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Maternal exercise during pregnancy modulates mitochondrial function and redox status in a sex-dependent way in adult offspring's skeletal muscle

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Original Article

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Abstract

Maternal exercise has shown beneficial effects on mother and child. Literature confirm progeny's cognition improvement, and upregulation in neurotrophins, antioxidant network, and DNA repair system. Considering that there is a lack of information demonstrating the impact of maternal exercise on offspring's skeletal muscle, we aimed to investigate the mitochondrial and redox effects elicited by maternal swimming. Adult female Wistar rats were divided into three groups: control sedentary, free swimming, and swimming with overload (2% of the body weight). Exercised groups were submitted weekly to five swimming sessions (30 min/day), starting 1 week prior to the mating and lasting to the delivery. Gastrocnemius and soleus muscle from 60-day-old offspring were analyzed. Our results clearly showed a sex-dependent effect. Male soleus showed increased mitochondrial functionality in the overload group. Female muscle from the overload group adapted deeply. Considering the redox status, the female offspring delivered to overload exercised dams presented reduced oxidants levels and protein damage, allied to downregulated antioxidant defenses. We also observed an increase in the mitochondrial function in the gastrocnemius muscle of the female offspring born from overload exercised dams. Soleus from female delivered to the overload exercise group presented reduced mitochondrial activity, as well as reduced reactive species, protein carbonyls, and antioxidant network, when compared to the male. In conclusion, maternal exercise altered the redox status and mitochondrial function in the offspring's skeletal muscle in a sex-dependent way. The clinical implication was not investigated; however, the sexual dimorphism in response to maternal exercise might impact exercise resilience in adulthood.

Introduction

The prenatal period is one of the most important developmental periods in life, being considered a window of vulnerability.[1] Environmental interventions, such as physical exercise and diets, are able to alter the offspring's susceptibility to the development of diseases later in life.[1–3] In accordance with the Developmental Origins of Health and Disease (DOHaD) concept, the development of several diseases during adulthood is related to the intrauterine environment to which the fetus was exposed, and is directly influenced by the mother's lifestyle, diet, physiological aspects, drugs, and infections.[3–6]

It is well established that physical exercise during pregnancy is safe for both, mother and fetus.[7,8] The *American College of Sports and Medicine* (2017) and the *American College of Obstetrics and Gynecologists*[8] (2020) suggest that pregnant women should exercise at least 30 min a day at moderate intensity during pregnancy. Moreover, there is a consistent number of reports demonstrating that maternal exercise during pregnancy is not just safe, but also beneficial to mother and child.[3,9] Among these beneficial and protective effects, some studies reported increased hippocampal neurogenesis, enhanced memory, and learning capacity, upregulated antioxidant defenses, and mitochondrial biogenesis on the offspring's brain.[10–12]

Improvement in oxidative capacity allied to mitochondrial biogenesis is a known adaptation of endurance exercise in skeletal muscle.[13,14] Conversely, several noncommunicable diseases, like obesity, cardiovascular diseases, and diabetes mellitus type 2, present diminished oxidative capacity and mitochondrial dysfunction as a common feature.[14–17] Since skeletal muscle has an important role in the systemic metabolism homeostasis, it is critical to maintain healthy muscles and to control and prevent metabolic disorders.[18] Amorim, dos Santos[19] show that rats who

exercised at moderate intensity during pregnancy increased the oxygen consumption by skeletal muscle. We sought whether maternal muscle programming could reverberate in the offspring. Skeletal muscles are very plastic tissues; they can adapt depending on the exercise type, intensity, substrate availability, and even under pathological circumstances.[20,21] However, little is known about the effects of maternal exercise in the offspring skeletal muscle, mainly on what mitochondrial aspects are concerned.

Mitochondria are responsible not only just for the energy supply to the cells, but they are also the main source of reactive oxygen species (ROS).[22,23] It is well established that exercise increases the production of ROS by mitochondria due to the high ATP demand required for muscle contraction. When the antioxidant network collapses, ROS can cause damage to DNA, lipids, and proteins resulting in oxidative stress.[24] However, in the last decade, several studies demonstrated that moderate ROS levels play an important role in cellular signaling pathways, causing beneficial modulations like inducing the expression of enzymatic and non-enzymatic antioxidant defenses in several tissues.[6,12,24–26] Despite such evidence, there are no studies demonstrating that maternal exercise can affect the redox homeostasis in the offspring's skeletal muscle.

The aim of our study is to verify the effects of two different intensity protocols of swimming exercise during pregnancy in the offspring: 2-month-old male and female rats. Our hypothesis is that maternal exercise could improve the offspring's skeletal muscle mitochondrial function and redox status.

