In our first series of experiments we studied male Wistar rats, which were randomly assigned to control and exercising groups. The exercising animals were placed individually into cages fitted with a running wheel and were allowed free access to the wheel for 24h per day for 6 weeks. The exercising protocol, defined as a voluntary wheel-running model, was selected in an effort exclude the additional stress associated with forced exercise protocols. During the exercising period, the average running distance was 3.91 ± 1.27km/day/animal. Control rats were placed in standard holding cages without a running wheel for the same period. All animals were housed in a temperature-controlled facility (23◦C) maintained on a 12:12 hlight-dark cycle with food and water provided ad libitum.

3.1.2.Measurement of MMP-2 Activity

Metalloproteinase activity (64kDa és 72kDa) in the serum and coronary effluent (CF) was detected by gelatin zymography and expressed results as intensity × mm2.

3.1.3. Measurement of surviving aorta ring contraction in rat

The isometric tension was measured through the transducer, which was connected to an ISOSYS computerized program system. We examined the

contraction as a response to 2.0 μg/ml arginine vasopressin (AVP) and results are expressed as the pressure of aorta ring (g/mg aorta ring).

3.1.4. Measurement of Basal Blood Pressure

The animals were anaesthetized with 30.0% urethane. Then the right carotid artery cannulation was connected to the HAEMOSYS computerized complex hemodynamic analysis system to record the mean blood pressure.

3.1.5. Determination of heart perfusion according to Langendorff

10-20 minutes prior to cervical dislocation the animals received heparin injection, then their hearts were placed to a Langendorff perfusion column. Heart perfusion as a response to AVP (1 μg) was measured following a stabilization period of 15 minutes. Results are expresed in % as compared to the basal value.

3.1.6. Experimental angina model evoked by adrenalin and phentolamine

Mean arterial blood pressure and surface II. ECG was measured and analyzed with the aid of HEMOSYS computerized system. The changes in ST depression was used for determining heart ischaemia. In epinephrine-phentolamine model, a single dose of epinephrine (10.0 μg/kg), and after 30 s an α-adrenoceptor antagonist phentolamine (15.0 mg/kg) was administered into the tail vein of the animals. Changes in ECG and blood pressure were monitored simultaneously.

3.1.7. Infarct Size Determination

Infarct size was measured after regional ischemia induced by left anterior descending artery (LAD) occlusion ex vivo. Infarct size was calculated as the percentage of the area at risk.

3.1.8. Statistics

The results shown in the figures are expressed as means ± S.E.M. Differences between groups were determined with two-tailed Student's *t*-test and *P* values less than 0.05 were considered a significant.

3.2. The effects of exercise training and high triglyceride diet in an estrogen depleted rat model

3.2.1. Animals and Experimental Design

In our second experiment 10-week-old female Wistar rats were divided into two groups and were subjected to sham-operation (SO group) or bilateral ovariectomy (OVX group) under anesthesia. After a 4-week recovery period serum levels of estrogen were measured using a quantitative enzyme-linked immunosorbent assay according to the manufacturer's directions to verify estrogen deficiency. At the end of week 4, both OVX and SO rats were randomly subdivided into two subgroups that would differ in the amount of regular daily activity and in diet for the next 12 weeks. The exercising (R) subgroups of rats were placed in cages mounted with running wheels. Training was defined as a voluntary wheel-running model, allowing the animals free access to the wheel for 24 h per day for 12 weeks. Physical activity was an inherent part of the animals' daily routine. Indeed, we have previously demonstrated that the average running distance for rats placed in cages mounted with running wheels highly exceeds that for the control group placed in standard holding cages during the exercising period. The animals in both

groups were maintained on either phytoestrogen free (CTRL) or high triglyceride 40% fat content phytoestrogen free chow (HT) and had free access to water. At the end of the 12-week exercising period the animals were killed.

