Ph.D. thesis

Cardiovascular changes induced by hormone deficiency and their therapeutic potential in a rat model

Denise Matvon

Supervisors:

Anikó Pósa Ph.D., habil., associate professor

Renáta Szabó Ph.D., senior lecturer

Ph.D School in Biology

University of Szeged

Faculty of Science and Informatics

Department of Physiology, Anatomy and Neuroscience

2022

Szeged

List of abbreviations

cGMP: cyclic guanosine monophosphate

Comt: catechol-O-methyltransferase

CVD: cardiovascular diseases

Esm1: endothelial cell specific molecule-1

GOT: glutamic oxaloacetic transaminase

GPT: glutamic-pyruvic transaminase

GSH: reduced glutathione

GSSG: oxidized glutathione

HO: heme oxygenase

HO-1: heme oxygenase 1

LAD: left anterior descending coronary artery

MPO: myeloperoxidase

Ogn: osteoglycine

Pcp4: Purkinje protein 4

TNF-α: tumor necrosis factor alpha

TTC: 2,3,5-Triphenyltetrazolium chloride

Introduction

Cardiovascular dysfunctions are the leading cause of morbidity and mortality worldwide, thus a deeper understanding of the underlying hormonal, inflammatory and oxidative processes may help to develop new therapeutic strategies. It is well known that young men are more likely to suffer from cardiovascular diseases (CVD) than women of a similar age, although after menopause this aforementioned cardiovascular risk of women increases sharply. Cholesterol-derived sex hormones have a wide range of biological effects. Androgens and estrogens play a vital role in cardiovascular health through their interactions with steroid receptors expressed in the cardiovascular system and through their metabolic effects as well. Their expression may decline with age and as a result of various pathological conditions, a process that can be associated with the risk of CVD. Estrogen deficiency in case of women as well as testosterone deficiency in men increase the risk of developing carbohydrate and lipid metabolism disorders and visceral obesity, thus the risk of atherosclerosis and hypertension. At the same time, studies support that physiological expression of sex hormones contributes to the prevention of CVD through modulating inflammatory and antioxidant processes. It can effectively reduce the level of inflammatory cytokines and increase numerous antioxidants in the body. The main antioxidant enzymes include superoxide dismutase (SOD), glutathione (GSH), and heme oxygenase (HO). Biliverdin and bilirubin, which are formed during the reaction catalysed by the HO enzyme, are considered non-enzymatic antioxidants. Besides its antioxidant effects, the HO system has also been shown to have anti-inflammatory properties. Unfortunately, the endogenous antioxidant defense mechanisms diminish as a result of age-associated hormonal, inflammatory and oxidative processes, consequently the incidence of noncommunicable chronic diseases increases. In addition to the sex differences in the etiology and clinical presentation of CVD, sedentary lifestyle is definitely a major predisposing factor as well. Elimination of risk factors and applying therapeutic strategies of co-morbidities and complications resulting from hormone deficiency, can effectively reduce mortality and morbidity in both sexes. Hormone replacement therapies can be a means of preventive therapy, but regular moderate-intensity exercise can also play a vital role in the prevention of CVDs.

Aims

Research focusing on the reduction of aging-related hormonal processes and their pathological consequences could open up important preventive and therapeutic targets for CVDs. The aim of our studies was to map the potential cardioprotective effects mediated by testosterone and to investigate whether cardiovascular changes with age can be mitigated by lifestyle changes in different sexes.

1. In our first series of experiments, we aimed to clarify the differences observed in systemic inflammatory and antioxidant parameters between young and aged male rats in response to testosterone deficiency or replacement.

2. In our second series of experiments, we sought to answer the question:

- How do regular exercise alter the protein concentrations that possess a pivotal role in cardiovascular homeostasis in aged male and female rats?

- How can exercise change the vulnerability of the heart to ischemia?

Materials and methods

1. Testosterone-mediated antioxidant and anti-inflammatory effects in the heart

Experimental protocol

In our first series of experiments 10-week-old and 24-month-old male Wistar rats were used. Within the young and old animal groups, castrated and sham-operated rats were separated. During surgical castration testes and epididymis were excised, while during sham surgery, testes of the rats were not removed. After a two-week resting period following the surgical procedures, one part of the animals received daily oral treatment with 2.5 mg/kg bw of

ciproterone acetate to completely block androgen production, while the other part of the animals received weekly *intramuscular (i.m.)* injections of testosterone undecanoate at a dose of 8 mg/kg bw. After the 6-week experimental period, glutathione (GSH+GSSG) levels, cyclic guanosine monophosphate (cGMP) expression, tumor necrosis factor-alpha (TNF-α) concentration, myeloperoxidase (MPO) and heme oxygenase (HO) enzyme activities, and HO-1 isoform concentration were determined from cardiac left ventricles. Together with these measurements, serum testosterone, glutamate-oxaloacetate aminotransferase (GOT) and glutamate-pyruvate aminotransferase (GPT) concentrations were also detected.

