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Original Article

Gender differences in nitric oxide and antioxidant response to physical stress in tissues of trained mice

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Abstract

Introduction: Nitric oxide (NO) is an important regulator involved in functional adaptation in all tissues to exercise, as shown in recent studies. The aim of this short-term study was to evaluate the hypothesis that the important factor of higher performance of trained females during exhaustive exercise can be the interaction between physiological effect of nitric oxide and oxidant/antioxidant response.

Methods: Males and females of trained mice were divided into three groups: basal, fasting and prolonged exercise. Parameters of oxidant/antioxidant state, including nitric oxide and glutathione were measured in blood, muscle, liver, heart, kidney, brain, small intestine, adipose tissue and thoracic aorta. Females in this animal model had better performance than males during exhaustive exercise.

Results: Females showed greater basal levels of nitric oxide, total antioxidant status and glutathione peroxidase in most tissues evaluated. Compared to fasting levels, the net effects of prolonged exercise included lipoperoxidation in liver, brain and kidney, and nitrosative stress in liver, muscle and heart only in males. The decrement of glutathione without significant changes in its grade of oxidation was observed in liver, intestine and adipose tissue only in females, confirmed possible redistribution of reduced glutathione during prolonged exercise.

Conclusion: It is possible that the gender difference that existed in the performance of the animals during exhaustive exercise was determined by NO modulation of the oxidant/antioxidant response in tissues, and particularly of the redistribution of glutathione from the liver to other tissues. NO- induced vasodilatation can be beneficial for ischemic tissues during prolonged or exhaustive exercise.

Keywords:
Gender difference;
Oxidative stress;
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Antioxidant;
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Introduction

All the record times for exhaustive swimming both in open waters and in pools have been set by women. It is known that female rodents perform better during exhaustive swimming than males (Venditti et al., 1996). It is possible that this difference is due to the greater capacity of females to counteract oxidative stress in tissues (Ilhan et al., 2004). Trained animals acquire adaptations that increase their capacity to resist oxidative stress in prolonged and exhaustive

exercise in particular (Nakao et al., 2000; Ravi Kiran et al., 2004; Liu et al., 2000; Yamamoto et al., 2002). There is a gender difference in the oxidant/antioxidant response during the stress provoked by exhaustive or prolonged swimming in animal model (Lew et al., 1985; Pepe, 2001) but this information is controversial and dispersed in various publications with different types of animals, tissues, measured parameters, training and exercise protocols (Qian et al., 2001; Ay et al., 2007; Powers et al., 2014; Xiao et al., 2003).

Nitric oxide (NO) is a relatively weak radical compared to peroxynitrite, the product of the reaction of NO with the superoxide radical (contribution to oxidative stress), and there is a second messenger involved in adaptation process in different tissues to exercise, as shown in various studies (Simoncini et al., 2000; Xiao et al., 2003; Lo Faro et al., 2014). The data about effects of exercise and NO production in cardiopulmonary system was analyzed in a recent review (Nosarev et al., 2015). Authors suggest that training improves functioning of the cardiovascular system through an increase in NO bioavailability, potentiation of antioxidant defense and decrease in oxidative stress. Principal physiological effects of NO in short-term study are vasodilatation that can be beneficial for tissue during prolonged or exhaustive exercise. The information about interaction between response of NO and antioxidant defense to stress in trained rodents is limited and focused principally on the heart (Santos et al., 2011; Farah et al., 2013; Roof et al., 2015; Zhang, 2017). Changes in vascular reactivity of coronary artery and thoracic aorta was in wild-type and null mutant mice were investigated in a recent study (Castillo-Hernandez et al., 2017).

There is controversial data on the effects of NO on glutathione (GSH) synthesis, from increase in cell-culture studies (Kuo et al., 1996) to decrease *in vivo* studies (Minamiyama et al., 1996; Payabvash et al., 2006). GSH is an abundant endogenous antioxidant synthesized in great quantities in the liver. It can be mobilized through the blood flow to tissues in the event of an imbalance between the production of ROS and the antioxidant defense. The aim of the present short-term study was to explore, in different tissues and blood of trained mice, the gender differences in the basal levels of NO and oxidant/antioxidant status (including GSH) and the net response during the physical stress provoked by prolonged exercise.

Materials and methods

Animals and training protocol

All procedures and handling of the animals were in accordance with Mexican Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico City, Mexico). This study was approved by the Institutional Laboratory Use and Care Committee (CICUAL) of the Escuela Superior de Medicina of Instituto Politecnico Nacional, N° 02/28-08-2015. This national regulation is in accordance with the international ethics standards of the scientific journal. The professional training in swimming of humans also begins in age 7-8 years that was reflected in the age of mice in this animal model. Two-month-old males (n=21) and females (n=21) Balb/C mice were maintained in transparent plastic cages at 20-25°C on a 12h light/dark cycle with food and water available *ad libitum*. All animals were trained for 14 weeks for unforced swimming program (flotation). The training started with a 2-week adaption program with swimming sessions 3 times per week. The time of exercising was gradually increased from 10 to 60min per session. Afterwards, the mice continued to be trained 3 times a week (60min per session) for another 12 weeks. Compared to other rodent studies involving swimming training, we consider that the present protocol represents moderate exercise training. Water temperature was 32±2°C. Swimming sessions were carried out in transparent tanks divided into cells (25×25cm), one animal per cell. After each exercise session, mice were dried and returned to their cages. All sessions were conducted between 11am and 1pm to avoid variations due to the circadian cycle. The pilot study demonstrated that females in this animal model had better performance than males during exhaustive swimming (297±12min vs 252±16min, $P<0.01$).

Study design

Trained animals of the same sex were divided into three groups (n=7): basal, fasting and prolonged swimming exercise. The fasting and prolonged swimming both lasted 4 hours. This time was chosen for prolonged exercise because the time of exhaustive swimming was between 4.5 and 5 hours

in this animal model. The fasting subgroup was included because of the following two reasons: during 4 hours the animals were obviously fasting and can be gender difference in response to fasting, thus permitting us to eliminate the effects by comparing post-exercise with post-fasting levels (net effect of exercise). The basal measurement was made by sacrificing the mice at 11am.