Methods

Animals and ethics

Male and female Wistar rats (90 days old) were obtained from the Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL), Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. The animal facility was under controlled light (12:12 h light/dark cycle), temperature (22 ± 1 °C), and humidity conditions (50–60%). Throughout the experiment, all animals had free access to a 20% (w/w) protein commercial chow and water *ad libitum*. All experimental procedures and animal care were conducted in accordance with the National Institutes for Health (NIH) guides for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and were approved by the : Local Ethics Commission of Universidade Federal do Rio Grande do Sul (CEUA/UFRGS; protocol number 33275). All efforts were made to minimize animal suffering and to keep their number at a minimum to demonstrate consistent effects.

Experimental design and exercise training model

The experimental design of the exercise protocol is demonstrated in Fig. 1. Female rats were divided into three groups: sedentary (control), free swimming exercise, and overload swimming exercise (Fig. 1a). The last group had a 2% (body weight) load addiction attached to the tail. The swimming protocol was previously used in our laboratory and was described by Marcelino et al..[12] Female rats from exercised groups swam 1 week prior to mating to habituate to the aquatic environment in a schedule of 5 days/week and 30 min/day. During the mating, two females were housed with one male. The pregnancy was confirmed by the presence of a vaginal plug and the conception day was noted as gestational day (GD) 0.

After the habituation, the free exercised and overload exercised groups swam throughout pregnancy, 5 days/week, lasting 30 min/day in a swimming pool (30 cm wide × 30 cm long × 90 cm deep) filled with 32 ± 1 °C water. The animals were left free to swim, being gently stimulated when not active; except the overload group, which naturally swam all the time. Control sedentary rats were immersed in the water, carefully dried, and returned to the housing boxes. The pregnant rats were weighed daily and the overload was increased in accordance with the rat body weight. This exercise protocol is considered as moderate intensity.

Pregnant females were housed individually on GD20 and allowed normal spontaneous vaginal delivery. We checked for birth twice a day (at 8 a.m. and 6 p.m.) to annotate the postnatal day (PD) 0. The offspring were weaned on PD21 and four rats, of the same gender, were housed per cage, according to the maternal intervention group. On PD60, males and females were euthanized by decapitation after a 4-hour stand fasting. Then, the soleus and lateral gastrocnemius muscles were isolated and used for further biochemical analyses (Fig. 1b).

Sample preparation

Flow cytometry assay

Flow cytometry was previously described in Marcelino et al.[12] Soleus and lateral gastrocnemius muscles were dissociated in PBS containing 1 mg% of collagenase IV (1:10 w/v). Dissociated cells were then filtered using a 40 µm pore size cell strainer (SPL Lifesciences Co., Naechon-Myeon Pocheon, South Korea), and then incubated at 37 °C with specific molecular probes. Cells were gated based on the FSC and SSC pattern of the sample cells and 20,000 events were acquired per sample in a FACScalibur flow cytometer (BD Biosciences, USA); a non-labeled sample was used as negative fluorescent control. Data were analyzed using software FlowJo.

Redox state parameters

Soleus and lateral gastrocnemius muscles were homogenized (1:10 w/v) in 20 mM sodium phosphate buffer, pH 7.4, containing 140 mM KCl, and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was used for the redox state assays.

Biochemical assays

Mitochondrial mass and membrane potential

Mitochondrial mass and membrane potential were assessed by flow cytometry using 100 nM MitoTracker® Green and 100 nM MitoTracker® Red, respectively, and incubated at 37 °C for 45 min with samples (Invitrogen, Molecular Probes, Eugene, OR, USA).[12]

Mitochondrial superoxide content

Mitochondrial superoxide was measured by flow cytometry using 1 µM MitoSOX® Red incubated at 37 °C for 20 min (Invitrogen, Molecular Probes, Eugene, OR, USA).[12]

Determination of total oxidants levels

The total content of oxidants was evaluated by incubating the samples with 240 µl of 2′,7′-dichlorofluorescein diacetate (H_{2D}CF-DA; Sigma Aldrich Co., St. Louis, MO, USA) (DCFH-DA) at 37 °C for 30 min in the dark. DCFH-DA is cleaved by cellular esterase and forms DCFH, which is oxidized by the reactive species present in the sample. The reaction can be measured fluorometrically at 488 nm excitation and 525 nm emission wavelength.[27,28]