Measurement of cardiac HO activity

The activity of HO enzyme was determined by spectrophotometric method based on the amount of bilirubin formed. HO activity was expressed as nmol/hour/mg protein.

Measurement of cardiac GSH+GSSG content

The cardiac GSH+GSSG levels were measured by spectrophotometry at 405 nm and the resulting GSH levels were expressed as nmol/mg protein.

Determination of cardiac HO-1, TNF- α and cGMP contenctrations

The levels of HO-1, TNF-alpha, cGMP were determined by ELISA following the manufacturer's instructions. HO-1 levels were expressed as ng/mg protein, TNF-α values were given as pg/mg protein, while cGMP concentrations were defined as pmol/mg protein.

Measurement of cardiac MPO activity

Cardiac MPO activity was detected spectrophotometrically. Values are expressed as μU/mg protein.

Determination of serum testosterone, GOT, GPT concentrations

Serum testosterone levels were determined using an Immulite 2000XPi chemiluminescent immunoassay, while GOT, GPT concentrations were determined using a Biolis 24i (Siemens) clinical chemistry analyzer. GOT and GPT values were expressed as U/L, while testosterone level was defined as ng/dl.

Protein determination by the Bradford method

The protein concentration of the samples was measured by spectrophotometry at 595 nm using the Bradford method. Results were defined as μg protein/ml.

Statistical analysis

Results are expressed as mean ± S.E.M. Differences between groups were determined by one-way ANOVA with Tukey's post-hoc test. Differences were considered significant at $p \leq 0.05$.

2. Effect of exercise on age-related changes in cardiac function in male and female rats

Experimental protocol

In our second series of experiments, 20-month-old female and male Wistar rats were used. Animals were divided to running and non-running groups. The non-exercising control animals (CTRL) were housed in standard cages, while running animals were kept in a running wheel-equipped cage. The exercise protocol was defined as recreational exercise, as the animals were allowed to run at any time of the day, 24 hours a day, according to their own biorhythms. At the end of the 12-week experimental period, the animals were terminated and their hearts were removed. The organs were either immediately subjected to *ex vivo* isolated cardiac perfusion or separated into right and left ventricles and placed in a freezer at -80°C, until biochemical analyses.

Implementation of ischemia-reperfusion injury by Langendorff perfusion system

Hearts were suspended on a Langendorff perfusion column via the aorta. After initiation of retrograde perfusion of the hearts, ischemic injury was induced by the ligation of left descending coronary artery (LAD) for 30 min. Ligation was followed by 120 min of reperfusion. The hearts were then perfused with 1% Evans blue solution and placed in a -20 °C freezer until further analysis.

Determination of infarcted area

Frozen hearts were sliced perpendicular to the apico-basal axis and incubated in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) and 10% formalin solution. The heart slices were evaluated between two glass slides using Image J program, and the size of infarcted area was expressed as a percentage of the area at risk.

Determination of cardiac Comt, Ogn, Pcp4 and Esm1 concentrations

The levels of Comt, Ogn, Pcp4 and Esm1 were determined by ELISA according to the manufacturer's instructions. Comt, Ogn and Pcp4 levels were expressed as pg/µg protein, while Esm-1 levels were expressed as pg/mg protein.

Protein determination by the Bradford method

The protein concentration of the samples was measured by spectrophotometry at 595 nm using the Bradford method. Results were defined as µg protein/ml.

Statistical analysis

Results are expressed as mean ± S.E.M. Differences between groups were determined by Student's t-test. Differences obtained were considered significant at $p \leq 0.05$.

