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Exercise before pregnancy attenuates the effects of prenatal stress in adult mice in a sex-dependent manner

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Abstract

The present study aimed to investigate the long-term effects of exercise before pregnancy on changes induced by prenatal stress. Female and male Balb/c mice were divided into three groups: control (CON), prenatal restraint stress (PNS), and exercise before the gestational period plus PNS (EX + PNS). As adult, fear/anxiety behavior, corticosterone secretion, expression of hypothalamic-pituitary-adrenal (HPA)-related genes, as well as epigenetic modifications were evaluated. Exercise before gestation did not prevent the increased fear/anxiety behavior in PNS mice. A nearly significant ($p = .06$) basal corticosterone increase was observed in PNS males and the exercise before pregnancy reduced the stress-induced corticosterone increase in PNS females. In addition, an increase on prefrontal cortex (PFC) CRHR1 gene expression was observed in PNS females, which was attenuated by the exercise before gestation. We have also found a glucocorticoid receptor (GR) gene expression decrease in the prefrontal cortex in PNS males, as well as a histone H3 acetylation decrease ($p = .06$) close to the significance level. In conclusion, pregestational exercise may attenuate developmental changes induced by prenatal stress in a sex-dependent manner.

KEYWORDS

early life stress, exercise, prefrontal cortex, sexual differences

1 | INTRODUCTION

Early life is characterized as a period of high plasticity, in which different organs and systems are molded according to the environment. An adverse environment in utero has the ability to program fetal development and growth, promoting changes in the offspring that persist throughout life (Seckl & Meaney, 2004). It is well established that offspring exposed to prenatal stress have altered hypothalamic-pituitary-adrenal (HPA) axis responses, with increased glucocorticoid secretion due to alterations in the negative feedback generated by the misbalance in key regulatory factors, such as glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and type 1 corticotrophin-releasing hormone receptor (CRHR1) (van Bodegom, Homberg, & Henckens, 2017). Moreover, evidence shows that prenatal exposure to maternal glucocorticoids may lead to permanent effects in the offspring

in a sex-dependent manner, including increased corticosterone levels in females (Liu, Li, & Matthews, 2001), reduced neurogenesis in males (Zuena et al., 2008), and impaired depression/anxiety behaviors in females (Schulz et al., 2011; Van den Bergh, Calster, Smits, Huffel, & Lagae, 2008; Weinstock, 2007). In addition, sexual differences are strongly related to the type, frequency, and period of the prenatal stress (Weinstock, 2007).

The prefrontal cortex (PFC) is a brain region with the longest period of cerebral maturation, presenting an important role in the postnatal neurodevelopment (Gogtay et al., 2004). Studies have reported that several early life stress alterations involve the PFC, including altered long-term potentiation, impaired memory, and alterations in sexual motivation (Baudin et al., 2012; Hernandez-Gonzalez et al., 2017). Thus, the PFC seems to be an important target in the search for mechanisms related to neuropsychiatric and cognitive disorders. Additionally, epigenetic mechanisms are known to change gene expression and thereby disrupt particular biological functions in the brain (Fagiolini, Jensen, & Champagne, 2009). Exposure to stress during the prenatal period is able to decrease acetylated histone H3 levels and increase histone deacetylases (HDACs) expression, which may trigger a lower expression of genes associated to the HPA axis regulation (Benoit, Rakic, & Frick, 2015; Zheng, Fan, Zhang, & Dong, 2016).

Exercise has already been implicated in promoting several beneficial effects in stress-related disorders, including the regulation of corticosterone levels and behavior (Kim & Leem, 2016; Li et al., 2013; Seo, 2018). However, little is known regarding preventive effects of exercise before pregnancy on long-term changes in the offspring. Previous studies have demonstrated that maternal exercise increases neurogenesis and cognition, although the relation of pregestational exercise and HPA axis response with behavior and epigenetic effects were not yet addressed (Gobinath et al., 2018; Marcelino et al., 2016). Thus, assessing important stress-related target genes, as well as the modification of HDACs activity and H3 acetylation would lend insight into the mechanism of the effects of pregestational exercise on prenatally stressed mice.