Tissues and blood processing

Animals were sacrificed immediately after the respective procedure by diethyl ether anesthesia, which affects oxidant/antioxidant parameters to a lesser extent compared to other procedures. Liver, skeletal muscle from the hind leg (*vastus lateralis*), heart, brain, small intestine (lamina propria and mucosa), kidney and visceral adipose tissue were extracted and stored at -80°C to await analysis. Samples were obtained by placing a tissue in 30mmol cold phosphate buffer solution (pH: 7.2) and adding 0.1% of Triton 100 (1mg of tissue per 10µl of buffer). Tissues were homogenized and centrifuged at 10000rpm for 15min (4°C) and the supernatants were stored at -80°C for no more than two weeks before being analyzed. Whole blood samples with heparin (for enzymes measurements) and plasma were stored at -80°C.

The Cayman chemical assay kit was employed for measurement of total proteins (TP, No.704002), nitric oxide (NO, nitrates/nitrites, No.780001), total reduced glutathione (GSH, No.703002), oxidized GSH (GSSG, No. 703002) and the activity of catalase (CAT, No. 707002) in homogenates of tissues. In the tissues with high presence of blood (liver, heart and kidney) homogenates was treated by Amicon Ultra-0.5 centrifugal filter devices (30K) before the determination of NO. The Randox chemical kits were adapted in order to measure total antioxidant status (TAS, No. NX2332) (Miller et al., 1993) as well as the activity of total superoxide dismutase (SOD, No.SD125) and total glutathione peroxidase (GPx, No. RS504) in homogenates of the tissues. Products of lipoperoxidation (thiobarbituric acid reactive substances, TBARS) were also measured (Hicks et al., 1995). The values of TBARS, NO, TAS, GSH and GSSG were expressed as nmol/mg of TP and enzymes in U/mg of TP for SOD, CAT and GPx. The degree of GSH oxidation was calculated: GSSG/2GSH×100%.

The corticosterone (CST, Enzo Life Sciences, No. ADI-901-097) level in both genders and estradiol (EST, Assay Designs, No. 0460818) level in females was determined in plasma by the enzyme-linked immunosorbent assay (ELISA). TAS, NO (nmol/ml), lactate (mmol/l, Randox, LC2389) were also measured in plasma. Enzymes activity and hemoglobin (HB) were also measured in whole blood and were expressed as U/g HB.

Statistical analyses

After collection of data, statistical analysis was performed on SPSS. Data are presented as the mean±SD. Statistical evaluation was performed using analysis of variances (ANOVA) followed by Tukey post hoc test. A P value <0.05 was considered significant. We analyzed the Pearson bivariate correlation between the different basal parameters measured in the all tissues in both genders and P value <0.05 was considered significant.

Results

Basal levels

The comparison of basal levels of oxidant/antioxidant parameters in all tissues of both genders were presented in Table 1. The basal levels of NO, TAS, GSH and GPx were presented according to the gender differences in majority of tissues in this animal model. The basal level of NO was significant higher in females than in males for all tissues, except brain (P=0.059). The level of NO in small intestine (lamina propria and mucosa) of females was at least 2 times more compared to other tissues and males. The maximal difference was presented in heart; 8 times more in females than males.

Basal value of TAS in liver and kidney was higher for at least 10 times in both genders, compared to other tissues. The females expressed significantly greater basal level of TAS in various tissues (except liver, heart and mucosa intestinal), while kidney of females showed lower level, compared to males. The basal level of GSH in liver for both genders was at least 50 times bigger than it was in other tissues without significant gender difference. Compared to males, in females a lower basal level of GSH was found in muscle and brain, while a higher level in heart, small intestine and adipose tissue (AT) was observed. Basal level of GPx in liver was at least 5 times higher

Table 1: Gender differences in basal levels.

	LIVER		MUSCLE		BRAIN		HEART	
	m	f	m	f	m	f	m	f
NO	3.6 ± 0.6	5.1 ± 0.7*	3.8 ± 1.1	6.3 ± 1.9*	3.8 ± 0.8	5.1 ± 1.1	0.8 ± 0.5	6.5 ± 4.0**
TBARS	2.0 ± 0.4	2.3 ± 0.6	3.5 ± 1.8	3.8 ± 1.1	8.1 ± 1.3	7.5 ± 0.8	2.8 ± 0.9	4.8 ± 2.3
TAS	3478 ± 989	3554 ± 312	83 ± 28	141 ± 37**	113 ± 19	174 ± 42*	116 ± 46	136 ± 71
SOD	83 ± 35	119 ± 29*	23 ± 13	27 ± 8	74 ± 18	75 ± 24	22 ± 9	22 ± 12
CAT	1166 ± 584	1266 ± 166	15 ± 8	48 ± 8**	33 ± 11	42 ± 19	61 ± 26	60 ± 13
GPx	1546 ± 678	2552 ± 143**	109 ± 26	518 ± 133**	94 ± 37	311 ± 68**	288 ± 116	489 ± 175**
GSH	219 ± 73	242 ± 23	5.3 ± 1.9	1.8 ± 0.8*	1.7 ± 0.9	0.86 ± 0.45*	0.60 ± 0.16	1.2 ± 0.8*
GPx/GSH	7 ± 1.5	10 ± 2.0	12 ± 3	277 ± 64**	93 ± 50	324 ± 93**	482 ± 284	393 ± 220
GSSG %	0.6 ± 0.3	0.3 ± 0.1*	6.2 ± 1.6	0.8 ± 0.5**	6.5 ± 4.8	1.4 ± 0.8*	2.2 ± 1.5	O.7 ± 0.1*
	KIDNEY		LAMINA PROPRIA		MUCOSA		ADIPOSE TISSUE	
NO	1.9 ± 0.5	6.3 ± 4.4*	4.2 ± 0.9	13.8 ± 4.2**	4.9 ± 1.9	13.1 ± 3.2*	1.4 ± 0.3	3.6 ± 0.8**
TBARS	2.0 ± 0.3	1.7 ± 0.3	3.2 ± 1.0	2.1 ± 0.6	2.4 ± 1.8	2.6 ± 0.4	1.3 ± 0.4	1.0 ± 0.1
TAS	2596 ± 782	1605 ± 376*	289 ± 21	349 ± 39C	262 ± 21	272 ± 19	25 ± 6	52 ± 15*
SOD	12.3 ± 1.2	12.1 ± 2.6	78 ± 9	98 ± 13*	92 ± 26	74 ± 7	0.2 ± 0.1	0.4 ± 0.2*
CAT	332 ± 102	254 ± 89	19 ± 3	21 ± 5	52 ± 16	42 ± 9	11 ± 2.4	9 ± 3.1
GPx	241 ± 104	582 ± 98*	319 ± 44	537 ± 115**	328 ± 664	493 ± 53*	44 ± 13	42 ±24
GSH	0.30 ± 0.01	0.29 ± 0.09	0.59 ± 0.10	1.04 ± 0.13*	0.55 ± 0.03	1.04 ± 0.24*	0.39 ± 0.21	1.08 ± 0.32**
GPx/GSH	569 ± 290	2064 ± 594**	522 ± 94	450 ± 60	465 ± 139	410 ± 67	121 ± 39	40 ± 13**
GSSG %	4.3 ± 1.1	3.9 ± 2.0	0.6 ± 0.3	0.4 ± 0.4	0.6 ± 0.4	0.4 ± 0.4	0.8 ± 0.3	12.1 ± 8.6*