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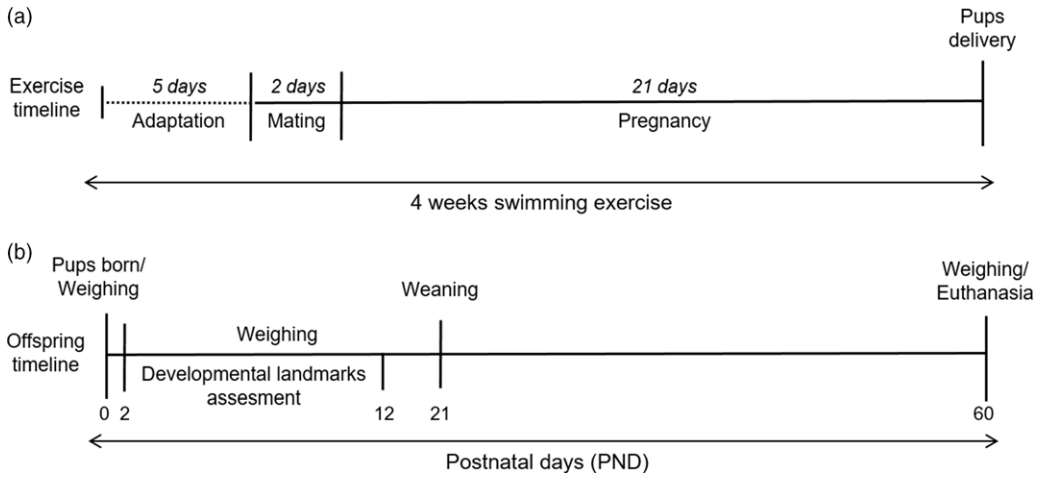

Fig. 1. Experimental designs. (a) Mothers exercise design timeline: the female rats were submitted to swimming exercise during pregnancy 30 mins/day, 5 days/week. (b) Offspring experimental design timeline: on PND 60, gastrocnemius and soleus muscles were dissected to further biochemical analyses.

Antioxidant enzymes activity

Superoxide dismutase

Superoxide dismutase (SOD) activity was evaluated by quantifying the inhibition of the autoxidation of epinephrine by SOD at 480 nm. Considering the protocol used in sample preparation, we measured total SOD activity, expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to one unit. The results were calculated as units/mg protein.[27]

Catalase

Catalase (CAT) activity was evaluated by measuring the decrease of hydrogen peroxide at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, and 10 mM potassium phosphate buffer, pH 7.0. One CAT unit is defined as 1 µmol of H_2O_2 consumed per minute and the specific activity as units/mg protein.[29]

Glutathione peroxidase

Glutathione peroxidase (GPx) activity was evaluated by the decrease of NADPH concentration at 340 nm. The reaction medium contained 100 mM potassium phosphate buffer, pH 7.7, containing 1 mM EDTA, 2 mM reduced glutathione, 0.15 U/ml glutathione reductase, 0.4 mM azide, 0.1 mM NADPH, and 0.5 mM tert-Butyl hydroperoxide as enzyme substrate. GPx unit is defined as 1 µmol of NADPH consumed per minute and the specific activity as units/mg protein.[30]

Glyoxalase 1

Glyoxalase 1 (GLO1) activity was measured by following the increase in the S-D-lactoylglutathione at 240 nm. The essay takes place in a reaction medium of 60 mM sodium phosphate buffer, pH 6.6, with reduced glutathione 0.01 M and methylglyoxal 0.01 M. GLO1 unit is defined as the amount of enzyme needed to catalyze the formation of 1 µmol of S-D-lactoylglutathione per minute, and the specific activity is represented as units/mg protein.[31]

Total reduced glutathione content

Initially, the proteins in the supernatant were precipitated with meta-phosphoric acid (1:1, v/v), and centrifuged at 5000 *g* for 10 min at 25 °C. Reduced glutathione (GSH) present in the supernatant reacts with the fluorophore o-phtaldialdehyde 7.5 mM prepared in 100 mM sodium phosphate buffer, pH 8.0, with 5 mM EDTA. The fluorescence was read at excitation and emission wavelengths of 350 and 420 nm, respectively, using the SpectraMax Gemini XS Fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA). A standard GSH curve ranging from 0.001 to 1 mM was prepared and a blank sample was performed in parallel. Data were expressed as nmol of GSH/mg protein.[32]

Protein carbonyl content

Carbonyl content was assessed according to Reznick and Packer.[33] Protein carbonyls react with dinitrophenylhydrazine forming dinitrophenylhydrazone, a yellow compound that was detected at 370 nm. For carbonyl determination, we first measured the protein content of the samples and used an interval of 0.7-1 mg of protein for carbonyl assay. Then, an equal volume of 20% TCA was added to the microtubes following 5 min incubation at 4 °C. After the 5-minute incubation, the samples were centrifuged at 4000 *g* for 5 min at 4 °C. Then, the supernatant was discarded and suspended with 100 µl of 0.2 M NaOH. Following, 100 µl of 2 M HCl was added to the control samples and 100 µl of 100 mM DNPH was added to the test samples, and both were incubated at room temperature for 1 h and shaken every 15 min during this period. Following the 1 h incubation, 100 µl of 20% TCA was added to the samples, and again incubated at 4 °C for 5 min and then centrifuged at 20,000 *g* for 5 min at 4 °C. The supernatant was discarded and the pellet was further washed three times with 500 µl of ethyl acetate:ethanol (1:1, v/v) and centrifuged again at 20,000 *g* for 5 min at 4 °C. The supernatant was discarded, and the pellets were resuspended in 8 M urea pH 2.3. The samples were incubated at 60 °C for 15 min and then centrifuged at 20,000 *g* for 3 min and the absorbance was measured at 370 nm. Protein carbonyl content was expressed as nmol/mg protein.