The objective of the present study, therefore, was to investigate the long-term effects of exercise before pregnancy on changes induced by prenatal stress in the adult offspring. For that, fear/anxiety behavior, corticosterone secretion, expression of HPA-related genes, as well as epigenetic modifications were evaluated. In addition, we have also aimed to explore possible sexual differences in these responses.

2 | MATERIALS AND METHODS

2.1 | Animals

Male and female Balb/c mice were purchased from the Center for Experimental Biological Models at PUCRS. Animals were kept in a controlled temperature environment (24 ± 2°C), light/dark cycle of 12 hr, with free access to water and food for at least 10 days before any experimental procedure.

2.2 | Experimental design

Balb/c females were divided into three experimental groups: CON—control (n = 8 dams), PNS—prenatal restraint stress (n = 9 dams), EX + PNS—physical exercise before the gestational period and PNS (n = 9 dams). Animals from the CON group were kept in their cages and only handled during cleaning routine.

During the fertile period, females were placed together with males during the dark cycle for 24 hr to allow mating. With the confirmation of mating, which was considered day 0 of gestation (G0), pregnant females were randomized according to the experimental groups. After birth, the litters were standardized in six animals and weaning was performed on the 21st day (PND 21). Animals were maintained until adulthood in order to perform the elevated plus maze test on day 60 of life. On the 74th day of life, animals were euthanized and samples collected (plasma and brain). All animals were only brought to the procedure room at the time of euthanasia, in order to avoid contact with any blood olfactory stimulus, which could influence stress related measurements. Brains were removed for the evaluation of CRHR1, GR, and MR gene expression in the PFC. The segmentation of brain tissue was performed by freehand dissection (Spijker, 2011). In addition, PFC HDAC activity and global acetylation of histone H3 were also evaluated. Blood samples were collected for the determination of plasmatic corticosterone. Figure 1 summarizes the experimental design.

2.3 | Prenatal restraint stress

A closed cylinder, made of acrylic crystal, with 34 mm of height, 42 mm of width, 100 mm of circumference, and 10 lateral holes of 6 mm for the entrance and exit of air was used. Females from PNS and EX + PNS groups were submitted to the stress protocol, which was performed from the 8th day of gestation, for 30 min, on intercalated days until the day of birth of the offspring. Females from CON group were not submitted to any experimental intervention during the prenatal period (Vargas et al., 2016).

2.4 | Exercise protocol

Animals from the EX + PNS group were submitted to an exercise protocol starting in the last hour of the light cycle on a

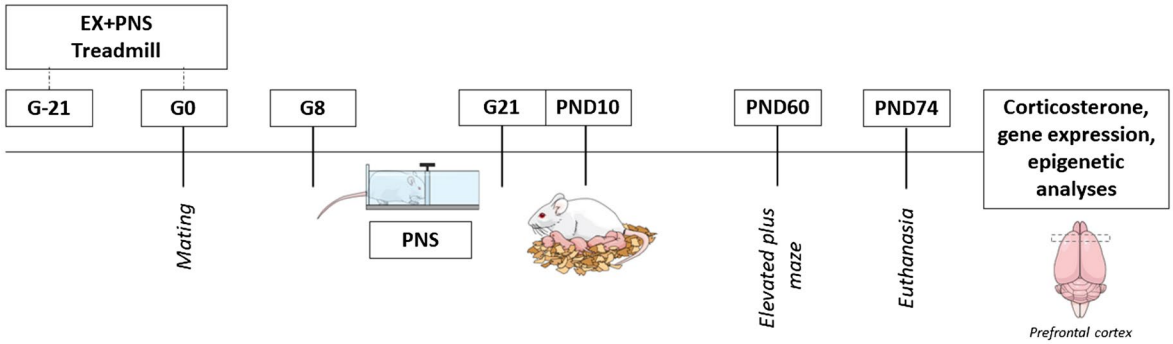

FIGURE 1 Experimental design of the study. The timeline is presented in days. G-21:21 days before G0; G0: gestational day 0/mating day; G8: gestational day 8; G21: gestational day 21; PND 0: post-natal day 0/offspring birth; PND 60: post-natal day 60; PND 74: post-natal day 74; PNS: prenatal restraint stress; EX + PNS: exercise before the gestational period and prenatal restraint stress [Colour figure can be viewed at wileyonlinelibrary.com]

