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RESEARCH ARTICLE

# Impact of maternal physical exercise on inflammatory and hypothalamic–pituitary–adrenal axis markers in the brain and lungs of prenatally stressed neonatal mice

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## Abstract

This study aimed to evaluate the effects of maternal exercise on alterations induced by prenatal stress in markers of the inflammatory process and the hypothalamic–pituitary–adrenal axis in the brain and lungs of neonatal mice. Female Balb/c mice were divided into three groups: control, prenatal restraint stress, prenatal restraint stress and physical exercise before and during the gestational period. On day 0 (PND0) and 10 (PND10), mice were euthanized for brain and lung analyses. The gene expression of GR, MR, IL-6, IL-10, and TNF in the brain and lungs and the protein expression of MMP-2 in the lungs were analyzed. Maternal exercise reduced IL-6 and IL-10 gene expression in the brain of PND0 mice. Prenatal stress and maternal exercise decreased GR, MR, IL-6, and TNF gene expression in the lungs of PND0 mice. In the hippocampus of PND10 females, exercise inhibited the effects of prenatal stress on the expression of MR, IL-6, and IL-10. In the lungs of PND10 females, exercise prevented the decrease in GR expression caused by prenatal stress. In the hippocampus and lungs of PND10 males, prenatal stress decreased GR gene expression. Our findings confirm the effects induced by prenatal stress and demonstrate that physical exercise before and during the gestational period may have a protective role on inflammatory changes.

**KEYWORDS**
HPA axis, inflammation, prenatal stress, treadmill

## 1 | INTRODUCTION

Chronic stress is considered a risk factor for the development and aggravation of inflammatory, neuroendocrine, and respiratory diseases, among others, being related to excessive activation of the hypothalamic–pituitary–adrenal (HPA) axis (Boersma & Tamashiro, 2015). The HPA axis contributes to the maintenance of homeostasis, acting as a regulator of the production and release of corticosteroids (Rothe et al., 2020; Sze & Brunton, 2020). Consequently, it also plays an important role in fetal lung maturation, inflammatory response, neurodevelopment, and metabolic development (Won & Kim, 2020; Wood & Walker, 2015).

Several studies highlight the importance of the gestational period, in which the fetus is more vulnerable and more susceptible to the action of external and internal stimuli, increasing the risk of developing diseases throughout life (Bangasser & Valentino, 2014; Iwasaki-Sekino et al., 2009; Munck et al., 1984). Stress during the prenatal period is characterized as fetal exposure to increased levels of glucocorticoids as a result of hyperactivation of the maternal HPA axis (Akbaba et al., 2018; Rakers et al., 2020). Prenatal stress may induce several changes

  

in the regulation of genes involved both in the inflammatory process and in the HPA axis reactivity in different organs, including the brain and the lungs (Buss et al., 2012). Lung development begins during the fetal period and is influenced by several factors, including the action of matrix metalloproteinases (MMP), which play an essential role in lung function, tissue damage repair, inflammatory process, and tissue remodeling (Hendrix & Kheradmand, 2017). Thus, changes in MMP levels in response to environmental stimuli, infections, or injury can cause pulmonary complications (Ling et al., 2019; Mok et al., 2021). On the other hand, the central nervous system is also highly relevant for the pre- and postnatal development of the fetus and is considered a tissue of great vulnerability to HPA axis imbalances and inflammatory responses to stressful stimuli (Delhaye-Bouchaud, 2001; Donovan & Dyer, 2005; Rodier, 1994).

Studies have shown that chronic stress also impacts the regulation of inflammatory processes (Du et al., 2017). The deregulation of the HPA axis caused by stress alters the inflammatory balance, which can generate increased pro-inflammatory cytokines levels, such as IL-6 and TNF (Khan et al., 2021; Kim et al., 2019). Cytokines are involved in different functions, including homeostatic regulation, injury neutralization, and tissue remodeling, in addition to participating in neural mechanisms of plasticity and regulation of neurotransmitters (Denney et al., 2021). Considering that cytokines are able to cross the blood–brain barrier, the repercussions of increased levels of inflammatory markers may also be more significant during stressful situations (Kim et al., 2019).

Convincing data demonstrate that physical exercise during the gestational period may be considered a form of treatment and prevention to the development of chronic diseases (Lee et al., 2016). Therefore, the use of physical exercise to counterbalance stressful situations may induce positive effects, such as a better ability to regulate glucocorticoid secretion, which can benefit neural, cardiovascular, and metabolic functions, in addition to helping to modulate the inflammatory process (Luft et al., 2020; Marcelino et al., 2015; Stranahan et al., 2008). However, few studies have examined the effects of maternal physical exercise on the response of inflammatory and HPA axis markers. Thus, the objective of this study was to analyze the effects of maternal physical exercise before and during the gestational period on changes in the inflammatory and HPA axis markers in both brain and lung tissues of the offspring.