*P<0.05 and **P<0.01 for gender differences. m: male; f: female; TBARS: thiobarbituric acid reactive substances; NO: nitric oxide; TAS: total antioxidant status; GSH: total reduced glutathione; GPx: total glutathione peroxidase; SOD: total superoxide dismutase; CAT: catalase and GSSG: oxidized GSH.

Units: NO, TAS, GSH and TBARS: nmol/mg proteins; GPx, SOD and CAT: U/mg proteins; GPx/GSH: U/nmol ×1000; GSSG%: GSSG/2GSH ×100. The basal values of measured parameters in different tissues of both genders (mean±SD).

compared to other tissues, with upper levels in females versus males. Basal levels of GPx were higher in females in all tissues (except AT), especially in muscle (7.4 times more).

It was not found gender differences in basal levels of TBARS for all tissues. Compared to other tissues, brain showed highest basal levels of TBARS (nmol/mg proteins) for both genders. Basal levels of total SOD showed gender differences in the liver and the lamina propria (higher in females). AT had minimum activity of this enzyme. There was at least 240 times more basal level of CAT in the liver and 10 times more in the kidney than in other tissues for both genders. It was observed higher basal levels of CAT in the muscles of females, compared to males.

The GPx/GSH ratio can reflect the efficiency of participation of GPx in hydroperoxide elimination that was recovered by GSH after its antioxidant action. Lower values of this ratio in liver of both genders were determined by higher levels of reduced GSH. The gender difference of this ratio was drastic in muscle; the basal value for females was at least 20 times higher, compared to males. Basal levels of this ratio in females were higher also in brain, kidney and lower only in AT. The basal levels of the degree of the GSH oxidation (GSSG%= GSSG/2GSH ×100) were 2-4 times higher than in males, compared to females in muscle and heart, while females showed higher levels of GSSG% in adipose tissue only. Finally, the females began the fasting and prolonged exercise with higher basal levels of antioxidant defense and elevated levels of NO in most of the tissues, especially in muscle and heart.

Fasting levels

It was a surprise that relatively short fasting (4h) affected significantly basal levels measured parameters in tissues of both genders but these changes were different in males and females (Table 2). The decrease of measured parameters during fasting was dominated in the response of females and an increase in males. The significant decrement of GSH in liver without change in GSSG% during fasting it was observed only in females. While males showed a decrease of GSH in muscle during fasting and a significant increase in the kidney and the mucosa. The basal concentration of GSH in the muscle of males was at least 10 times higher compared to the kidney and the mucosa (Table 1). The fast was drastically increased GSSG% in brain of males that coincided with a decrease of GSH and confirmed its importance in antioxidant defense in this organ of males. While in females an increase of GSSG% in muscle and heart was coincided to

Table 2: Direction of significant changes during fasting in different tissues of both genders.

during fasting	TBARS		NO		TAS		SOD		CAT		GPX		GSH		GSSG%	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
liver				D										D		
muscle					D					I			D		D	I
heart							D			I		D				I
kidney		I		D								D	I		I	
lamina propria																
mucosa			I		I								I			
brain	D											D			I	
adipose tissue				D										I		
blood																

D: a decrease and I: an increase, significant compared fasting to basal levels; empty: no significant change. m: males, f: females, lamina propria and mucosa of small intestine; TBARS: thiobarbituric acid reactive substances; NO: nitric oxide; TAS: total antioxidant status; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GSH – total reduced glutathione and GSSG: oxidized GSH. GSSG%= GSSG/2GSH ×100