motorized treadmill. Exercise protocol was performed daily, during the 3 weeks that preceded the day of mating (G0). First, animals were familiarized with the treadmill at 5 m/min speed sessions for 10 min, in the 3 days prior to the start of the protocol. After that, exercise sessions at a speed of 10 m/min for 60 min, 5 days a week, were used. No stimulus, such as electric shock, was applied to stimulate animals to perform the activity. Mice from the CON and PNS groups performed only spontaneous activities in their cages (Wearick-Silva et al., 2017).

2.5 | Elevated plus maze

The test consists of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) joined equidistantly producing a common central field (5 × 5 cm), in a height of 66 cm from the ground. At the start of the test, the animals were placed on the central platform with the head directed toward the closed arm and remained in the apparatus for 10 min. The time spent on closed and open arms was assessed. At the end of the test, the animals were removed, returned to their boxes and the surface of the apparatus was cleaned with 70% of alcohol. The parameters were analyzed using the software ANY-maze™ (Stoelting, USA).

2.6 | Euthanasia

After 2 weeks of the behavioral test, the animals were euthanized by decapitation. The brains were removed and stored in RNA-later (Applied Biosystems, USA) for 24 hr at 4°C and then transferred to −80°C until final processing. Blood was collected, stored in tubes containing ethylenediaminetetraacetic acid and centrifuged at 3,000 rpm for 10 min at 4°C for plasma collection.

2.7 | Corticosterone response to stress

In order to evaluate the corticosterone response to a stressor in adulthood, animals were subdivided into two groups: euthanized at baseline or subjected to a restraint stress protocol, with the same apparatus described above, for 30 min and immediately euthanized. The commercial enzyme immunoassay kit (ELISA) (Mouse/Rat Cortisol ELISA, Sigma-Aldrich, USA) was used for the determination of plasma corticosterone concentrations. The final results were expressed as ng/mL. The lowest limit of detection was 0.82 ng/mL.

2.8 | Gene expression

The gene expression of CRHR1, GR, and MR in the PFC was evaluated. Total cellular RNA was extracted by the Trizol method (ThermoFisher—Scientific, USA) according to the manufacturer's instructions. The RNA was resuspended in 20 μL of nuclease-free water (Ambion®—ThermoFisher—Scientific, EUA) and converted to complementary deoxyribonucleic acid (cDNA) (GoScript™ Reverse Transcription System Protocol—Promega, USA), according to the protocol indicated by the manufacturer. The final concentration of cDNA was analyzed by fluorimetric method (Qubit®—ThermoFisher—Scientific, USA) from a commercial kit (Qubit® dsDNA HS Assay—ThermoFisher—Scientific, USA).

Gene expression was performed in real-time quantitative PCR (Step One Plus—Applied Biosystems) using 16 ng of cDNA. The samples were prepared in duplicate and the relative expression of messenger RNA (mRNA) was calculated by the Delta-Delta Ct method (ΔΔCt) using the male CON group as reference. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was adopted as the reference endogenous

gene. A negative CON for each primer was used on each plate to check for possible contamination. Amplification reagent measurements were calculated based on incorporation of the SYBR® Green fluorescence marker (ThermoFisher—Scientific, USA) into the double cDNA ribbon for each amplification reaction.

The set of specific primers for each gene were: CRHR1 (forward 5′TGAGTGTTAGCGATGCCTTG 3′; reverse 5′TCCTACCACTGAGGACTGG 3′), GR (forward 5′GGA ATAGGTGCCAAGGGTCT 3′, reverse 5′GAGCACACCA GGCAGAGTTT 3′), MR (forward 5′CCAGTTCTCCG TTCTCTGTA 3′; reverse 5′CTTGAGCACCAATCCGGTAG 3′), and GAPDH (direct 5′GGGGAGCCAAAAGGGTCATC 3′; reverse 5′GACGCCTGCTTCACCACCTTCTTG 3′).

