EVIDENCES THAT MATERNAL SWIMMING EXERCISE IMPROVES ANTIOXIDANT DEFENSES AND INDUCES MITOCHONDRIAL BIOGENESIS IN THE BRAIN OF YOUNG WISTAR RATS

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Abstract—Physical exercise during pregnancy has been considered beneficial to mother and child. Recent studies showed that maternal swimming improves memory in the offspring, increases hippocampal neurogenesis and levels of neurotrophic factors. The objective of this work was to investigate the effect of maternal swimming during pregnancy on development of the offspring. Adult female Wistar rats were submitted to five swimming sessions (30 min/day) prior to mating with adult male Wistar rats, and then trained during the pregnancy (five sessions of 30-min swimming/week). The litter was sacrificed when 7 days old, when cerebellum, parietal cortex, hippocampus, and striatum were dissected. We evaluated the production of reactive species and antioxidant status, measuring the activities of superoxide-dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GPx), as well as non-enzymatic antioxidants. We also investigated a potential mitochondrial biogenesis regarding mitochondrial mass and membrane potential, through cyto-metric approaches. Our results showed that maternal swimming exercise promoted an increase in reactive species levels in cerebellum, parietal cortex, and hippocampus, demonstrated by an increase in dichlorofluorescein oxidation. Mitochondrial superoxide was reduced in cerebellum and parietal cortex, while nitrite levels were increased in cerebellum, parietal cortex, hippocampus, and striatum. Antioxidant status was improved in cerebellum, parietal cortex, and hippocampus. SOD activity was increased in parietal cortex, and was not altered in the remaining brain structures. CAT and GPx activities, as well as non-enzymatic antioxidant potential, were increased in cerebellum, parietal cortex, and hippocampus of rats whose mothers were exercised. Finally, we observed an increased mitochondrial mass and membrane potential, suggesting mitochondrial biogenesis, in cerebellum and parietal cortex of pups subjected to maternal swimming. In conclusion, maternal swimming exercise induced neuroprotective and neurorestorative effects in the offspring that could be of benefit to the rats against future cerebral insults. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: metabolic programing, oxidative/nitrative stress, redox status, antioxidants, mitochondrial biogenesis, maternal swimming exercise.

INTRODUCTION

Regular physical activity may prevent the onset of a large range of pathological conditions, from heart diseases, and psychiatric disorders, to ischemia and neurodegenerative diseases (Dishman et al., 2006; Powell et al., 2011; Wolff et al., 2011). A recent review from Vina et al. (2012) reports the benefits of exercise promoting health and lifespan, and proposes an effective dose to obtain the best effect of exercise. Whether it is difficult to find the best level of exercise in health people, the better level of physical activity to persons with some special need, such as pregnant women, is much more distant to close. The American College of Obstetricians and Gynecologists recommends 30 min/day of moderate-intensity exercise, all days of the week, similar to the British recommendations (Artal and O’Toole, 2003). For pregnant women, aquatic exercise is preferable, in order to maintain the blood flow with nutrients to the fetus, protect the joints and against falls, as well as control of the body temperature (Hartmann and Bung, 1999; Artal and O’Toole, 2003; Lynch et al., 2003).

Exercise benefits are mediated by the metabolic adaptation elicited, which could be identified including in the CNS, such as increments in neurogenesis and neurotrophic factors, improvement of cognitive function (Vina et al., 2012), raises in glycogen stores (Dalsgaard, 2006; Matsui et al., 2012), glucose uptake, oxidative capacity, and the activity of the electron transport chain (Dishman et al., 2006; Kinni et al., 2011), besides promoting mitochondrial biogenesis (Steiner et al., 2011;
Zhang et al., 2012). Although the brain maintains its oxygen supplement virtually unchanged during exercise, the cellular adaptations to oxidative stress might be observed, such as upregulation of antioxidant enzymes and DNA repair enzymes, increasing the resistance to oxidative stress (Radak et al., 2001, 2007, 2008).