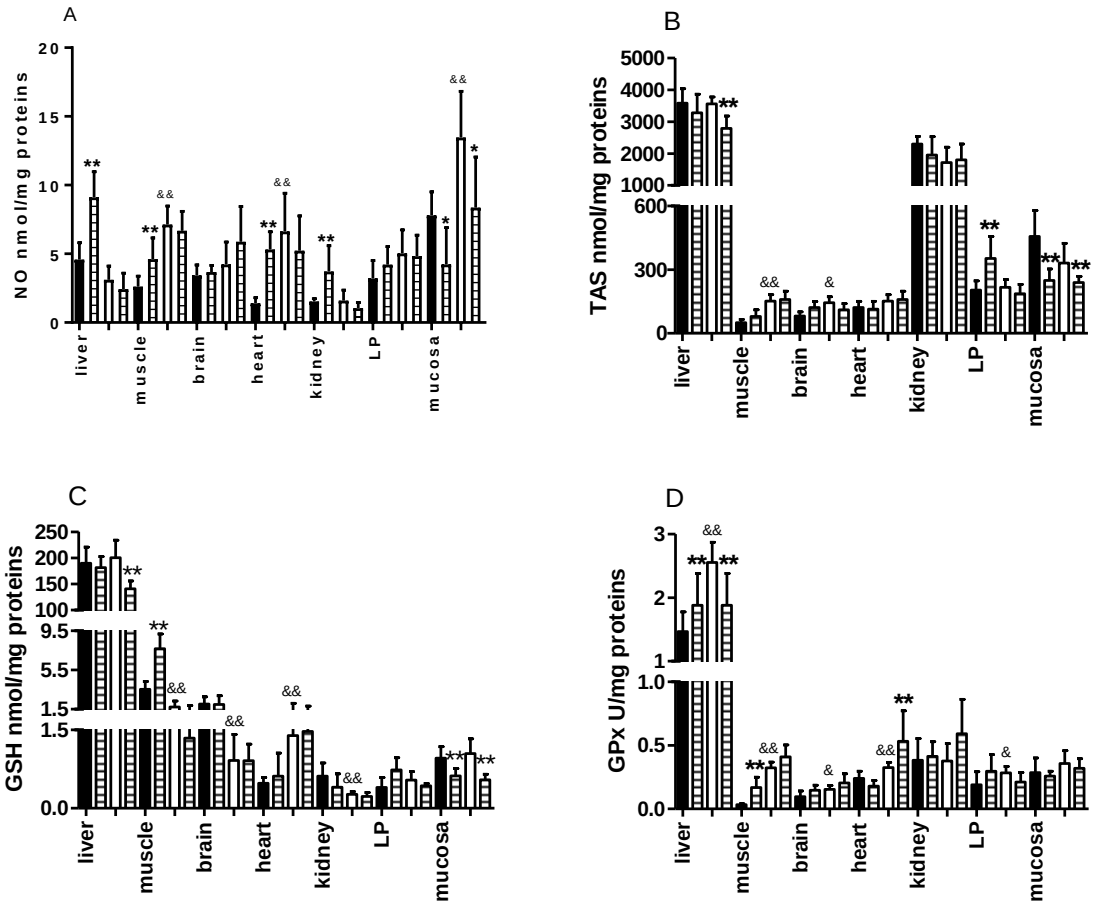

Fig.1. Net response to prolonged exercise of NO (A), TAS (B), GSH (C) and GPx (D) in different tissues. Black columns, fasting levels of males and white columns for females; Pattern columns, post-exercise levels in both genders. LP: lamina propria of small intestine. $^{*}P<0.05$ and $^{**}P<0.01$ compared exercise levels to fasting levels. $^{\&}P<0.05$ and $^{\&\&}P<0.01$ gender differences in fasting levels.

Table 3: Gender differences in blood.

	Basal		Fasting		Exercise	
	male	female	male	female	male	female
TBARS	4.8 ± 2.2	6.5 ± 3.0	4.6 ± 1.7	8.2 ± 4.2	4.8 ± 1.5	5.0 ± 1.6 #
NO	8.2 ± 3.1	16.8 ± 4.9**	7.6 ± 2.6	18.7 ± 2.1**	5.3 ± 3.0	13.0 ± 6.3**
TAS	749 ± 285	732 ± 283	715 ± 285	761 ± 488	600 ± 257	846 ± 263
SOD	2515 ± 234	2437 ± 756	2266 ± 174	2347 ± 552	2331 ± 615	2846 ± 681
CAT	197 ± 53	140 ± 59	182 ± 64	133 ± 50	193 ± 89	127 ± 36
GPx	1231 ± 168	1489 ± 171*	1299 ± 208	1460 ± 270	1328 ± 125	1596 ± 136*
HB	14.6 ± 0.6	14.5 ± 1.1	15.0 ± 0.2	13.8 ± 1.0	14.4 ± 0.5	14.0 ± 1.1
LA	3.4 ± 0.8	6.2 ± 1.5**	3.2 ± 2.6	4.8 ± 1.2 **	2.6 ± 1.6	2.8 ± 0.9 ##
CST	24.9 ± 12.9	30.5 ± 10.1	23 ± 11	23 ± 15.1	47.5 ± 5.5 ##	53.1 ± 8.5 ##
EST		127 ± 28		132 ± 31		90 ± 27##

The values of measured parameters (mean±SD) are indicated for basal, post-fasting and post-exercise levels of both genders. *P<0.05 and **P<0.01, gender differences in basal, post-fasting and post-exercise levels. ##P<0.01, compared post-exercise to post-fasting levels of the same gender (net response). TBARS: thiobarbituric acid reactive substances; NO: nitric oxide; TAS: total antioxidant status; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; HB: hemoglobin; LA: lactate; CST: corticosterone and EST: estradiol. Units: nmol/ml (TBARS, NO and TAS), U/g HB (enzymes), g/dl (HB); mmol/l (LA); ng/ml (CST) and pg/ml (EST).