Results

1. Testosterone-mediated antioxidant and anti-inflammatory effects in the heart

As a result of testosterone deficiency, a significant increase in the cardiac activity of MPO and in the level of TNF-α was detected compared to control animals. Furthermore, our results underpin that testosterone deprivation also results in a reduced antioxidant protection of the heart, as evidenced by the activity of HO enzyme measured from the left ventricle, the level of HO-1 and GSH. Elevated levels of GOT and GPT parameters measured from serum also demonstrate the harmful effects of testosterone deficiency. Hormone replacement therapy significantly reduced oxidative damage and inflammation in the hearts of both young and aged animals. Based on our results, it can be concluded that testosterone therapy protected the cardiovascular system through multiple targets.

2. Effect of exercise on age-related changes in cardiac function in male and female rats

In our second series of experiments, we demonstrated that regular physical activity has beneficial effects on the cardiovascular system, as evidenced by the concentration of proteins associated with cardiovascular risk and the size of the infarcted area. While a significant decrease in Comt and Ogn proteins was observed in the hearts of male running rats, the concentration of Pcp4 and Esm-1 proteins increased in the hearts of both sexes. Consistent with these results, the extent of ischemic injury in response to LAD ligation was also greatly reduced in running animals. The differences between the results of female and male animals are likely to be as a consequence of the genomic effects manifested via different steroid receptor. Therefore, further studies are needed to elucidate the exact underlying mechanisms of the results observed.

Summary

- In male animals, partial or absolute testosterone deficiency reduced the biological activity of HO pathway-associated anti-inflammatory and antioxidant end-products, which contributed to the elevation of inflammatory parameters.

- Our results suggest that in our hormone-deficient models, pharmacological reuptake of testosterone contributed greatly to normal cardiac function by inducing antioxidant and anti-inflammatory processes via the HO pathway.

- However, in addition to pharmacological therapy, regular moderate intensity exercise can also reduce cardiovascular risk factors associated with aging in both sexes. The hormonal changes and deficiencies associated with aging are a high risk factor for cardiovascular disease; however, with proper attention and lifestyle changes, life expectancy can be significantly improved.

List of publications:

MTMT code of Denise Matvon: 10062102

Publications directly related to the PhD thesis

Szabó, R., **Börzsei, D.*,** Kupai, K., Hoffmann, A., Gesztelyi, R., Magyariné Berkó, A., Varga, C., & Pósa, A. (2019). ***Spotlight on a New Heme Oxygenase Pathway: Testosterone-Induced Shifts in Cardiac Oxidant/Antioxidant Status***, Antioxidants (Basel, Switzerland), 8(8), 288. https://doi.org/10.3390/antiox8080288

co-first author

IF: 4,520

Börzsei, D. *, Priksz, D., Szabó, R., Bombicz, M., Karácsonyi, Z., Puskás, L. G., Fehér, L. Z., Radák, Z., Kupai, K., Berkó, A. M., Varga, C., Juhász, B., & Pósa, A. (2021). ***Exercise-mitigated sex-based differences in aging: from genetic alterations to heart performance.*** American journal of physiology. Heart and circulatory physiology, 320(2), H854–H866. https://doi.org/10.1152/ajpheart.00643.2020

*** co-first author***

IF: 4,026

Publications not directly related to the PhD thesis

Börzsei, D. *, Sebestyén, J., Szabó, R., Lesi, N. Z., Pálszabó, A., Pálszabó, P., Szász, A., Priksz, D., Juhász, B., Veszelka, M., Turcsán, Z., Deim, Z., Varga, C., Pósa, A. (2022) ***Resveratrol as a Promising Polyphenol in Age-Associated Cardiac Alterations,*** https://doi.org/10.1155/2022/7911222

***co-first author**

IF: 6,543

Börzsei, D. *, Szabó, R., Hoffmann, A., Harmath, A., Sebestyén, J., Osman, J., Juhász, B., Priksz, D., Varga, C., & Pósa, A. (2021). ***Multiple Applications of Different Exercise Modalities with Rodents***. Oxidative medicine and cellular longevity, 2021, 3898710. https://doi.org/10.1155/2021/3898710

***first author**

IF: 6,543

Börzsei, D.*, Szabó, R., Hoffmann, A., Veszelka, M., Pávó, I., Turcsán, Z., Viczián, C., Kupai, K., Varga, C., & Pósa, A. (2020). ***Distinct Approaches of Raloxifene: Its Far-Reaching Beneficial Effects Implicating the HO-System***. Biomolecules, 10(3), 375. https://doi.org/10.3390/biom10030375