The effect of exercise during pregnancy has been studied in a few animal models, suggesting promising changes in the CNS of offspring. Pups, delivered from rat dams subjected to involuntary swimming during pregnancy, were evaluated in the Morris water maze task. They presented an improvement in spatial memory allied to the rise in the number of cells in the hippocampus, CA1, and dentate gyrus (Akhavan et al., 2008). In agreement, Lee et al. (2006) showed that maternal swimming improves memory, measured by inhibitory avoidance test, and increases neurogenesis and brain-derived neurotrophic factor (BDNF) in the hippocampus of 29 postnatal day (PND) rats. Recently, Dayi et al. (2012) reported that maternal running during pregnancy increased the number of neurons in the hippocampus of pups, which have better scores in the Morris water maze, indicating a positive effect of maternal exercise on spatial memory even in adult pups.

The mechanism by which exercise elicited the brain metabolic adjustment is still unknown. Memory improvement appears to be related to BDNF increment, neuroplasticity, and neurogenesis (Lee et al., 2006; Kim et al., 2007). Akhavan et al. (2008) revealed that noradrenergic and serotonergic systems collaborate with the mnemonic positive effects of maternal exercise. Furthermore, energy metabolism might be altered by exercise in brain regions associated to motor performance, suggesting a connection between muscle and brain (Dishman et al., 2006). Park et al. (2013) reported that treadmill (40 min daily/3 weeks) performed by mice during pregnancy improves brain mitochondrial function in the offspring, in a mechanism related to adaptive counterresponse to eliminate reactive species. In addition, it has been showed that redox-sensitive transcription factors, such as CREB, could also mediate exercise changes in the brain (Radak et al., 2007, 2008). Redox status seem to have a fundamental role in exercise effect, reducing oxidative damage markers and increasing the antioxidant network in the brain, although the literature is not unanimous (Radak et al., 2007).

Taking these data into account, we assessed the effect of maternal swimming on the redox status, mitochondrial mass, and membrane potential, measured in the litter’s cerebellum, parietal cortex, hippocampus, and striatum. In order to accomplish the landscape of the redox status in offspring brain, we evaluated dichlorofluorescein (DCF) oxidation, mitochondrial superoxide, total nitrite levels, antioxidant enzyme activities (superoxide-dismutase (SOD), catalase (CAT), and glutathione-peroxidase (GPx)), and non-enzymatic antioxidant potential. Mitochondrial mass and mitochondrial membrane potential were determined using the probes MitoTracker® Green and Red, respectively.

### EXPERIMENTAL PROCEDURES

#### Animals and reagents

Twenty female and 10 male Wistar rats (adult, 90 days of age) were obtained from the Central Animal House of Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12/12-h light/dark cycle in an air-conditioned constant temperature (22 ± 1 °C) colony room. During the exercise protocol we housed five female rats per cage, except for mating (one male per two females). One day before the birth of the pups, we isolated the pregnant dams (one/cage). After the delivery, we leave the mother and offspring together, until the euthanasia of pups, on the 7th day of life. Rats had free access to a 20% (w/w) protein commercial chow and water. The experiments were approved by the local Ethics Commission (Comissão de Ética no Uso de Animais/Universidade Federal do Rio Grande do Sul – CEUA/UFRGS) under the number 19481, and followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996). We further attest that all efforts were made to minimize the number of animals used and their suffering.

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA, except MitoTracker® and MitoSOX®, obtained from Invitrogen.

#### Experimental design

The experimental design is presented in Fig. 1. Female Wistar rats were subjected to the swimming protocol 1 week previous to mating the males, in order to habituate to the aquatic environment. In mating, one male rat was placed in contact with two females for 12 h. Pregnancy was diagnosed by the presence of a vaginal plug. The pregnant rats were submitted to the swimming exercise protocol during all of the pregnancy. From the 20th day after the onset of pregnancy, the rats were observed twice a day (at 9 and 18 h), to verify the litter’s birth. The day corresponding to the birth of offspring is defined as postnatal day (PND) 0. Table 1 shows reproductive data, where we could see that control- and swimming-exercised rat dams have approximately the same number of implantations and the number of pups delivered. The sex ratio was 0.60 to control litter against 0.49 to maternal swimming exercise litter, and the viability index in the PND 7 was 96.7% for control and 100% for the litter subjected to maternal swimming exercise. We observed a modest reduction in body weight in rats subjected to maternal swimming exercise, on PND1 ($p < 0.001$) and PND7 ($p < 0.05$). We used one pup for each offspring for each technique, in order to eliminate the litter effect (total = 85 animals from both sex).