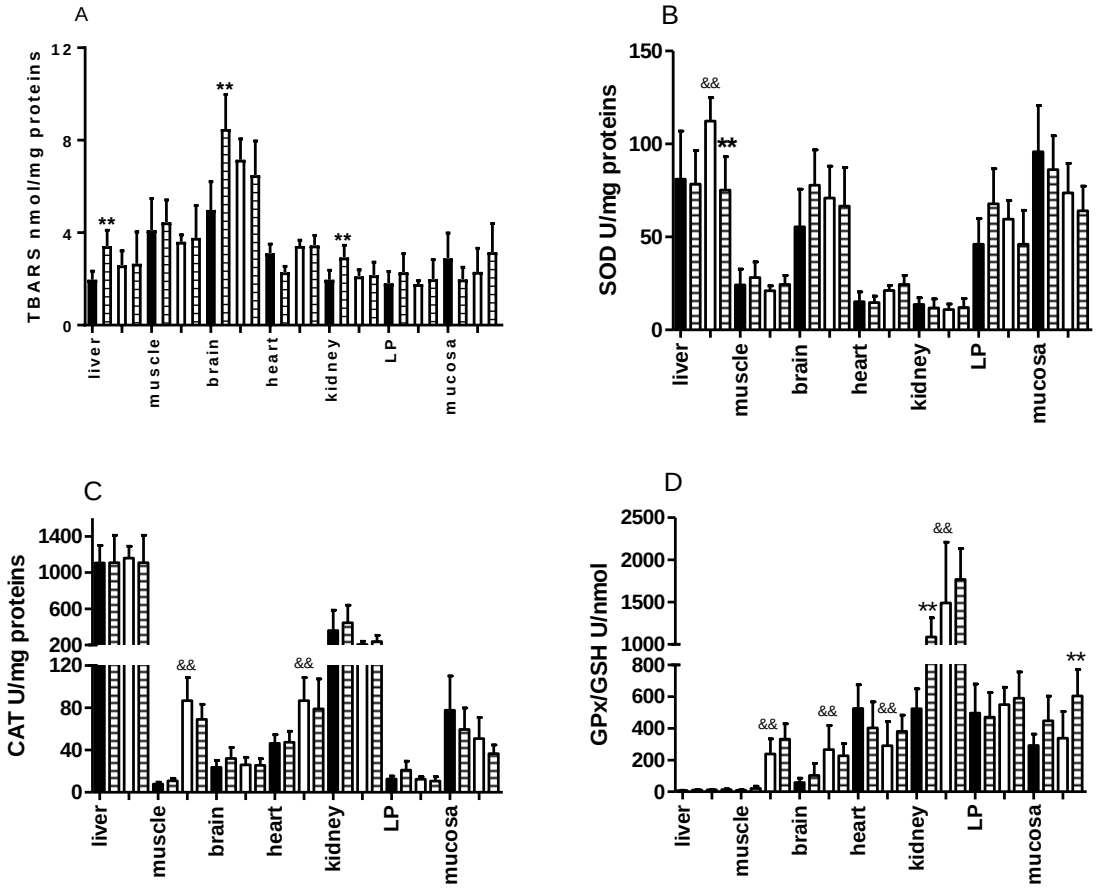

Fig.2. Net response to prolonged exercise of TBARS (A), SOD (B), CAT (C) and the ratio GPx/GSH (D) in different tissues. Black columns, fasting levels of males and white columns for females; Pattern columns, post-exercise levels in both genders. LP: lamina propria of small intestine. *P<0.05 and **P<0.01 compared exercise levels to fasting levels. &P<0.05 and &&P<0.01 gender differences in fasting levels.

decrease in levels of GPx and an increase of CAT in these tissues. The ratio GPx/GSH was not affected significantly by fasting in all the tissues of both genders.

Net response during prolonged exercise

The net response of NO, TAS, GSH and GPx in different tissues was presented in Figure 1. The gender differences in fasting levels of NO (Fig. 1A) were observed in the muscle, the heart and the mucosa (higher levels in females). Compared exercise levels to fasting levels (net response), the liver, the muscle, the heart and the kidney showed an increase of NO in males only, while intestinal mucosa showed a decrease in both genders. The gender differences in fasting levels of TAS (Fig. 1B) was found in the muscle and the brain (higher in females). Net response of TAS during prolonged exercise included an increase in the lamina propria of males, a decrease in the liver of females, while the mucosa of both genders showed a decrease (similar to response of NO).

Gender differences in fasting levels of GSH (Fig. 1C) showed the muscle, the brain and the kidney (lower in females) and the heart (higher in females). The net response of GSH during prolonged exercise showed significant decrease in the liver, the mucosa and the adipose tissue only in females. Males showed strong increase of GSH in muscle and a decrease in mucosa. Gender differences in fasting levels of GPx (Fig. 1D) was observed in the liver, the muscle, the brain, the heart and the lamina propria of small intestine (higher in females). During prolonged exercise males expressed significant an increase of GPx activity in the liver while females a decrease in the liver and the muscle, and an increase in the heart.The net response of TBARS, SOD, CAT and ratio GPx/GSH was presented in Figure 2. Net response of TBARS during prolonged exercise included an increase in the liver, the brain and the kidney in males only (Fig. 2A). Females contrarily did not show any changes of TBARS in all the tissues. SOD showed gender difference in fasting levels (higher in females) only in the liver (Fig. 2B). The net response of SOD during exercise showed a decrease only in the female's liver. Fasting duplicated the level of CAT in the muscle and the heart of females, resulting in gender differences that confirm the high sensibility of this enzyme in females to relatively short fasting (Fig. 2C). The level of CAT during exercise was not changed. The fast did not affect basal levels of ratio GSH/GSH in the tissues of both genders, maintaining higher levels in females in the muscle, the brain and the kidney, and lower level in the heart. Males showed an increase during prolonged exercise in the kidney, and females also in the mucosa (Fig. 2D), while the majority of tissues showed the stability of this ratio during exercise.

The lower fasting levels of GSSG% were observed in the muscle and brain of females compared to males (not presented). The net response of GSSG % during exercise showed strong decrease in muscle and brain, and an increase in heart of males. Females showed stability of this parameter during prolonged exercise in the majority of the tissues. The net response to exercise in adipose tissue was not presented because the lower levels of most of the parameters that were measured, compared to the other tissues, except GSH in females that was decreased significantly during exercise.

The oxidant/antioxidant, metabolic and hormonal response in the plasma and the whole blood was presented in the Table 3. When comparing basal values of males and females in the blood, they were higher for female mice, with a significant difference for NO and GPx. Basal level of lactate in females was double, compared to males, confirming higher basal level of anaerobic glycolysis in females. The similar pattern of gender differences was maintained after fasting (NO and lactate) or prolonged exercise (NO and GPx). Plasmatic corticosterone in females decreased during fasting compared to basal levels, while there was no change in the estradiol level during fasting in females. There was a similar increase in plasmatic the corticosterone during prolonged exercise in both genders (compared to fasting levels), confirmed the same grade of physical stress during exercise in this animal model.

We analyzed the bivariate correlation coefficients between the different basal parameters measured in both genders in the various tissues presented in the Table 4, excluding the liver and the kidney tissue (where the majority of parameters were over 10-fold greater than in other tissues). Multiple bivariate correlations between basal parameters measured in different tissues is an indicator that there is a close relation between some parameters in all the tissues. In the case of males, there was a correlation between

Table 4: Pearson correlation analysis.