2.9 | HDAC activity

The total HDAC activity was evaluated using the EpiQuik HDAC Activity Assay Kit (Epigentek, USA). Briefly, nuclear extracts of the PFC were obtained with the EpiQuik™ Nuclear Extraction Kit (Epigentek, USA), incubated with substrate and assay buffer at 37°C for 1 hr, followed by the incubation with a capture antibody at room temperature for 60 min. The detection antibody was incubated at room temperature for 30 min. The absorbance was read on a microplate spectrophotometer at 450 nm. The HDAC activity was measured according to the manufacturer's instructions.

2.10 | Global acetylation of histone H3

The levels of global histone H3 acetylation were evaluated in the PFC using the EpiQuiK Global Histone H3 Acetylation Assay Kit (Epigentek, USA). Briefly, the histones were extracted with the extraction buffer, incubated with blocking buffer at 37°C for 30 min, followed by the incubation with a capture antibody at room temperature for 60 min on an orbital shaker. After, the detection capture antibody was incubated at room temperature for 30 min. The absorbance was read on a microplate reader at 450 nm. The amount of acetylated histone H3 was quantified according to the manufacturer's instructions.

2.11 | Statistical analysis

The normality of data was tested using the Shapiro–Wilk test. Data were expressed using mean and standard error of the mean (*SEM*). In order to evaluate differences between the experimental groups (CON, PNS, and EX + PNS) and the interaction with sex (males and females), two-way ANOVA followed by the LSD post-test was used. In order to evaluate differences and the interaction between groups, sex, and the second-hit stress, a three-way ANOVA followed by the LSD post-test was used. In all cases, the level of significance was set at 5%. Data were analyzed using the software SPSS 18.0 (SPSS Inc., USA) and graphs were made using Prism GraphPad (version 5.0, GraphPad Software Inc., USA).

3 | RESULTS

3.1 | Pregestational exercise does not prevent fear/anxiety effects in PNS mice

The effect of exercise before pregnancy on preventing the well-known effects of prenatal stress on fear/anxiety was evaluated through the elevated plus maze test. No significant differences were observed between group and sex when the time in the closed arms was analyzed (Figure 2a). When evaluating the time spent in the open arms, a significant effect for group ($F_{(2,55)} = 13.74$; $p = .000014$) was observed. In the pairwise analysis, both PNS (males $p = .016$ and females $p = .003$) and EX + PNS (males $p = .00005$ and females

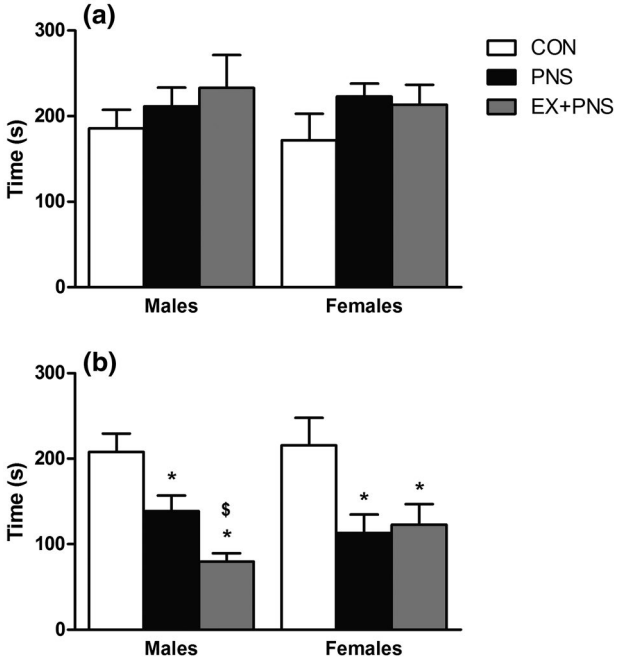

FIGURE 2 Behavioral evaluation in the elevated plus-maze apparatus. The time spent in the closed arms (a) and in the open arms (b) were evaluated in males and females. Data are shown as mean and *SEM*, and were compared by two-way ANOVA followed by the LSD *post-hoc* test. Number of animals for each group are: CON = 10, PNS = 13, and EX + PNS = 11 for males; and CON = 7, PNS = 9, and EX + PNS = 11 for females. *significant differences compared to the CON group in the same sex; $^{\$}$significant difference compared to the PNS group in the same sex

$p = .006$) animals showed significantly less time when compared to the CON group (Figure 2b). In addition, EX + PNS males ($p = .036$) also showed significantly less time in the open arms compared to the PNS group (Figure 2b). No other significant differences were found.