Total protein concentration

Protein concentration was measured according to Lowry et al..[34]

Statistical analysis

All data were tested for normality and analyzed by two-way ANOVA to evaluate the effect of two different independent variables, maternal exercise and offspring sex, followed by Tukey's *post hoc* test. GraphPad Prism 6.0 software was used to perform all statistical analyses. Results were expressed as mean ± standard error of the mean (SEM) and were considered statistically significant when $p < 0.05$.

Results

Maternal exercise increases offspring functional mitochondria in a sex-dependent way on different muscular fiber types

The number of functional mitochondria was accessed using Mitotracker Green and Mitotracker Red double-labeled events

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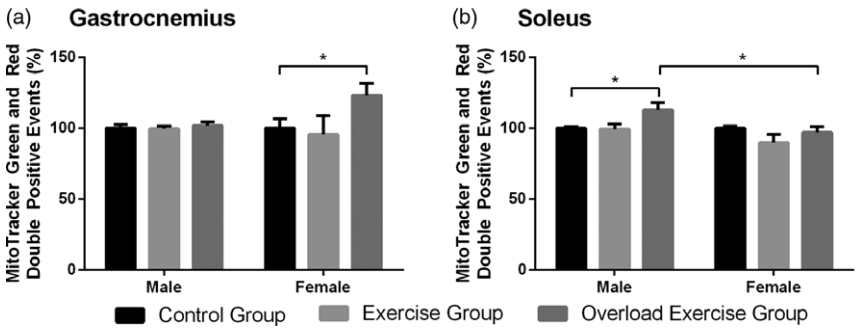

Fig. 2. Biochemycal analysis. Percentage of double-positive MitoTracker Green and MitoTracker Red-labeled cells, (a) Gastrocnemius muscle. (b) Soleus muscle. Data were evaluated by two-way ANOVA and Tukey's test was used to compare groups and sex. $^{*}p < 0.05$. Data were presented as mean + SEM (7–12 animals per group, from distinct breeds).

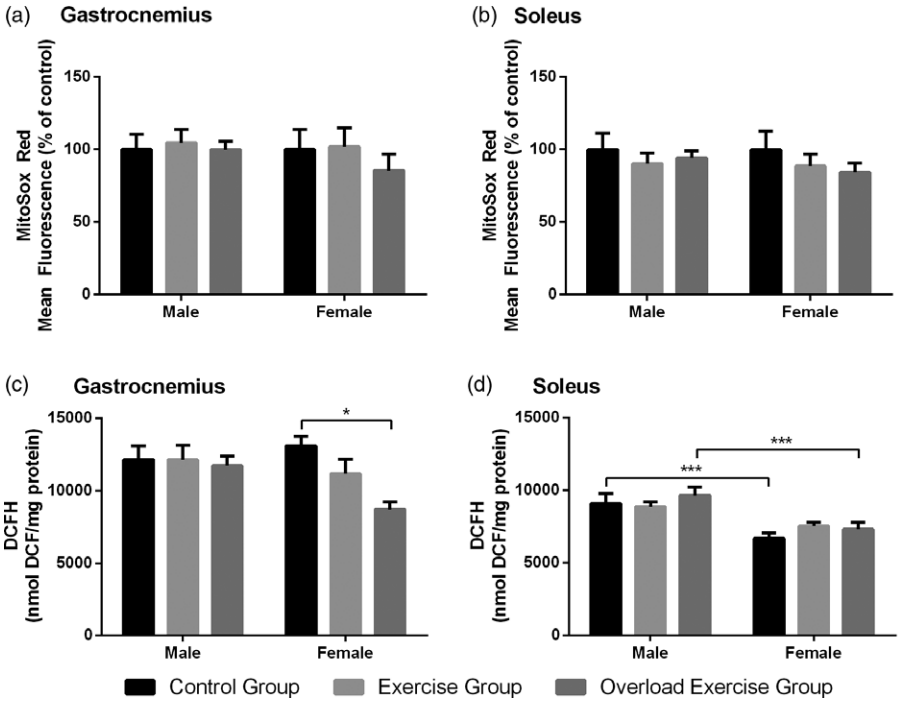

Fig. 3. Biochemycal analysis. Mitochondrial superoxide levels by MitoSox Red fluorescence was evaluated in (a) Gastrocnemius muscle and (b) Soleus muscle. DCFH oxidation was evaluated in (c) Gastrocnemius muscle and (d) Soleus muscle. Data were evaluated by two-way ANOVA and Tukey's test was used to compare groups and sex. $^{*}p < 0.05$, $^{***}p < 0{,}001$. Data were presented as mean + SEM (7–12 animals per group, from distinct breeds).