Pearson correlation in tissues		
parameters	males	females
TBARS-GSSG%	0.77*	
GPx-GSSG%		-0.87*
NO-TAS	0.72	0.96**
NO-CAT		
NO-SOD	0.73*	0.75*
TAS-SOD	0.85*	0.89**
TAS-CAT		
TAS-GPx	0.74*	
CAT-GPx		0.71

Bivariate correlation coefficients (starting with 0.7) between values of basal parameters, measured in both genders in the various tissues (except liver and kidney). $^{*}P<0.05$ and $^{**}P<0.01$ for statistical significance of the correlation coefficients.

the changes in TBARS and the degree of oxidation of GSH (GSSG %) in all the tissues, confirming the role of this parameter as an indicator of oxidative stress in the males of this animal model. The females showed a negative correlation between GPx and GSSG % in all tissues, confirming the known relation between GPx and GSSG. It is important that the level of NO positively correlated with TAS and SOD in both genders, which suggests a close relation between these parameters. Variations in TAS positively correlated with the changes in enzymes of both genders (except with CAT). This coincides with our previously reported data in the human ocular tissue (Kormanovski et al., 2014) and confirms that enzymes together with nonenzymatic antioxidants contribute to TAS.

Discussion

In this short-term study it was explored the hypothesis that higher performance of trained females in exhaustive exercise is due to their greater capacity to counteract oxidative stress in multiple tissues that includes the interaction between the antioxidant defense and the physiological effect of NO (vasodilatation). The oxidant/antioxidant response during the physical stress of trained rodents in short-term study depends on at least three key factors: 1) the basal levels of the antioxidant defense and NO in the tissues; 2) the capacity of the mobilization of endogenous resources to the strengthening of antioxidant defense and the production of NO during a stress and 3) possible modulation of antioxidant response by physiological effect of NO.

It was not found significant gender differences in basal levels of TBARS in majority of tissues that confirmed similar level of oxidative stress (lipoperoxidation) in tissues of both genders at rest. In this study it was observed a higher basal levels of NO in most tissues and the small intestine and plasma of females showed double basal level of NO compared to males. Females showed greater basal levels than males of TAS and GPx in the majority of the analyzed tissues and GPx in blood. Each of them, in the case of females, has at least one other parameter (besides GPx) of the basal antioxidant defense with a greater value than that found in males. The participation of GPx in elimination of hydroperoxide depends on the concentration of GSH that recovers the activity of enzyme during its antioxidant activity. Females showed by higher basal levels of GSH in the heart and the intestine compared to males, but lower in muscle and brain that probably was determined by highest basal levels of GPx in these tissues. Drastically higher basal ratio GPx/GSH in the muscle of females, determined by higher basal level of GPx, and lower level of GSH, compared to males. A similar situation was observed in brain and kidney of females. Therefore, females started prolonged exercise with a higher basal levels of NO and the antioxidant defense including GSH in most tissues.

Despite the decrease in estradiol in the plasma of females during prolonged exercise, decrement in oxidative stress in females was only observed in the blood, as evidenced by a decrease of TBARS and NO and coincided to decrease of lactate. At the same time it was observed an increase of GPx in females only. There was controversial data about antioxidant protection mechanism of estrogens. The pros and cons of this relation were discussed (Tiidus and Enns, 2009). It is possible that the mechanisms of the protective effect of estrogens, can be indirect through the up-regulation of NO synthases (NOS) (Simoncini et al., 2000; Prorock et al., 2003). But interaction of estrogens with antioxidant system and activation of NOS was investigated principally in long-term studies (Siow et al., 2007).

The increase of NO during prolonged exercise was

observed in most tissues of males only that coincided with their lower basal levels. The liver, the muscle, the heart and the kidney of males showed greater increase (approximately 100%) that probably also reflects nitrosative stress. The lipoperoxidation appearance during prolonged exercise was observed also in the liver, the brain, the kidney of males and was not present in females. This data confirmed a higher capacity of females to prevent oxidative stress during exercise in tissues in this animal model that coincided with the elevated basal levels of antioxidant defense and NO. Both genders showed the relative stability of TAS, SOD and CAT in the tissues during prolonged exercise, while GPx showed an increase during exercise principally in the liver and the muscle in males, while in females a decrease in liver and increase in the muscle and the heart.

Only females showed during prolonged exercise the decrement in GSH in liver, the intestinal mucosa and adipose tissue, did not present any significant change in its grade of oxidation that coincided with a stability of GSH in the muscle, the brain as well as the heart and it can reflect the stress-induced redistribution of GSH from these tissues in females. The decrement of GSH concentration in the liver was approximately 50nmol/mg of total proteins that there at least 10 times higher of GSH level in majority of tissues analyzed. It is interesting that a significant decrease of GSH in the liver of females was observed also during fasting, that is a weaker trigger compared to prolonged exercise. It is possible that the possible mechanism of liberation of GSH from the liver in the blood flow in females is more sensible compared to males and the level of NO can be important in this sensibility.

In a study (Payabvash et al., 2006) was found that increased synthesis of NO in liver of endotoxemic mice (males) caused decrease of hepatic GSH synthesis, GPx activity and inhibition of nitric oxide synthesis prevented both of this changes. In our study, prolonged exercise was not affected elevated level of NO in liver of females and diminished GPx activity, while in males contrarily level of NO resulted 3 times lower with the same level of GPx. But females showed significant decrement in GSH concentration in liver during exercise, and males not. The liberation of GSH from liver can be contrary process to its synthesis, probably it is necessary inhibition of synthesis for the facilitation of its liberation in blood stream. Extracellular GSH is not able to pass through cell membranes. However, after being enzymatically divided into its constituent aminoacids, the latter was indeed able to cross this barrier. Once inside the cell, these aminoacids are available for the resynthesis of GSH. It is possible that the gender difference that existed in the performance of the animals during exhaustive exercise was determined by NO modulation of the oxidant/antioxidant response in tissues and particularly of the redistribution of GSH from the liver to other tissues.