3.2 | Pregestational exercise prevents stress-induced plasmatic corticosterone increase in PNS females

Plasmatic levels of corticosterone, both at basal and after a restraint stress, were evaluated. We observed a significant effect for stress ($F_{(1,63)} = 258.37$; $p = <0.0001$) and interaction between group, stress, and sex ($F_{(11,63)} = 3.30$; $p = .043$). When basal levels were analyzed, PNS males presented a nearly significant ($p = .061$) increase when compared to the CON group, although no effects for exercise were demonstrated. As expected, after 30 min of restraint stress, animals from all experimental groups showed significantly ($p < .05$) increased levels of plasmatic corticosterone. In females, maternal exercise significantly reduced ($p = .011$) the stress-induced increase in corticosterone, as demonstrated by the decrease seen when EX + PNS was compared to the PNS group (Figure 3).

Regarding sexual differences, CON females presented a nearly significant ($p = .053$) corticosterone increase at basal when compared to CON males. Similarly, after restraint stress in adulthood, PNS females ($p = .010$) showed increased corticosterone when compared to PNS males (Figure 3).

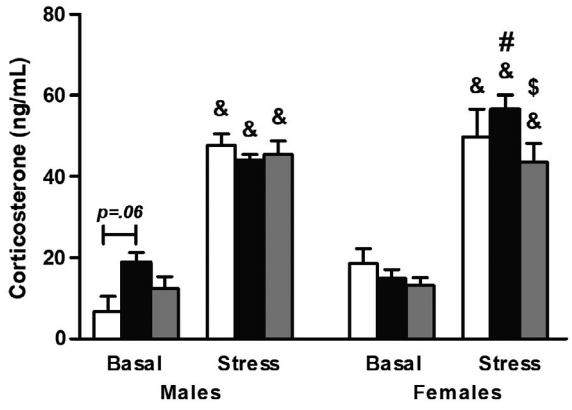

FIGURE 3 Evaluation of corticosterone secretion. Plasmatic corticosterone concentrations was evaluated at basal and after a 30 min restraint stress in males and females. Data are shown as mean and *SEM*, and were compared by three-way ANOVA followed by the LSD *post-hoc* test. $n = 5–7$ males per group and $n = 6–7$ females per group. *significant differences compared to the CON group in the same sex; $^{\$}$significant differences compared to the PNS group in the same sex; $^{\&}$significant second-hit stress differences within the same group; $^{\#}$significant sex differences within the same group

3.3 | Pregestational exercise attenuates PFC CRHR1 gene expression in PNS females

Considering the results found in corticosterone, gene expression of MR, GR, and CRHR1 in the PFC were evaluated. In the analysis of CRHR1 gene expression, significant effects for group ($F_{(2,26)} = 4.53$; $p = .02$), sex ($F_{(1,26)} = 8.89$; $p = .006$), and interaction between group and sex ($F_{(5,26)} = 4.45$; $p = .022$) were found. *Post-hoc* analysis revealed that prenatally stressed females (PNS group) showed an increase in the expression of CRHR1 ($p = .002$) compared to the CON group. Exercise before pregnancy prevented the PNS induced increase in the CRHR1 mRNA expression ($p = .002$) (Figure 4a).

When sexual differences were evaluated, we observed that PNS females presented increased CRHR1 mRNA expression ($p = .0002$) when compared to PNS males (Figure 4a).

3.4 | Prenatal stress decreases PFC GR gene expression in males

Significant effects for sex ($F_{(1,22)} = 7.63$; $p = .011$) were found in the GR gene expression analysis. Pairwise comparisons for the GR mRNA expression revealed that PNS ($p = .035$) males showed a significant decrease when compared to CON. Comparisons between sexes demonstrated a significant decrease in the GR gene expression in CON females ($p = .006$) when compared to CON males. No other significant differences were found (Figure 4b).