The litter was left with the mother until PND7, when the rats were weighted and decapitated without anesthesia. Cerebellum, parietal cortex, hippocampus, and striatum were dissected, and stored at −80 °C until...
the completion of the biochemical assays or processed on the same day for cytometric analyses.

Swimming exercise protocol

Adult female Wistar rats were submitted to swimming in a pool filled with 32 ± 1°C water; 5 days/week for 4 weeks; 30 min/day. Control rats were immersed in water, carefully dried, and returned to the housing boxes. This protocol has moderate intensity exercise, and was adapted from Lee et al. (2006) and Akhavan et al. (2008).

Biochemical assays

Sample preparation. For oxidative stress assays, cerebellum, parietal cortex, hippocampus, and striatum were homogenized in 10 volumes (1:10, w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl. Homogenates were centrifuged at 1000 g for 10 min at 4°C, to discard nuclei and cell debris. The pellet was discarded and the supernatant was taken for biochemical assays. The homogenates used were from individual animals, and they were never pooled.

For flow cytometric assay, cerebellum, parietal cortex, hippocampus, and striatum were used freshly and prepared as described below.

**DCF assay.** The reactive oxygen species (ROS) and nitrogen reactive species (RNS) production was assessed through the 2′,7′-DCF oxidation method (LeBel et al., 1992). Briefly, 60 lL of the biologic sample was incubated at 37°C, in the dark, for 30 min, with the addition of 240 lL of 2′,7′-DCF diacetate (H2DCFDA) in a 96-well plate. H2DCFDA is cleaved by cellular esterases and form H2DCF that is oxidized by ROS and RNS which are present in the sample, producing a fluorescent compound, DCF. DCF oxidation was measured fluorimetrically by using a 488 nm excitation and 525 nm emission wavelength. A standard curve, using standard DCF (0.25–10 mM), was performed in parallel with the samples.

Determination of nitrite levels. Nitrite is the stable endproduct of the autoxidation of NO in aqueous solution (Ignarro et al., 1993). Nitrite levels were measured using the Griess reaction; 100 µL of supernatant was mixed with 100 µL Griess reagent (1:1 mixture of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water) and incubated in 96-well plates for 10 min at room temperature. The absorbance was measured on a microplate reader at a wavelength of 543 nm. Nitrite concentration was calculated using sodium nitrite standards (Green et al., 1982).

Antioxidant enzymes activity. SOD activity was evaluated by quantifying the inhibition of superoxide-dependent autoxidation of epinephrine, verifying the absorbance of the samples at 480 nm (Misra and Fridovich, 1972). SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. The data were expressed as Units/mg protein.
CAT activity was assayed according to Aebi (1984) by measuring the absorbance decrease at 240 nm in a reaction medium containing 20 mM H$_2$O$_2$, 0.1% Triton X-100 and 10 mM potassium phosphate buffer, pH 7.0. One CAT unit is defined as 1 μmol of hydrogen peroxide consumed per minute and the specific activity is reported as units/mg protein. The data were expressed as Units/mg protein.

GPx activity was measured according to the method described by Wendel (1981) using tert-butyl hydroperoxide as substrate. NADPH disappearance was monitored spectrophotometrically at 340 nm in a medium containing 2 mM glutathione, 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.5 mM tert-butyl hydroperoxide and 0.1 mM NADPH. One GPx unit is defined as 1 μmol of NADPH consumed per minute and the specific activity is represented as units/mg protein.