***first author**

IF: 4,694

Szabó, R., **Börzsei, D.*,** Karácsonyi, Z., Gesztelyi, R., Nemes, K., Berkó, A. M., Veszelka, M., Török, S., Kupai, K., Varga, C., Juhász, B., & Pósa, A. (2019). ***Postconditioning-like effect of exercis: new paradigm in experimental menopause.*** American journal of physiology. Heart and circulatory physiology, 316(2), H400–H407. https://doi.org/10.1152/ajpheart.00485.2018

*** co-first author**

IF: 3,569

Osváth, P., Szűcs, M., **Börzsei, D**., Szabó, R., Lesi, Z. N., Turcsán, Z., Veszelka, M., Sebestyén, J., Juhász, B., Priksz, D., Varga, C., & Pósa, A. (2022). ***Andrological Aspects of Exercise: Moderate Swimming Protects against Isoproterenol Induced Testis and Semen Abnormalities in Rats***. Antioxidants (Basel, Switzerland), 11(3), 436. https://doi.org/10.3390/antiox11030436

IF: 6,313

Szabó, R., Szabó, Z., **Börzsei, D**., Hoffmann, A., Lesi, Z. N., Pálszabó, P., Pálszabó, A., Dvorácskó, S., Gesztelyi, R., Kupai, K., Priksz, D., Juhász, B., Altmayer, A., Varga, C., & Pósa, A. (2022)**. *Potential Implications of Rimonabant on Age-Related Oxidative Stress and Inflammation.*** Antioxidants (Basel, Switzerland), 11(1), 162. https://doi.org/10.3390/antiox11010162

IF: 6,313

Szabó, R., **Börzsei, D.,** Hoffmann, A., Lesi, Z. N., Gesztelyi, R., Juhász, B., Szebeni, G. J., Osman, J., Sebestyén, J., Nagy, A., Szegedi, S., Varga, C., & Pósa, A. (2021***). Lifestyle-Induced Redox-Sensitive Alterations: Cross-Talk among the RAAS, Antioxidant/Inflammatory Status, and Hypertension***. Oxidative medicine and cellular longevity, 2021, 3080863. https://doi.org/10.1155/2021/3080863

IF: 6,543

Szabó, R., Hoffmann, A., **Börzsei, D**., Kupai, K., Veszelka, M., Berkó, A. M., Pávó, I., Gesztelyi, R., Juhász, B., Turcsán, Z., Pósa, A., & Varga, C. (2021). Hormone Replacement Therapy and Aging: A Potential Therapeutic Approach for Age-Related Oxidative Stress and Cardiac Remodeling. Oxidative medicine and cellular longevity, 2021, 8364297. https://doi.org/10.1155/2021/8364297

IF: 5,076

Szabó, R., **Börzsei, D.,** Szabó, Z., Hoffmann, A., Zupkó, I., Priksz, D., Kupai, K., Varga, C., & Pósa, A. (2020). A Potential Involvement of Anandamide in the Modulation of HO/NOS Systems: Women, Menopause, and "Medical Cannabinoids". International journal of molecular sciences, 21(22), 8801. https://doi.org/10.3390/ijms21228801

IF: 4,556

Szabó, R., Ménesi, R., H Molnár, A., Szalai, Z., Daruka, L., Tóth, G., Gardi, J., Gálfi, M., **Börzsei, D.**, Kupai, K., Juhász, A., Radács, M., László, F. A., Varga, C., & Pósa, A. (2019). New Metabolic Influencer on Oxytocin Release: The Ghrelin. Molecules (Basel, Switzerland), 24(4), 735. https://doi.org/10.3390/molecules24040735

IF: 3,06

Varga, C., Veszelka, M., Kupai, K., **Börzsei, D.,** Deim, Z., Szabó, R., Török, S., Priksz, D., Gesztelyi, R., Juhász, B., Radák, Z., & Pósa, A. (2018). ***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 & medicine, 17(4), 580–588.

IF: 1,774

Szabó, R., Karácsonyi, Z., **Börzsei, D.,** Juhász, B., Al-Awar, A., Török, S., Berkó, A. M., Takács, I., Kupai, K., Varga, C., & Pósa, A. (2018***). Role of Exercise-Induced Cardiac Remodeling in Ovariectomized Female Rats***. Oxidative medicine and cellular longevity, 2018, 6709742. https://doi.org/10.1155/2018/6709742

IF: 4,593

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 Mediatedcby Heme Oxygenase in Estrogen-Deficient Rat***, Oxid Med Cell Longev, 2017;2017:2176749. doi: 10.1155/2017/2176749

IF: 4,593

Cumulative IF: 72,716