via flow cytometry. Double-labeled events indicate functional respiring mitochondria,[35] in this case, showing an increased number of functional mitochondria in the offspring skeletal muscle.

An increase in the mitochondrial mass and membrane potential was observed in the female gastrocnemius muscle from the overload exercised group when compared to the control females ($p < 0.05$). No alteration was identified in the males (Fig. 2a). On the other hand, the mitochondrial mass and membrane potential on soleus muscle were increased on male pups from the overload exercised group when compared with both the male control group ($p < 0.05$) and the female from overload exercised group ($p < 0.05$) (Fig. 2b).

Maternal exercise affects the redox status on lateral gastrocnemius muscle in offspring in a sex-dependent way

Despite no alteration in mitochondrial superoxide levels were observed on lateral gastrocnemius ($p > 0.05$) (Fig. 3a), we did observe a reduction in DCFH oxidation on female pups from the overload exercised group when compared to the female control group ($p < 0.05$) (Fig. 3c).

Considering the antioxidant enzymes, we observed a decreased activity of SOD (Fig. 4a) and GPx (Fig. 4C) in the female pups born from overload exercised dams versus males from the same group ($p < 0.01$). No alteration was observed on CAT activity ($p > 0.05$) (Fig. 4b). We also observed the inhibition of GLO1 activity in the female pups from the overload exercised group when compared to the female control group ($p < 0.05$), while no alterations were seen on male pups (Fig. 4d). GSH, the substrate for GPx and GLO1, was decreased in female pups from both exercised groups (regular and overload) when compared with the female control group ($p < 0.05$). Besides that, we observed a decreased GSH content in the female pups from the overload exercised group when compared with the male pups from the same group ($p < 0.05$) (Fig. 4e).

Carbonyl concentration, a marker for protein damage, was decreased in the female pups from the overload exercised group when compared to the control females ($p < 0.05$), while no alteration was identified on male pups (Fig. 6a).

Maternal exercise alters total oxidant production and redox status in a sex-dependent way on soleus muscle

Although no alteration on mitochondrial superoxide levels was observed on soleus muscle ($p > 0.05$) (Fig. 3b), DCFH oxidation was reduced in the female pups born from control and overload exercised groups when compared with the male pups in the same groups ($p < 0.001$) (Fig. 3d).

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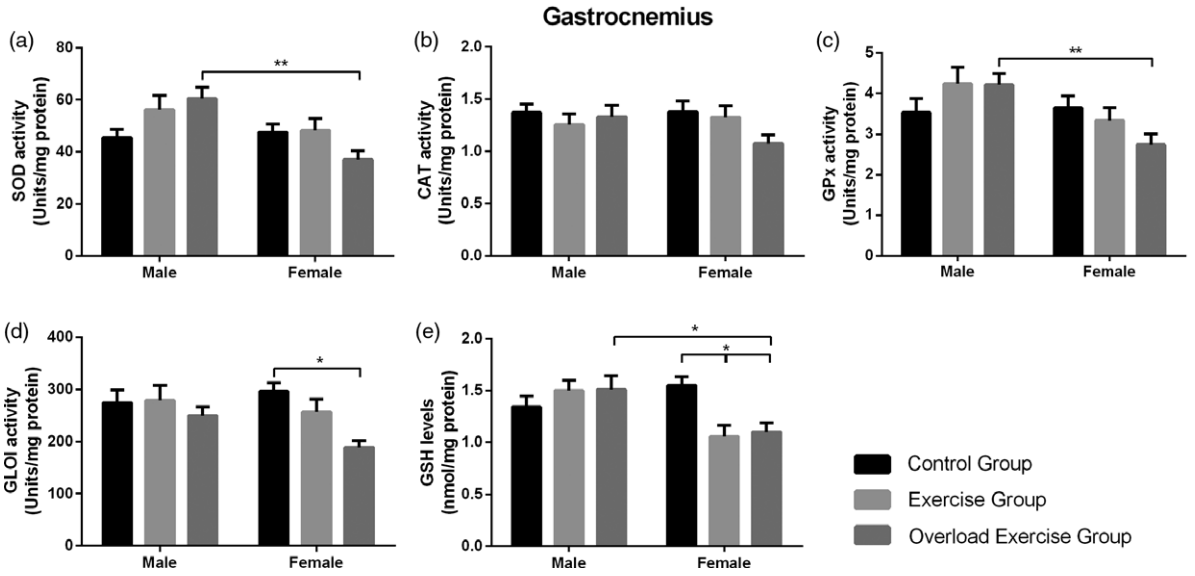