3.5 | Prenatal stress does not alter PFC MR gene expression

Significant effects for sex ($F_{(1,24)} = 14.21$; $p = .001$) were found in the MR gene expression analysis. When MR mRNA expression was analyzed, there were no significant differences in the comparisons between groups (Figure 4c). On the contrary, when sex differences were evaluated, an increased MR gene expression was observed in females from CON ($p = .035$) and PNS ($p = .025$) when compared to males from the same experimental groups (Figure 4c).

3.6 | Prenatal stress decreases PFC histone H3 acetylation in males

Epigenetic mechanisms were evaluated by measuring HDAC activity and H3 acetylation. No significant effects for group, sex, and interaction between group and sex were found in HDAC and H3 analyses. When the HDAC activity was assessed, no significant differences were found between all experimental groups (Figure 5a). On the contrary, pairwise comparisons

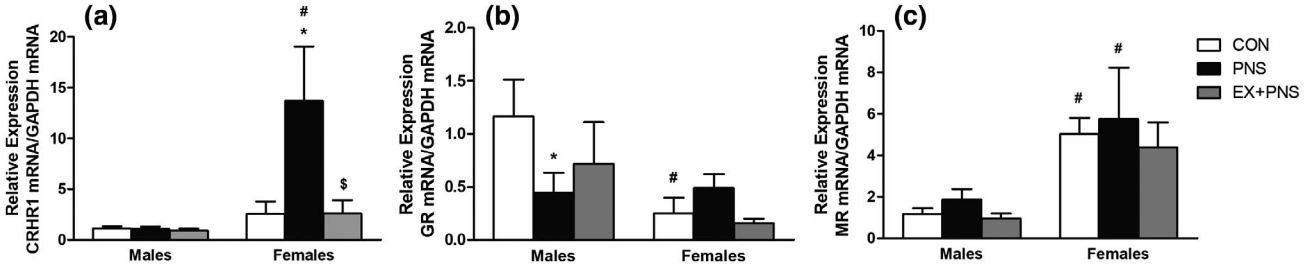

FIGURE 4 CRHR1, GR, and MR mRNA expression. The prefrontal cortex gene expression of CRHR1 (a), GR (b), and MR (c) were evaluated in both males and females. Data are shown as mean and *SEM*, and were compared by two-way ANOVA followed by the LSD *post-hoc* test. $n = 4–6$ animals per group for both males and females. *significant differences compared to the CON group in the same sex; #significant sex differences within the same group

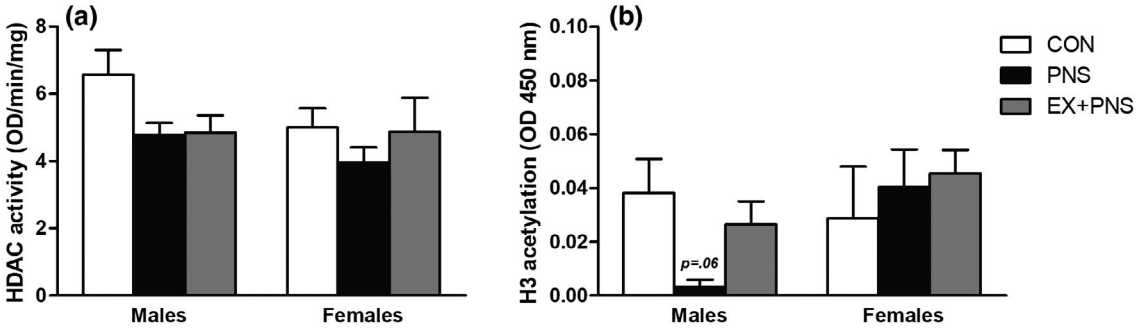

FIGURE 5 Epigenetic regulation. The HDAC activity (a) and histone H3 acetylation (b) were evaluated in the prefrontal cortex in males and females. Data are shown as mean and *SEM*, and were compared by two-way ANOVA followed by the LSD *post-hoc* test. $n = 4–8$ males per group and $n = 4–7$ females per group. *significant differences compared to the CON group in the same sex; #significant sex differences within the same group

reveled a nearly significant ($p = .062$) histone H3 acetylation decrease in PNS males when compared to CON (Figure 5b).