Higher ratio GPx/GSH in the muscles can be beneficial for females in the elimination of hydroperoxide that coincided to the stability of lipoperoxidation during exercise in this gender. Something similar was observed in females' heart. During prolonged exercise females showed the stability of GSSG% in the majority of tissues, while males showed a decrease of elevated initial levels of this parameter in the muscle, the brain and the kidney. It is possible that the redistribution of GSH during exercise in females is the reason of this gender differences in the grade of GSH oxidation.

According to recent reviews (Lo Faro et al., 2014; Cortese-Krott et al., 2015; Zhang, 2017), NO is derived from endogenous NOS and exogenous sources including the nitrite reduction in the presence of acidosis (stomach), hypoxia or exercise. Values of NO production from specific sources that contribute to the bioavailability of NO remain unclear. NO regulates diverse downstream effector proteins through three principal mechanisms: sGC/cGMP/PKG-dependent phosphorylation, S-nitrosation and transnitrosation. S-nitrosoglutathione is one of main nitrosothiols are carriers and donors of NO in tissues (Ganzarolli, 2016).

There was controversial data about the modulation of NOS by training. Numerous studies suggest that exercise stimulates NO production by the modulation of activity of NOS and lower part of nitrites can be recycled to NO under determinant conditions (Laughlin et al., 2001; Gielen et al., 2005; Shur et al., 2013). There was evidence that training on swimming had no effect on changing in basal levels of eNOS in mice (Pellegrin et al., 2011) and nNOS in brain of rats (Park et al., 2012), but improves blood vessel function in porcine coronary arterioles (Xie et al., 2012) and potentiates vasodilatation and activation of

nNOS in lungs and aorta (Tatchum-Talom et al., 2000). Trained mice showed an increase in NO production on heart (Calvert et al., 2011). The data about effects of exercise and NO production in cardiopulmonary system was analyzed in a recent reviews (Nosarev et al., 2015). Authors suggest that training improves functioning of the cardiovascular system through an increase in NO bioavailability, potentiation of antioxidant defense and decrease in indicators of oxidative stress.

The paper of exercise-induced improvements of oxidant/antioxidant state parameters in cardioprotection was investigated recently (Powers et al., 2014; Roof et al., 2015). Interaction between ROS and NO was investigated also principally in the heart (Zhang et al., 2009; Santos et al., 2011; Zhang, 2017) and concluded that only lowering levels of ROS are not beneficial for myocyte contraction and it is necessary positive shift in nitrous-redox balance; an increase of NO and a decrease of ROS for restored contraction. In the study (Farah et al., 2013) was investigated whether modulation the level of eNOS during reperfusion could participate in the exercise-induced cardioprotection. Authors concluded that in the hearts of trained animals, eNOS uncoupling associated with the improved myocardial antioxidant capacity prevents excessive NO synthesis and limits the production the cytotoxic peroxynitrite. Despite eNOS uncoupling, exercised hearts had more S-nitrosylated proteins after early reperfusion and also less nitro-oxidative stress, compared to sedentary hearts. The information about this interaction in other tissues is limited.

Conclusion

In regard to the higher performance of females during exhaustive swimming in the current animal model, an important role may have been played by the interaction of the antioxidant response in tissues with the modulatory mechanisms of NO. The latter include the possible vasodilator effect of NO in ischemic tissues as well as the capacity of this radical to affect the redistribution of GSH from the liver to other tissues.

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Conflict of interest

The authors have no conflicts of interest and declare that this article has not been published.

References

Ay H, Topal T, Uysal, B, Ozler M, Oter S, Kokmaz A, et al. Time-dependent course of hyperbaric oxygen-induced oxidative effects in rat lung and erythrocytes. Clin Exp Pharmac Physiol 2007; 34: 787-91.

Calvert JW, Condit ME, Aragón JP, Nicolson CK, Moody BF, Hood RL. Exercise protects against myocardial ischemia–reperfusion injury via stimulation of β3-adrenergic receptors and increased nitric oxide signaling: role of nitrite and nitrosothiols. Circ Res 2011; 108: 144-58.

Castillo-Hernandez MC, Guevara-Balcazar G, Kormanovski A, Rubio Gayosso I, Coral-Vazquez RM. Changes in vascular reactivity of the coronary artery and thoracic aorta in the delta sarcoglycan null mutant mice. Physiol Pharmac 2017; 21: 322-30.

Cortese-Krott MM, Fernandez BO, Kelm M, Butler AR, Feelisch M. On the chemical biology of nitrite/sulfide interaction. Nitric Oxide 2015; 46: 14-24.

Farah, C, Kleindienst A, Bolea, G,Meyer G, Gayard S, Geny B, et al. Exercise-induced cardioprotection: a role for eNOS uncoupling and NO metabolites. Basic Res Cardiol 2013; 108: 389.

Ganzarolli de Oliveira M. S-nitrosothiols as platforms for topical nitric oxide delivery. Basic Clin Pharmacol Toxicol 2016; 119: 49-56.

Gielen S, Adams V, Linke A, Erbs S, Möbius-Winkler S, Schubert A, et al. Exercise training in chronic heart failure: correlation between reduced local inflammation and improved oxidative capacity in the skeletal muscle. Eur J Cardiovasc Prev Rehabil 2005; 12: 393-400.

Hicks JJ, Medina-Navarro R. Inhibitory capacity of human serum on induced microsomal lipoperoxidation. Arch Med Res 1995; 26: 169-72.

Ilhan N, Kamanli A, Ozmerdivenli R, Ilhan N. Variable effects of exercise intensity on reduced glutathione, thiobarbituric acid reactive substances levels, and glucose concentration. Arch Med Res 2004; 35: 294-300.

Kormanovski A, Parra F, Jarillo-Luna A, Lara-Padilla E, Pacheco-Yepez J, Campos-Rodriguez R. Oxidant/antioxidant state in tissue of primary and recurrent pterygium. BMC Ophthalmol 2014; 14: 149-52.