Total radical-trapping antioxidant potential (TRAP) and total antioxidant reactivity (TAR). TRAP represents the total non-enzymatic antioxidant capacity of the tissue and was determined by measuring the luminal chemiluminescence intensity induced by 2,2’-azo-bis(2-amidinopropane) (ABAP) at room temperature (Evelson et al., 2001). Two hundred and forty microliters of 10 mM ABAP dissolved in 50 mM sodium phosphate buffer pH 8.6 plus 5.6 mM luminol were added to the microplate and the background chemiluminescence was measured. Ten microliters of 300 μM trolox or supernatant were added and chemiluminescence was measured until it reached the initial levels. The addition of trolox or sample to the incubation medium reduced the chemiluminescence. The time necessary to return to the levels presented before the addition was considered to be the induction time, which is directly proportional to the antioxidant capacity of the tissue and was compared to the induction time of trolox. The results are reported as nmol of trolox per mg of protein.

In the same assay, we measured TAR (Lisi et al., 1995), which represents the quality of the tissue antioxidants. The chemiluminescence value was measured after 1 min after adding ABAP plus luminol. Ten microliters of trolox or brain supernatant, which decrease light intensity, were then added and chemiluminescence was measured after 60 s (final chemiluminescence). The ratio between the initial and the final chemiluminescence values is used to calculate TAR measurement. TAR values were expressed as nmol trolox/mg of protein.

Flow cytometry assay. Mitochondrial superoxide was measured using the probe MitoSOX® Red (Invitrogen, Molecular Probes, Eugene, OR - USA), while mitochondrial mass and membrane potential were evaluated using MitoTracker® Green and MitoTracker® Red (Invitrogen, Molecular Probes, Eugene, OR - USA), respectively, in a FACScalibur flow cytometer (BD Biosciences). The tissue samples (100 mg) were dissociated with 1 mL of phosphate-buffered saline (PBS) pH 7.4 containing 1 mg% of collagenase IV, filtered and incubated with the probes. One hundred microliters of each sample was incubated in a bath at 37 °C, in the dark, for 10 min in a final concentration of 1 μM MitoSOX; or 45 min in a final concentration of 100 nM Mitotracker green + red. After that, 10,000 cells were evaluated per sample. Data were analyzed using the software FlowJo.

Protein determination. Protein concentration was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Statistical analysis

Biochemical data were analyzed by Student’s t test (parametric data) or Mann–Whitney test (non-parametric data), using GraphPad prism 5.0. Differences were considered statistically significant if $p < 0.05$.

RESULTS

Maternal swimming exercise modified reactive species production in brain of rats

It is known that exercise increases the reactive species production, at least in individuals that are carrying out the exercise. Here, we showed that maternal swimming exercise augmented the oxidation of DCF, an indicative of reactive species production, in cerebellum ($t(8) = 4.60$, $p = 0.0018$), parietal cortex ($t(10) = 2.71$, $p = 0.0218$), and hippocampus ($t(10) = 2.68$, $p = 0.0233$) (Fig. 2) obtained from offspring. Striatum was not altered [$t(8) = 1.75$, $p = 0.1176$]. Considering that DCF oxidation is not specific for a single reactive species, we evaluated the contribution of mitochondrial superoxide and nitric oxide (NO) on this effect. Table 2 shows that maternal swimming exercise increased the number of events with low fluorescence [cerebellum: $U = 14.00$, $p = 0.0052$; parietal cortex: $U = 17.00$, $p = 0.0115$] and reduced the events with high fluorescence [cerebellum: $U = 5.00$, $p = 0.0002$; parietal cortex: $U = 8.00$, $p = 0.0007$] measured with MitoSox® by cytometry, indicating a reduction in mitochondrial superoxide levels in the cerebellum and parietal cortex of pups born from exercised mothers. Hippocampus [low fluorescence $U = 43.50$, $p = 0.6500$; high fluorescence $U = 35.00$, $p = 0.2799$] and striatum [low fluorescence $U = 37.00$, $p = 0.3527$; high fluorescence $U = 37.00$, $p = 0.3527$] were not altered. As an index of RNS levels, we measured a stable final product of NO, nitrite. Table 3 shows that cerebellum [$t(10) = 2.74$, $p = 0.0209$], parietal cortex [$t(8) = 3.26$, $p = 0.015$], hippocampus [$t(10) = 2.82$, $p = 0.0182$], and striatum [$t(10) = 4.41$, $p = 0.0013$] present a significant increase in nitrate levels. Although there was an increment in DCF oxidation, we have not found any change in carbonyl content ($p > 0.05$; data not shown).