3.2.2. Determination of aortic and cardiac HO enzyme activity

We measured the amount of bilirubin, which is formed during the conversion of heme using spectrophotometric method. The results are expressed in nM bilirubin/h/mg protein.

3.2.3.Measurement of HO-1, TNF-α and IL-6 concentrations

The concentrations of TNF-α and IL-6 of the plasma were determined by quantitative enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's directions. The values are expressed in ng/mg protein (HO-1) pg/ml protein (IL-6) and ng/ml protein (TNF-α).

3.2.4.Cardiac and aortic MPO activity

MPO activity was detected by O-dianisidine dihydrochloride and hydrogen peroxide. It was measured spectrophotometrically and the values are expressed as mU/mg protein.

3.2.5.Statistical analysis

Normality of data sets were verified with Shapiro-Wilk test. Data of the eight experimental groups (for each biological variable investigated) were compared using one-way ANOVA (with Geisser-Greenhouse correction) followed by Tukey post-hoc-test. Alterations in body weight were compared using linear regression supplemented with an F test. P values less than 0.05 were considered as significant. All data are presented as mean ± SEM.

4.RESULTS

4.1.The effects of physical exercise on the cardiovascular system in male rats

The results of our study showed that 6-week voluntary wheel-running provides protection against I/R injury by reducing the myocardial infarct size in rats. The voluntary exercise diminished the ST segment depression and therefore reduced the susceptibility of the heart against ischemia. Further examination we utilized AVP, which can regulate the hemodynamic parameters by inducing moderate vasoconstriction. In a response to 1.0 μg AVP, a significant improvement was observed in the heart perfusion after exercise training. After voluntary exercise training, no differences were observed in aorta contraction and basal blood pressure between the control and the exercising animals. In this current study, wheel-running exercise decreased the activities of 64 kDa MMP-2 from the heart into the coronary effluent as a consequence of 30-minute LAD occlusion. Our zymography analyses revealed that 6 weeks of voluntary exercise training in male rats decreased the serum levels of the 64- and the 72 kDa MMP-2 isoforms compared to the control group. Similar to the decreases in the MMP-2 values, the infarct size was also reduced. In summary, such a training period seems to be a potent stimulus for functional recovery in favour of the myocardial perfusion and ischemic susceptibility of the heart.

4.2. Effects of exercise training and high triglyceride diet in an estrogen depleted rat model

Through the measuring of the aortic and cardiac HO activity and HO-1 concentrations, we found a significant decrease in all of the NR OVX and SO HT groups. However, the 12-week physical exercise was able to improve HO-1 values. Plasma IL-6 concentrations were higher in the NR OVX animals and rats fed with HT diet compared to SO CTRL rats. TNF-α concentrations were significantly elevated in the NR OVX groups. 12 weeks of exercise significantly reduced the concentrations of both TNF-α and IL-6 compared to the NR group. The activity of MPO was significantly increased as a result of OVX and HT diet, however voluntary wheel-running exercise was able to restore the elevated values.

5.DISCUSSION

In summary, our results proved the adaptive and cardioprotective effects of voluntary wheel-running exercise. The reduced activity of serum MMP-2 might be a contributing factor to the multiple adaptation mechanisms of 6-week physical exercise. The fact that the infarct size is decreased suggests that the release of MMP-2 into the perfusate could be a part of the observed cardioprotective effects. Moreover, our training program was able to improve the angina susceptibility of the heart and causes functional recovery detected by AVP-induced perfusion changes.

In the second study we clarified how estrogen depletion and overnutrition related to the disruption of HO enzyme system and thus to an elevation in inflammatory processes. Decrease in the concentration and activity of HO-1 and increase in the levels of TNF-α, IL-6 and MPO enzyme activity are the major causes of life expectancy in postmenopausal women. Similar changes were observed on the effect of HT diet. However, 12 weeks of voluntary wheel-running exercise seems to be a potential non-pharmacological strategy to improve the deteriorated HO and to ease inflammatory parameters.