Kuo PC, Abe KY, Schroeder RA. Interleukin-1-induced nitric oxide production modulates glutathione synthesis in cultured rat hepatocytes. Am J Physiol 1996; 271: C851-62.

Laughlin MH, Pollock JS, Amann JF, Hollis ML, Woodman CR, Price EM. Training induces nonuniform increases in eNOS content along the coronary arterial tree. J Appl

Physiol 2001; 90: 501-10.

Lew H, Pyke S, Quintanilla A. Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rats. FEBS Lett 1985; 185: 262-6.

Liu J, Yeo HC, Overvik-Doukl E, Hagen T, Doniger SJ, Chyu DW, et al. Chronically and acutely exercise rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 2000; 89: 21-8.

Lo Faro ML, Fox B, Whatmore JL, Winyard PG, Whiteman M. Hydrogen sulfide and nitric oxide interactions in inflammation. Nitric Oxide 2014; 41: 38-47.

Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond) 1993; 84: 407-12.

Minamiyama Y, Takemura S, Koyama K, Yu H, Miyamoto M, Inoue M. Dynamic aspects of glutathione and nitric oxide metabolism in endotoxemic rats. Am J Physiol 1996; 271: G575-81.

Nakao C, Ookawara S, Kizaki T, Oh-Ishi S, Miyazaki H, Haga S, et al. Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissue. J Appl Physiol 2000; 88: 649-54.

Nosarev AV, Smagliy LV, Anfinogenova Y, Popov SV, Kapilevich LV. Exercise and NO production: relevance and implications in the cardiopulmonary system. Front Cell Dev Biol 2015; 2: 73.

Park S, Shin J, Hong Y, Kim S, Lee S, Park K, et al. Forced exercise enhances functional recovery after local cerebral ischemia in spontaneously hypertensive rats. Brain Sci 2012; 2: 483-503.

Payabvash S, Ghahremani MH, Goliaei A, Mandegary A, Shafaroodi H, Amanlou M, et al. Nitric oxide modulates glutathione synthesis during endotoxemia. Free Radic Biol Med 2006; 41:1817-28.

Pellegrin M, Miguel-Alfonsi C, Berthelot A, Mazzolai L, Laurant P. Long-term swimming exercise does not modulate the Akt-dependent endothelial nitric oxide synthase phosphorylation in healthy mice. Can J Physiol Pharmac 2011; 89: 72-6.

Pepe H. The effects of gender and exercise on malondialdehyde, nitric oxide and total glutathione levels in rat liver. Afr J Pharmac Pharmac 2011; 5: 515-21.

Powers SK, Sollanek KJ, Wiggs MP, Demirel HA, Smuder AJ. Exercise-induced improvements in myocardial antioxidant capacity: the antioxidant players and cardioprotection. Free Radic Res 2014; 48: 43-51.

Prorock AJ, Hafezi-Moghadam A, Laubach VE, Liau JK, Ley K. Vascular protection by estrogens in ischemia-reperfusion injury requires endothelial nitric oxide synthase. Am J Physiol Heart Circ Physiol 2003; 284: H133-40.

Qian ZM, Xiao DS, Ke Y,Liao QK. Increased nitric oxide is one of causes of changes of iron metabolism in strenuously exercised rats. Am J Physiol Regul Integr Comp Physiol 2001; 280: R739-43.

Ravi Kiran T, Subramanyam MV, Asha Devi S. Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: relationship to swim intensity and duration. Comp Biochem Physiol B Biochem Mol Biol 2004; 137: 187-96.

Roof SR, Ho HT, Little SC, Ostler JE, Brundage EA, Periasamy M, et al. Obligatory role of neuronal nitric oxide synthase in the heart's antioxidant adaptation with exercise. J Mol Cell Cardiol 2015; 81: 54-61.

Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. Free Radic Biol Med 2011; 50: 777-93.

Shur F, Gehlert S, Grau M, Bloch W. Skeletal muscle function during exercise-fine-tuning of diverse subsystems by nitric oxide. Int J Mol Sci 2013; 14: 7109-39.

Simoncini T, Hafezy-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK. Interaction of estrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 2000; 407: 538-41.

Siow RC, Li FY, Rowlands DJ, Winter P, Mann GE. Cardiovascular target for estrogens and phytoestrogens: transcriptional regulation of nitric oxide synthase and antioxidant defense genes. Free Radic Biol Med 2007; 42: 909-25.

Tatchum-Talom R, Schulz R, McNeil IR, Khadour FH. Upregulation of neuronal nitric oxide synthase in skeletal muscle by swim training. Am J Physiol Heart Circ Physiol 2000; 279: H1757-66.

Tiidus PM, Enns DL. Point:Counterpoint: Estrogen and sex do/do not influence post-exercise indexes of muscle damage, inflammation, and repair. J Appl Physiol 2009; 106: 1010-2.

Venditti P, Piro MC, Artiaco G, Di Meo S. Effect of exercise on tissue anti-oxidant capacity and heart electrical properties in male and female rats. Eur J Appl Physiol Occup Physiol 1996; 74: 322-9.

Xiao DS, Jiang L, Che LL, Lu L. Nitric oxide and iron metabolism in exercised rats with L-arginine supplementation. Mol Cell Biochem 2003; 252: 65-72.

Xie W, Parker JL, Heaps CL. Effect of exercise training on nitric oxide and superoxide/H_2O_2 signaling pathways in collateral-dependent porcine coronary arterioles. J Appl Physiol 2012; 112: 1546-55.

Yamamoto T, Ohkuwa T, Itoh H, Sato Y, Naoi M. Effect of gender differences and voluntary exercise on antioxidant capacity in rats. Comp Biochem Physiol C Toxicol Pharmacol 2002; 132: 437-44.

Zhang QJ, McMillin SL, Tanner JM, Palionyte M, Abel ED, Symons JD. Endothelial nitric oxide synthase phosphorylation in treadmill-running mice: role of vascular signaling kinases. J Physiol 2009; 587: 3911-20.

Zhang YH. Nitric oxide signaling and neuronal nitric oxide synthase in the heart under stress. F1000Res 2017; 6: 742.