Maternal swimming exercise improves antioxidant parameters in brain of pups

We measured the activities of SOD, CAT, and GPX, which is an efficient system responsible by eliminating ROS. We verified that maternal swimming exercise increased CAT and GPX activities in the cerebellum.
Fig. 2. Effect of maternal swimming exercise on dichlorofluorescein oxidation in cerebellum (A), parietal cortex (B), hippocampus (C), and striatum (D) of rats from different offspring. Results are expressed as mean ± S.D. for five to six animals in each group. Different from control, *p < 0.05; **p < 0.01 (Student’s t test).

Table 2. Effect of maternal exercise on mitochondrial superoxide levels, measured by MitoSOX Red probe, in cerebellum, parietal cortex, hippocampus, and striatum from offspring

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maternal exercise</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cerebellum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fluorescence</td>
<td>1221 (992–1357)</td>
<td>1612 (1339–2070)**</td>
<td>0.0052</td>
</tr>
<tr>
<td>High fluorescence</td>
<td>1375 (997–1836)</td>
<td>556 (332–815)**</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Parietal cortex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fluorescence</td>
<td>1425 (759–1813)</td>
<td>1873 (1793–2806)</td>
<td>0.0115</td>
</tr>
<tr>
<td>High fluorescence</td>
<td>2147 (2006–2732)</td>
<td>1120 (425–1714)**</td>
<td>0.0007</td>
</tr>
<tr>
<td><strong>Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fluorescence</td>
<td>2035 (1915–2130)</td>
<td>2210 (1716–2449)</td>
<td>0.6500</td>
</tr>
<tr>
<td>High fluorescence</td>
<td>1607 (1348–1679)</td>
<td>1301 (1009–1790)</td>
<td>0.2799</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fluorescence</td>
<td>2078 (1780–2237)</td>
<td>1687 (886–2373)</td>
<td>0.3527</td>
</tr>
<tr>
<td>High fluorescence</td>
<td>1462 (1225–2122)</td>
<td>1925 (1327–2376)</td>
<td>0.3527</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile ranges) for 10 experiments (animals).
* p < 0.05 (Mann–Whitney test).
** p < 0.01 (Mann–Whitney test).

Table 3. Effect of maternal exercise on nitrite levels in cerebellum, parietal cortex, hippocampus, and striatum from offspring

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maternal exercise</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cerebellum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.50 ± 0.58</td>
<td>9.75 ± 0.95*</td>
<td>0.0209</td>
<td></td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>5.54 ± 0.88</td>
<td>7.10 ± 0.61*</td>
<td>0.0115</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>5.40 ± 0.60</td>
<td>6.75 ± 1.01*</td>
<td>0.0182</td>
</tr>
<tr>
<td>Striatum</td>
<td>7.20 ± 0.30</td>
<td>10.96 ± 1.86**</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D. for five to seven experiments.
* p < 0.05 (Student’s t test).
** p < 0.01 (Student’s t test).

[CAT: t(9) = 3.35, p = 0.0085; GPx: t(12) = 5.66, p = 0.0001; Fig. 3] and hippocampus [CAT: t(10) = 3.61, p = 0.0047; GPx: t(10) = 3.97, p = 0.0027; Fig. 5], and did not affect SOD activity [cerebellum: t(10) = 2.02, p = 0.070; hippocampus: t(10) = 1.43, p = 0.1834]. Parietal cortex presented an increase in all enzymes evaluated [SOD: t(10) = 10.69, p < 0.0001; CAT: t(10) = 3.18, p = 0.0098; GPx: t(10) = 2.34, p = 0.0410; Fig. 4], while striatum was not affected by the maternal swimming exercise [SOD:
We also evaluated non-enzymatic antioxidant indexes (Table 4). TRAP, that represents the non-enzymatic antioxidant potential, was significantly increased in cerebellum \( t(10) = 3.50, p = 0.0044 \), parietal cortex \( t(10) = 4.68, p = 0.0009 \), and hippocampus \( t(10) = 3.19, p = 0.0097 \), while the striatum was not affected \( t(12) = 0.39, p = 0.7015 \). A complementary antioxidant parameter, TAR, which represents the antioxidant reactivity, was improved in the cerebellum \( t(12) = 3.82, p = 0.0034 \), and in a borderline way in the hippocampus \( t(10) = 2.22, p = 0.0506 \). In order to detect whether the increment in TRAP and TAR was related to reduced glutathione (GSH) content; we measured the total level of GSH. Surprisingly, we did not find any alteration in this parameter in either structure evaluated \( p > 0.05 \); data not shown).