LIST OF PUBLICATIONS

Médea Veszelka, MTMT code: 10053090

Publications connected to the thesis:

Médea Veszelka[*], Csaba Varga[*], Krisztina Kupai, Denise Börzsei, Zoltán Deim, Renáta Szabó, Szilvia Török, Dániel Priksz, Rudolf Gesztelyi, Béla Juhász, Zsolt Radák, and Anikó Pósa.

The effects of exercise training and high triglyceride diet in an estrogen depleted rat model: the role of the heme oxygenase system and inflammatory processes in cardiovascular risk, *Journal of Sports Science and Medicine, 2018*

[*] ***co-first author***

IF: 1,99

Pósa A, Szabó R, Kupai K, Baráth Z, Szalai Z, Csonka A, **Veszelka M,** Gyöngyösi M, Radák Z, Ménesi R, Pávó I, Magyariné Berkó A, Varga C: ***Cardioprotective effects of voluntary exercise in a rat model: role of matrix metalloproteinase-2***, *Oxid Med Cell Longev*, 2015;2015:876805., doi: 10.1155/2015/876805.

IF: 4,593

Cumulative impact factor of the publications based on the dissertation:*6,583*

Publications not directly related to the PhD thesis

Pósa A, Szabó R, Kupai K, Berkó AM, **Veszelka M**, Szűcs G, Börzsei D, Gyöngyösi M, Pávó I, Deim Z, Szilvássy Z, Juhász B, Varga C:

Cardioprotective Effect of Selective Estrogen Receptor Modulator Raloxifene Are Mediated by Heme Oxygenase in Estrogen-Deficient Rat, *Oxid Med Cell Longev,* 2017;2017:2176749. doi: 10.1155/2017/2176749

IF: 4,593

Al-awar Amin, Kupai Krisztina, **Veszelka Médea**, Szűcs Gergő, Attieh Zouhair, Murlasits Zsolt, Török Szilvia, Pósa Anikó, Varga Csaba

Experimental Diabetes Mellitus in Different Animal Models

JOURNAL OF DIABETES RESEARCH 2016: Paper 9051426. 12 p. (2016)

IF: 2.717

Pósa A, Szabó R, Csonka A, **Veszelka M,** Magyariné Berkó A, Baráth Z, Ménesi R, Pávó I, Gyöngyösi M, László F, Kupai K, Varga C:

Endogenous estrogen-mediated heme oxygenase regulation in experimental menopause*, Oxid Med Cell Longev*, 2015;2015:429713., doi: 10.1155/2015/429713.

IF: 4,593

Kupai K, Szabó R, **Veszelka M,** Awar AA, Török S, Csonka A, Baráth Z, Pósa A, Varga C: ***Consequences of exercising on ischemia-reperfusion injury in type 2 diabetic Goto-Kakizaki rat hearts: role of the HO/NOS system.***, *Diabetol Metab Syndr.* 2015 Oct 6;7:85. doi: 10.1186/s13098-015-0080-x.

IF: 2.347,

Pósa A, Szabó R, Kupai K, Csonka A, Szalai Z, **Veszelka M**, Török S, Daruka L, Varga C: ***Exercise training and calorie restriction influence the metabolic parameters in ovariectomized female rats***, *Oxid Med Cell Longev*, 2015;2015:787063., doi: 10.1155/2015/787063.

IF: 4,593

Zita Szalai, Krisztina Kupai, **Médea Veszelka**, Anikó Pósa, Szilvia Török, Anikó Magyariné Berkó, Zoltán Baráth, Ferenc A László, Csaba Varga

Novel features of the rat model of inflammatory bowel disease based on 2,4,6-trinitrobenzenesulfonic acid - induced acute colitis

ACTA BIOLOGICA SZEGEDIENSIS 58:(2) pp. 127-132. (2014)

IF: 0.66

Cumulative impact factor of the publications related to the dissertation: ***12,193***

Cumulative impact factor: ***18,776***