Fig. 4. Lateral gastrocnemius muscle antioxidant status analysis. Biochemical assays: (a) SOD activity, (b) CAT activity, (c) GPx activity, (d) GLO1 activity, and (e) GSH content. Data were evaluated by two-way ANOVA and Tukey's test was used to compare groups and sex. *$p < 0.05$, **$p < 0.01$. Data were presented as mean + SEM (9–12 animals per group, from distinct breeds).

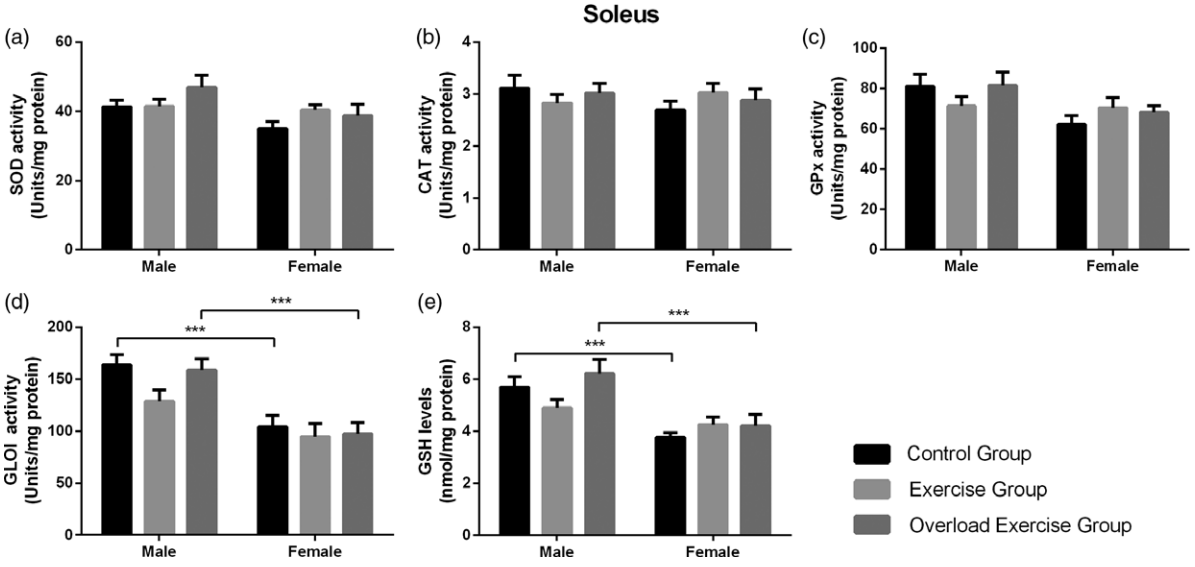

Fig. 5. Soleus muscle antioxidant status analysis. Biochemical assays: (a) SOD activity, (b) CAT activity, (c) GPx activity, (d) GLO1 activity, and (e) GSH content. Data were evaluated by two-way ANOVA and Tukey's test was used to compare groups and sex. ***$p < 0.001$. Data were presented as mean + SEM (9–12 animals per group, from distinct breeds).

We also evaluated the main antioxidant enzymes, and no alteration was observed on SOD, CAT, and GPx activities ($p > 0.05$; Fig. 5a-c, respectively). On the other hand, reduced GLO1 activity was observed both in control and overload exercised female pups when compared to the males from the same groups ($p < 0.001$) (Fig. 5d). In addition, GSH content from the control female and overload exercised pups was reduced in comparison to males in the same groups ($p < 0.001$), while no alterations were observed between other groups (Fig. 5e). Finally, we observed a reduced carbonyl content in the female pups from the overload exercised group when compared with the male pups from the same group ($p < 0.001$) (Fig. 6b).