**Maternal swimming exercise induces mitochondriogenesis in the brain of pups**

Mitochondrial mass and membrane potential were evaluated in a flow cytometer by MitoTracker® Green and Red probes, respectively. Table 5 shows that cerebellum [MitoTracker green-high fluorescence: \( U = 18.00, p = 0.0147 \); Mitotracker red-high fluorescence: \( U = 22.00, p = 0.0355 \)] and parietal cortex [MitoTracker green-high fluorescence: \( U = 20.00, p = 0.0232 \); Mitotracker red-high fluorescence: \( U = 17.00, p = 0.0115 \)] from pups subjected to maternal swimming exercise presented significantly increased fluorescence provided by these two probes,
suggesting an increase in mitochondrial mass and membrane potential, an indicative of mitochondrial biogenesis. Offspring's hippocampus [Mitotracker green-high fluorescence: $U = 48.00$, $p = 0.9118$; Mitotracker red-high fluorescence: $U = 36.00$, $p = 0.3150$] and striatum [Mitotracker green-high fluorescence: $U = 30.00$, $p = 0.1431$; Mitotracker red-high fluorescence: $U = 36.00$, $p = 0.3150$] were not affected by maternal swimming exercise.

**DISCUSSION**

In the present study, we showed that maternal exercise, performed previously and during pregnancy, induced metabolic changes in the offspring's brain. Antioxidant defenses were increased in cerebellum, parietal cortex, and hippocampus of rats, probably triggered by the production of reactive species, as NO. In addition, we found an indicative of mitochondrial biogenesis in cerebellum and in parietal cortex, evidenced by a rise in mitochondrial mass and membrane potential.

Manipulation of environmental factors during pregnancy might define the susceptibility to diseases onset in the offspring during adulthood. Metabolic programing could be modulated by hormones, nutrition, stress, and physical activity (Barker, 1995; Hanley et al., 2010; Ferraro et al., 2012; Seki et al., 2012), even by epigenetic modifications (Donovan and Miller, 2011). Considering the recent studies showing the beneficial role of maternal exercise on cognitive aspects in animal models, promoting neurogenesis and increasing hippocampal neurotrophins (Lee et al., 2006; Akhavan et al., 2008, 2012; Dayi et al., 2012), in the present study we intend to evaluate the brain redox status of pups subjected to maternal swimming. Remarkably, the brain is vulnerable to oxidative stress, largely by its high oxygen consumption, high iron and polyunsaturated fatty-acid side-chains contents, as well as by low antioxidant levels compared to other organs (Halliwell, 

**Fig. 5.** Effect of maternal swimming exercise on superoxide dismutase (A), catalase (B), and glutathione peroxidase (C) in hippocampus of rats, from different offspring. Results are expressed as mean ± S.D. for six animals in each group. Different from control, **$p < 0.01$** (Student’s $t$ test).

**Fig. 6.** Effect of maternal swimming exercise on superoxide dismutase (A), catalase (B), and glutathione peroxidase (C) in the striatum of rats, from different offspring. Results are expressed as mean ± S.D. for six to seven animals in each group. There is no difference from control, $p > 0.05$ (Student’s $t$ test).
In this sense, if a maternal intervention could diminish the brain susceptibility to oxidative stress, it might generate pups more resistant to brain insults in perinatal period or even in adulthood.