## 2 | MATERIAL AND METHODS

### 2.1 | Animals

Male and female Balb/cByJ mice were obtained from the Center for Experimental Biological Models (CEMBE) at PUCRS. These animals were kept in a controlled temperature environment (24 ± 2°C), 12-h light/dark cycle, with free access to water and food. All the experiments were performed in agreement with international ethical standards and following the local animal protection guidelines. The experimental protocol was approved by the Ethics Research Committee (protocol number 9923) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

### 2.2 | Experimental design

Adult females with approximately 60 days of life were divided into three experimental groups: (I) control (CONT); (II) prenatal restraint stress (PNS); (III) prenatal restraint stress and physical exercise before and during the gestational period (PNS+EX). The CONT group was kept in their cages and only handled during the cleaning routine. All females were housed in groups of five until mating. The exercise protocol started 3 weeks before mating. Then, females were kept together with males of the same breed to allow mating. With the confirmation of mating, considered as day 0 of gestation (G0), females were relocated individually in cages separate from the males and, on the eighth day of gestation (G8), the prenatal restraint stress protocol was started. In addition, on G8, the females were returned to the exercise protocol until the 21st day of life (G21/PND0). A cesarean section was performed on females at G21 (PND0) and the pups were removed to obtain the brain and lung tissues. The segmentation of brain tissue was performed by free-hand dissection (Spijker, 2011). Briefly, after decapitation, scissors were used to open the skull and remove the layers of meninges from the brain. In the midline, a division of the brain was performed and layers were removed until the visualization of the hippocampus. Finally, the left and right hippocampi were very carefully manually removed using tweezers. In addition, for PND0 experiments, males and females were combined, as sexual differentiation in mice at this age is challenging due to the small size of their internal sex organs and the small difference in the anogenital distance. Another cohort of animals was used to perform the same analyses on the 10th day of life (PND10). This day was chosen based on the key milestones in rodent postnatal central nervous system development. The gene expression of GR, MR, IL-6, IL-10, and TNF-$\alpha$ was evaluated in the brain and lungs. The protein expression of MMP-2 was evaluated in the lungs.

### 2.3 | Prenatal stress

Females from PNS and PNS+EX groups were submitted to the prenatal restraint stress protocol starting on G8. The animals were kept immobile in a closed acrylic cylinder for 30 minutes per day, every other day, until the day of birth of the offspring (Du et al., 2017).

### 2.4 | Exercise protocol

Females from the PNS+EX group were submitted to a protocol of physical exercise on a treadmill. The exercise was performed during the 3 weeks that precede the mating day and returned from G8 to G21. Physical exercise was performed at a speed of 10 m/min, for