Discussion

In this study, we aimed to demonstrate the impact of two different maternal exercise intensities, performed during pregnancy, in the skeletal muscle of male and female offspring. To our knowledge, it is the first study to access the redox status in the offspring skeletal muscle. We demonstrated that moderate-intensity swimming exercise (with overload) during pregnancy can affect the skeletal muscle mitochondria and redox status both in male and female offspring differently at PND 60. Females are more affected, presenting reduced ROS, antioxidant activity, and protein damage in gastrocnemius muscle, while female soleus muscle presents

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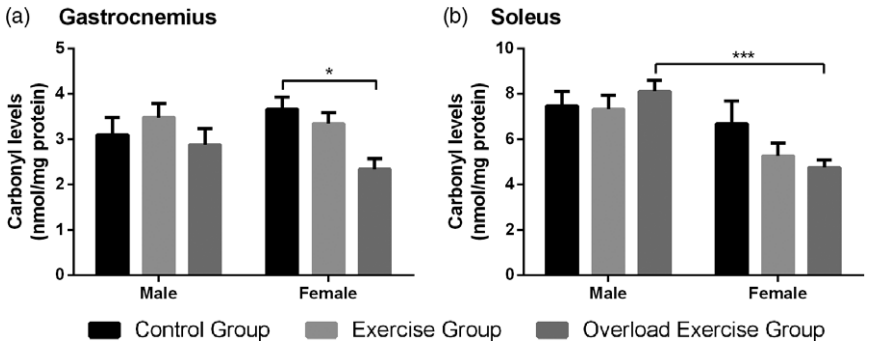

Fig. 6. Biochemical assay. Carbonyl levels. (a) Gastrocnemius muscle. (b) Soleus muscle. Data were evaluated by two-way ANOVA and Tukey's test was used to compare groups and sex. $^{*}p < 0.05$, $^{***}p < 0.001$. Data were presented as mean + SEM (7–12 animals per group, from distinct breeds).

similar metabolic adaptation as gastrocnemius when compared to the male overload group, but not with female control. Regarding the mitochondrial function and content, we observed an increased mitochondrial mass and membrane potential in the females' gastrocnemius muscle, which is in accord with reduced ROS in this muscle. Interestingly, we observed an increased mitochondrial mass and membrane potential in the males' soleus muscles. This was the only adaptive effect observed in males' muscle, suggesting that the sex-dependent effect is powerful.

We have used soleus and lateral gastrocnemius muscles in order to obtain most specifically type I and II muscle fibers, known by their distinct metabolic characteristics as oxidative and glycolytic metabolism, respectively, aiming to demonstrate the effects of maternal exercise in both male and female offspring skeletal muscle.[20,36–38] In this case, we demonstrated that maternal exercise affected mitochondria mainly in type II on female offspring while on male offspring it affected type I fibers. In a study, Liu et al.[39] demonstrated that pups born to exercised dams have increased expression of myosin heavy chain I (MHC I) in plantaris muscle and increased expression in troponin T and I, on lateral gastrocnemius muscle. This can partly explain the differences found in the offspring skeletal muscle, demonstrating that maternal exercise can alter the expression of muscular proteins and in consequence, its metabolism, since type I fiber possess increased oxidative capacity due to the greater mitochondrial content.[20,26]

In previous works, our research group already demonstrated that maternal exercise can improve antioxidant defenses and increase the number of functional mitochondria, revealed by increased α-ketoglutarate dehydrogenase (α-KGDH) and complex IV activities, as well as immunocontent of protein mitofusin-1 (Mfn-1), early and late in life on the brain of pups born to exercised dams.[12,40] Human and rodent studies have already demonstrated that Mfn-1/2, proteins related to mitochondrial fusion, are increased in the skeletal muscle as a response to physical exercise,[41–43] which can be related to our result on increased mitochondrial content found in female gastrocnemius and male soleus. In addition, Liu et al.[39] reported that maternal exercise increased mitochondrial volume density and length and increased citrate synthase activity in plantaris muscle. In the quadriceps femoris muscle, the pups also presented increased cytochrome *c* oxidase activity and cellular energy levels. Furthermore, their study also demonstrated increased immunocontent of mitochondrial fission protein 1 (FIS1), Lon protease (LON), and the mitochondrial transcription factor A (TFAM), which is responsible for the mitochondrial DNA replication, leading to mitochondrial biogenesis in response to exercise.[44,45] In accord, Siti, Dubouchaud[9] demonstrated that pups born from exercised mother during pregnancy present increased complex IV activity and reduced hydrogen peroxide (H_2O_2) release by the mitochondria in offspring skeletal muscle, in accord with our data.

The majority of the medical and exercise studies are conducted only in males.[46] Notwithstanding, some studies already demonstrated sexual dimorphic adaptations in the skeletal muscle of male and female rats. Farhat and colleagues[47] showed that aerobic exercise increased the mitochondrial function on gastrocnemius muscle in both male and female exercised rats; however, the effects are more pronounced in male than female rats. Other studies also show that females possess a greater proportion of type I muscle fiber, increased intramuscular lipid storage, and lipid oxidation in the skeletal muscles than males.[13,48] In the same way, females appear to consume less carbohydrate and protein as substrates during endurance exercise than males,[13,48,49] which demonstrate greater oxidative capacity, which is mainly characteristic of type I fibers. With this in mind, it is possible to infer that the female offspring born from exercised dams show increased proportion of type I fibers or metabolic properties changes in the lateral gastrocnemius compared to the males since gastrocnemius is a more heterogeneous muscle than soleus in the proportion of fiber type's proportion and metabolic properties.[37]