First of all, we verified the effect of maternal swimming exercise on ROS and RNS production, evaluated by indirect detection of these species. We measured DCF oxidation, which has been shown to be sensitive to ROS and RNS, such as hydroxyl, peroxy, hydrogen peroxide, superoxide, NO, and peroxynitrite (Halliwell and Whiteman, 2004; Jackson, 2009). Cerebellum, parietal cortex, and hippocampus presented increased DCF oxidation, suggesting an increment in ROS and/or RNS. In order to detect which reactive species could be responsible for this effect, we measured mitochondrial superoxide production and nitrite levels. We found a significant increment in nitrite levels in all the structures evaluated, suggesting an increase in NO production in these brain structures. Although NO might react with biomolecules and other reactive species, being responsible by DCF oxidation verified; it should be related to exercise benefits (Stranahan et al., 2009; Esch and Stefano, 2010; Pietrelli et al., 2011). Surprisingly, superoxide was reduced in cerebellum and parietal cortex, suggesting a reduced production or an increased consumption, such as by reaction with NO generating...
peroxynitrite. Thereby, we could not discard the relevance of superoxide from nonmitochondrial sources, as well as other ROS contribution. A “leak” in the electron transport chain is responsible by superoxide generation, which yields hydrogen peroxide by dismutation. The presence of transition metals catalyzes the non-enzymatic synthesis of the hydroxyl radical, the most harmful oxidant produced cellullarily (Halliwell and Gutteridge, 2007). During the exercise, muscle is particularly affected by reactive species provided by respiratory chain, phagocytes activation, and xanthine oxidase (Vina et al., 2000; Sachdev and Davies, 2008; Gomez-Cabrera et al., 2008b; Jackson, 2011). Although the CNS appears to be less affected by changes in blood flow during exercise, metabolic adaptation has been verified in the brain of exercised individuals (Radak et al., 2001, 2007, 2008). Radak et al. (2006) performed an intense protocol of 8 weeks of swimming in rats (5 times/week, 2 h/day) and found a reduction in free radical production in the cerebellum, measured by electron spin resonance, which is in agreement with our results.

We also measured carbonyl levels in cerebellum, parietal cortex, hippocampus, and striatum, and did not find any alteration in this damage marker index. These data are relevant, because they suggest that reactive species production occurs but not damage to biomolecules, such as proteins. In agreement with our results, Toldy et al. (2005) subjected adult Wistar rats to chronic exercise training, swimming for 6 weeks, and did not find any alteration in carbonyl levels, an index of protein modification.

Considering the vast literature showing that antioxidant enzymes regulation could be mediated by ROS and RNS, we performed the evaluation of antioxidant defenses from cerebral structures. We clearly showed an increment in the activities of CAT and GPx in the cerebellum, parietal cortex, and hippocampus. Parietal cortex seems to be more affected, since SOD activity was also increased. In opposite, striatum’s antioxidant enzymes were not altered. The enzymes evaluated here took part in an efficient ROS removing system (Halliwell, 2007; Halliwell and Gutteridge, 2007). It is known that increments in ROS synthesis could induce antioxidant enzymes by transcription modulation, even by regular exercise (Sachdev and Davies, 2008). The muscle adaptive response to physical activity is well characterized, and comprises mitochondrial biogenesis, increased expression of stress genes/proteins, named heat shock protein 70 (HSP70), and induction of antioxidant enzymes, such as SOD (Sachdev and Davies, 2008; Gomes et al., 2012), CAT, and GPx (Muthusamy et al., 2012). Moreover, our results are supported by other authors, who have shown that brain antioxidant enzymes were activated by exercise training in rats (Somani and Husain, 1996; Marosi et al., 2012). Hippocampal SOD and GPx densities were increased following a moderate intensity running protocol applied to female adult Wistar rats (Marosi et al., 2012). In addition, brain CAT and GPx activities were also increased in trained rats (Somani and Husain, 1996).