4 | DISCUSSION

Results from the present study have demonstrated, for the first time, the preventive effects of exercise before the gestational period on HPA axis changes due to prenatal stress on male and female adult mice. Several parameters were assessed and data suggests that exercise may modulate the expression of GR and CRHR1 in the PFC, in addition to the corticosterone response, in a sex-specific manner.

Considering the well-recognized long-term effects of stress during gestation on behavior, fear, and anxiety were evaluated (van Bodegom et al., 2017). Epidemiological studies in humans have shown that individuals subjected to an adverse environment during the gestational period present an increased risk of developing psychiatric disorders (O'Connor et al., 2005). The present study aimed to evaluate the effects of exercise before gestation on fear/anxiety behavior (elevated plus maze) in mice submitted to prenatal stress. Data showed that prenatal stress-induced an increase in fear/anxiety behavior, although no effects of pregestational exercise were found. It is possible that intensity and type of exercise may have influenced results, although no other studies evaluating the effects of pregestational exercise on behavior were found for comparisons. In a work using voluntary exercise in a running wheel, an anxiolytic effect with decreased markers of depression was observed in mice (Duman, Schlesinger, Russell, & Duman, 2008). On the contrary, there is evidence showing that forced exercise in rodents is able to activate the HPA axis and develop anxiety changes by inducing the secretion of corticosterone (Chang et al., 2008). In addition, it is not possible to rule out the possibility that the elevated plus maze test used in the present study was not sensitive enough to detect possible effects of pregestational exercise.

During pregnancy, corticosterone concentrations are naturally higher. However, stress during this period may further increase corticosterone secretion, which can reach the fetus leading to developmental alterations (Kou et al., 2014). Our group recently demonstrated that PNS animals, from both sexes, when exposed to stress in adult life, present an increase in corticosterone secretion (Vargas et al., 2016). Likewise, our results demonstrated that basal levels of corticosterone in PNS males were increased compared to CON mice and, although not statistically significant, physical exercise before gestation also seems to have an effect. In contrast, in females,

exercise showed a protective effect on the increased corticosterone levels after 30 min of restraint stress. In a rodent study, exercise for 3 weeks decreased serum corticosterone and hippocampal concentrations 20 days after the end of the protocol, demonstrating a long-term effect of the treadmill use (Radahmadi, Alaei, Sharifi, & Hosseini, 2015). It is possible that protective long-term effects of exercise may have contributed to the results presented here in an effect that may reflect an interaction with specificities of the stressor and additional modulations from sexual differences.

The corticotrophin releasing hormone (CRH) is essential for the stress response and the CRHR1 subunit is related to increased fear and anxiety behavior under a regulatory process mediated by glucocorticoids (Bale & Vale, 2004). Several authors have demonstrated increased CRH gene expression in the paraventricular nucleus (Makino et al., 1995), amygdala (Hsu, Chen, Takahashi, & Kalin, 1998), and PFC (Meng, Chen, Tong, & Zhou, 2011) in response to stress. Our results demonstrated that exercise before pregnancy prevented the increased CRHR1 mRNA expression in the PFC of PNS females. The protective effect of exercise only in females may indicate hormonal influences on the regulation of this response. There are few studies that addressed sexual differences in the CRH regulation after a stressful event in the gestational period. An increase in the paraventricular nucleus CRH expression, accompanied by changes in the HPA axis in response to stress was demonstrated only in females prenatally stressed during the last week of gestation (Zohar & Weinstock, 2011). To the best of our knowledge, this is the first study to evaluate the PFC CRHR1 expression in Balb/c mice undergoing prenatal stress. Likewise, no study to date has evaluated PFC CRHR1 response to exercise, although an attenuated stress response has already been described with decreased paraventricular nucleus CRHR1 mRNA expression in animals who practiced voluntary exercise (Droste et al., 2003).