Related to the redox status, we previously showed that maternal interventions during pregnancy increased the enzymatic and non-enzymatic antioxidant defenses in the pups' brain.[6,12] In the present study, the most intense alteration in redox status was observed in the female lateral gastrocnemius muscle. We found reduced levels of ROS allied to reduced antioxidant enzyme activity. The inhibition of GPx and GLO1 is probably related to diminished GSH levels. Reduced antioxidant status can have several detrimental effects on health, which can lead to a pro-oxidant environment and cause damage to proteins, lipids, and DNA, inducing oxidative stress and cell death.[24,50] Oxidative stress is a known feature of several noncommunicable diseases such as diabetes mellitus type 2, obesity and even limiting exercise capacity due to mitochondrial dysfunction.[50] However, despite the reduction in the antioxidant network found in our study, the ROS and carbonyl content, an index of protein damage, were reduced in the female pups born to overload exercised dams. These findings correlate to each other and are in accord with the increased functionality of mitochondria, preventing the leak of electrons from the respiratory chain and ROS production. Interestingly, the redox adaptation found in soleus from female offspring is similar to that found in gastrocnemius, when compared to male offspring.

Mitochondria seem to be the center of all alterations that we observed since it is the largest producer of ROS in cells.[22,23] Moreover, studies already reported that the most relevant differences in the antioxidant defenses between males and females are found in mitochondria.[22,51,52] It has been reported that females produce less H_2O_2 than males, and, consequently, are less prone to

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oxidative damage.[53,54] This data are in accord with what we observed in oxidant levels and carbonyl content from overloaded exercised females in our study. Farhat et al.[47] also demonstrated that trained female rats are more resistant to mitochondrial function reduction when exposed to ROS than males and, in the same study, the females showed less antioxidant defenses and reduced malondialdehyde (MDA) content in the gastrocnemius muscle than males, similar to our findings to both muscles.

The sex-dependent alteration found in our study is abysmal, and probably has participation in, but is not limited to, the sexual hormones. Despite that estrogen hormone is found in males, it is mainly a female sex hormone.[55] The protective effects of estrogen are well reviewed by Enns and Tiidus,[55] reporting its antioxidant effect, membrane stabilizer, as well as regulating the expression of several genes. Moreover, studies also demonstrated that exercise increases the expression of estrogen receptors alpha (ERα) that are found in skeletal muscle, including in mitochondria, which is associated with the increase in mitochondrial function.[22,56,57] In this way, estrogen may be potentially acting in cell signaling and stimulating the expression of Mn-SOD, or simply acting as an oxidant scavenger, due to its structural similarity to other antioxidants like vitamin E,[55] which can explain the reduced oxidant content and also the antioxidant network, found in the females in our model. Unfortunately, we did not evaluate the estrous cycle on females and the hormonal profile of the groups to answer this specific hypothesis in our study.

Finally, in order to check if the maternal exercise during pregnancy could cause any harm to mother or fetus, we evaluated several gestational parameters on the dams and litter, as well as postnatal neuromotor and anatomical developmental parameters on the postnatal period on the offspring, which are disposed in the Supplementary material. In this way, we observed a decrease in the pregnancy rate on the overload exercised dams. We believe that it was the first mention in literature for this effect, and has to be confirmed in future studies. We did not observe any other negative effects in the evaluated parameters on the dams or offspring, which presented normal weight at birth and normal neuromotor and anatomical development in the neonatal period, demonstrating that overload exercise does not cause any harmful effects on the pups' postnatal development.

Conclusion

Taken together, our results add important evidence to the DOHaD literature, demonstrating sex-specific adaptations found in offspring born from exercised mothers. We demonstrated that both males and females showed increased functional mitochondria in the skeletal muscle as an adaptation to dams moderate exercise. The exercise affected pups' skeletal muscle fiber, types I and II, in a sex-dependent way, concerning to redox status, where female pups show less oxidants production, reduced antioxidant defenses, and diminished protein damage. Gastrocnemius was more deeply affected than soleus. In conclusion, we demonstrate that the maternal exercise during pregnancy can affect male and female pups' skeletal muscle and that sex is a very important factor that must be taken into account for future investigations.

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Conflicts of interest. The authors declare no conflict of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (National Institutes for Health (NIH) Guides for the Care and Use of Laboratory Animals (NIH publication No. 80–23, revised 1996) and has been approved by the Local Ethics Commission of Universidade Federal do Rio Grande do Sul (CEUA/UFRGS; protocol number 33,275).

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