It has been shown that reactive species trigger these metabolic adaptations in cells, via the activation of several signaling pathways, such as nuclear factor κ B (NFκB) (Ji et al., 2007; Sachdev and Davies, 2008; Gomez-Cabrera et al., 2008b), nuclear erythroid 2 p45-related factor 2 (Nrf2) (Osburn and Kensler, 2008; Muthusamy et al., 2012), and peroxisome-proliferator-activated receptor-c coactivator-1α (PGC-1α) (Gomez-Cabrera et al., 2008a; Jackson, 2009; Derbre et al., 2012; Gomes et al., 2012; Zhang et al., 2012). One of the most important metabolic adaptations is the increment in antioxidant enzyme activities, responsible for ROS elimination. Given this concern, Sachdev and Davies (2008) extensively reviewed the adaptive response to free radicals in exercise, and showed that SOD has its expression regulated by the redox-sensitive transcription factor NFκB, specially mitochondrial-SOD. Hydrogen peroxide or another ROS could activate NFκB in the cytosol, which translocates to the nucleus, resulting in increased expression of antioxidant enzymes. In addition, Nrf2 has been shown as a broad antioxidant transcription factor, regulating the expression of glutathione reductase, GPx, peroxiredoxin, thioredoxin, thioredoxin reductase, CAT, and Cu/Zn−SOD (Osburn and Kensler, 2008). In this context, GPx and CAT have their expression regulated by Nrf2 in mice myocardium, following acute treadmill exercise as a trigger of ROS production (Muthusamy et al., 2012).

Reactive species, produced during exercise, may perhaps mediate additional benefits to cells, leading to mitochondrial adaptations (Lee and Wei, 2005; Wright et al., 2007). Mitochondrial biogenesis is well characterized in skeletal muscle from animals subjected to physical exercise (Gomez-Cabrera et al., 2008a; Jackson, 2009; Steiner et al., 2011), and has been related to the benefits of exercise on brain (Steiner et al., 2011; Zhang et al., 2012). We verified a noticeable potential of maternal swimming exercise to induce mitochondriogenesis in our model, showed by a raise in the cytometric signal in cortical and cerebellar cells labeled with Mitotracker® green and red, suggesting an increment in the mitochondrial mass and membrane potential. PGC-1α has been reported as the master regulator of mitochondrial biogenesis, activating nuclear transcription factors leading to mitochondrial protein expression, as well as regulating mitochondrial growth and division in response to environmental stress factors, such as oxidative stress, exercise and caloric restriction (Ventura-Clapier et al., 2008; Marques-Aleixo et al., 2012). PGC-1α has been implicated as the main muscle adaptation mediator, eliciting mitochondriogenesis in response to ROS and RNS produced in aerobic exercise training (Gomez-Cabrera et al., 2008a; Jackson, 2009). Besides the well-established effect of exercise on muscle mitochondrial biogenesis, a couple of authors have shown a similar effect on brain from rodents (Steiner et al., 2011; Zhang et al., 2012). Seven
days of treadmill training, following ischemia, were sufficient to increase mitochondrial proteins and DNA (mtDNA), in a PGC-1α-dependent mechanism, as well as to improve cognitive performance and reduce the cerebral infarction volume, reversing the ischemic injury in rats (Zhang et al., 2012). Exercise benefits were also demonstrated by Steiner et al. (2011), in mice submitted to a treadmill run for 1 h/day, 6 days/week at 25 m/min. The mice presented increased PGC-1α mRNA, and mtDNA in several brain regions, such as cerebellum, cortex, and hippocampus.

Akhavan et al. (2008) measured corticosterone levels 21 days after the mating, and showed an increment of 100% in the hormone levels after 10 min of exercise. Possibly, this hormone can modulate the metabolism of the litter and be part of the mechanism by which maternal exercise works. Meanwhile, we cannot discard the participation of other chemical mediators, produced by placenta, or by the mother, that can trespass the placental barrier; or even synthesized by the pup. BDNF, nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) have been considered as key mediators of exercise effects (Carro et al., 2000; Bick-Sander et al., 2006; Ding et al., 2006; Lee et al., 2006; Kim et al., 2007; Akhavan et al., 2008, 2012; Uysal et al., 2011; Bustamante et al., 2013), and might be related to maternal exercise effects on offspring. Certainly, the knowledge of the factor responsible by the metabolic modulation found in the brain of progeny is a central element in this effect, and is an important limitation of our study.

CONCLUSION

We showed some evidences that maternal swimming exercise induced a biochemical programing in the offspring’s brain, eliciting mitochondrial biogenesis and improving the antioxidant network, probably triggered by local reactive species production, such as NO. The metabolic adjustment verified in the pups subjected to maternal swimming exercise possibly may bring benefits, conferring neuroprotection against future brain insults.

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