The effects of pregestational exercise in genes related to the CON of HPA axis were also evaluated. GR and MR are the main regulators of HPA axis CON, participating in the stress response modulation (van Bodegom et al., 2017). We have demonstrated that prenatal stress reduced PFC GR mRNA expression in males and that pregestational exercise prevented this effect. To date, no study has evaluated GR expression in response to a stressor stimulus in treadmill-trained animals. However, although the long-term effects of exercise on GR expression are not known yet, Chang et al. demonstrated an unchanged expression 24 hr after a moderate treadmill exercise protocol (Chang et al., 2008). We hypothesized that the observed responses in GR mRNA expression would be directly related to basal corticosterone secretion in males, since it is possible to observe an attenuation of the prenatal stress effect by physical exercise. Furthermore, the affinity of MR for corticosterone is about ten times greater than GR. Thus, basal corticosterone concentrations predominantly occupy MR, whereas during a stressor event, increased corticosterone levels lead to the co-activation of GR (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998). Our study did not demonstrate significant differences in the PFC MR mRNA expression for both stress and exercise. Still, studies have reported that prenatal stress is able to decrease MR expression throughout life (Grundwald & Brunton, 2015; Tamura, Sajo, Kakita, Matsuki, & Koyama, 2011). The relationship between exercise and MR response has never been investigated in the PFC of mice after forced exercise. Conversely, a study has shown that hippocampal MR levels may decrease at the end of a treadmill exercise period, probably as a consequence of increased corticosterone (Chang et al., 2008). Possibly, differences in the exercise protocols and the species used may contribute to the different responses found.

It has been already established that exposure to an adverse prenatal environment can lead to lifelong epigenetic changes (Babenko, Kovalchuk, & Metz, 2015). The PFC has an important role in emotional and cognitive functions, besides being key in the physiological CON of the stress response (Arnsten, 2009). Thus, we have also analyzed PFC HDAC activity and histone H3 acetylation. Evidences have shown that early life stress decreases histone H3 acetylation in both male and female hippocampus (Benoit et al., 2015). In addition, it has been reported that the expression of HDAC is increased in prenatally stressed male rats (Van den Hove et al., 2013). Similarly, our data shows that prenatal stress decreased histone H3 acetylation only in males and exercise before gestation seems to block this effect. Studies have already reported the exercise potential to decrease HDAC activity, in the PFC, and to increase histone H3 acetylation in the hippocampus and cerebellum (Abel & Rissman, 2013; Spindler et al., 2014). The balance between HDAC and histone acetyltransferase (HAT) is important for neuronal homeostasis and, thus, for normal brain functioning (Saha & Pahan, 2006). Our study did not find significant differences between groups in the assessment of HDAC, so it is possible that the use of a total HDAC activity assay for this enzyme may mask specific dysfunctions of its subunits or HATs.

One limitation of the present study is the lack of gonadal hormone assessment. Studies have reported important sexual differences generated by the effects of prenatal stress in the offspring, including the regulation of the HPA axis (Weinstock, 2007). Females are generally more sensitive to maternal hormones than males, with changes in HPA axis markers in both basal and stress conditions. In accordance with our results, females generally report a greater response to stress (corticosterone) in animals prenatally stressed (Schoonman et al., 2007; Veenema et al., 2007; Zohar & Weinstock, 2011).

In conclusion, our findings indicate that exercise before pregnancy may attenuate some developmental changes

induced by prenatal stress, evidenced mainly by the modulation of corticosterone and CRHR1 in females, besides the GR mRNA expression and histone H3 acetylation in males. The findings of present study may contribute to a better understanding on the preventive long-term effects of exercise before pregnancy and its role on stress-related diseases.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICS APPROVAL

All the experiments were performed in agreement with the international ethical standards and following the local animal protection guidelines. The experimental protocol was approved by the Ethics Research Committee (protocol number 15/00446) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

AUTHOR CONTRIBUTIONS

Carolina Luft conceived the work, acquired data, drafted the paper, performed data analysis, and approved the final version. Isadora Perez Levices, Mariana Severo da Costa, and Gabriela Viegas Haute acquired data, revised the article and approved the final version. Rodrigo Grassi-Oliveira and Jarbas Rodrigues de Oliveira conceived the work, revised the paper, and approved the final version. Márcio Vinícius Fagundes Donadio conceived the work, acquired funding, performed data analysis, revised the article, and approved the final version.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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