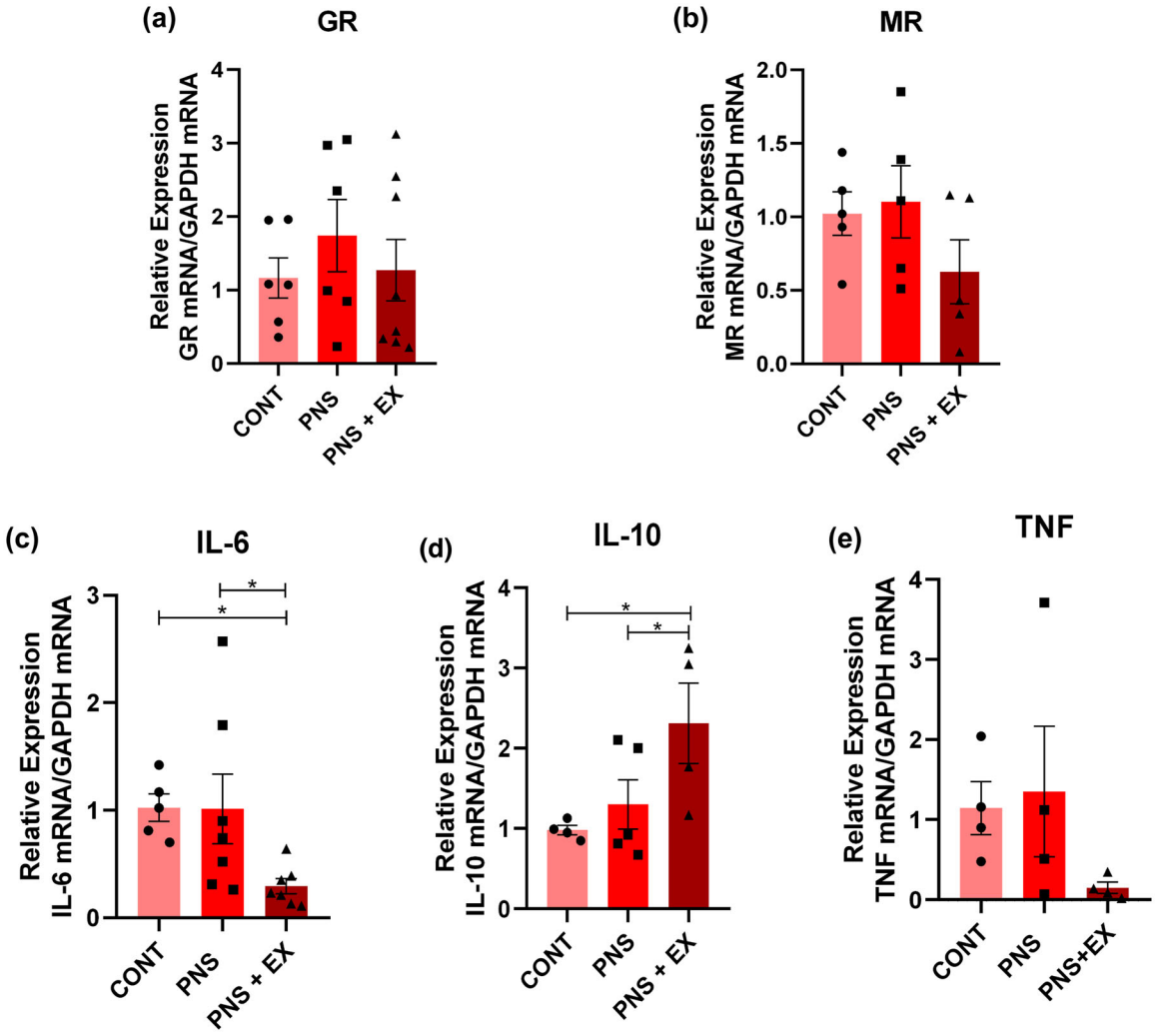


**FIGURE 1** Effects of prenatal stress and maternal physical exercise on the brain of animals on day 0 (PND0). The graphs show the gene expression of GR (a), MR (b), IL-6 (c), IL-10 (d), and TNF (e). The number of animals is presented in each bar graph. Results were evaluated by one-way ANOVA followed by the LSD posttest. Data are presented as mean and standard error of the mean. Asterisk (*) indicates significant difference with $p \leq .05$ in comparisons between groups

60 minutes, 5 days a week. In the last week of pregnancy, the speed was reduced to 6 m/min. No stimulus, such as electric shock, was applied to stimulate animals to perform the activity. Animals that refused to practice physical exercise spontaneously were excluded from the study ($n = 5$). Females from CONT and PNS groups only performed spontaneous activities in their cages (Kim et al., 2019).

## 2.5 | Euthanasia

The euthanasia of the animals (pregnant females and offspring) was performed by decapitation to allow the rapid removal of the tissues. Anesthetics were not used due to the well-known influence on the stress-induced response. Subsequently, lung and brain samples were removed and stored in RNA-Later (Thermo Fisher Scientific) for 24 h at 4°C and then transferred to a freezer at −20°C until final processing.

## 2.6 | Gene expression

The total RNA from brain and lung tissues was extracted using Trizol (Thermo Fisher Scientific), according to the manufacturer's instructions. The RNA was resuspended in 20 $\mu$l of nuclease-free water (Ambion-Thermo Fisher Scientific) and converted into complementary DNA (cDNA) (GoScript™ Reverse Transcription System Protocol - Promega), following the manufacturer's instructions. The quantification of cDNA was performed using spectrophotometry (Nanodrop Spectrophotometer-Model 1000, Thermo Fisher Scientific). Then,

gene expression was evaluated by real-time quantitative PCR (Step One Plus – Applied Biosystems – Thermo Fisher Scientific). The relative expression of mRNA was calculated using the Delta-Delta Ct (ΔΔCt) formula, using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous reference gene. To identify contamination, a negative control of each primer was used. The calculation of the proportions of the amplification components was based on the inclusion of the fluorescent marker SYBR® Green (Applied Biosystems – Thermo Fisher Scientific) in the cDNA for each amplification response. The set of specific primers for each gene as follows: GR (forward 5′ GGAATAGGTGCCAAGGGTCT 3′ and reverse 5′ GAGCACACCAGGCAGAGTTT 3′), MR (forward 5′ CCAGTTCTCCGTTCTCTGTA 3′ and reverse 5′ CTTGAGCACCAATCCGGTAG 3′), IL-6 (forward 5′ CTGACCACAGTGAGGAATGTCCAC 3′ and reverse 5′ TGGAGTCACAGAAGGAGTGGCTAA 3′), IL-10 (forward 5′ CCCTTTGCTATGGTGTCCTT 3′ and reverse 5′ TGGTTTCTCTTCCCAAGACC 3′), TNF (forward 5′ CACCCCGAAGTTCAGTAGACA 3′ and reverse 5′ ATAGCDHAGAA3′), and GAPDH (forward 5′ GGGGAGCCAAAAGGGTCATC 3′ and reverse 5′ GACGCCTGCTTCACCACCTTCTTG 3′).

## 2.7 | Protein expression

The proteins from lung samples were extracted using Tris-HCl 10 mM, pH 7.5, $MgCl_2$ 1 mM, 1 mM EDTA, PMSF 0.1 mM, B-mercaptoethanol 5 mM, Chaps 0.5%, and 10% glycerol. Final quantification was verified by spectrophotometry (Nanodrop Spectrophotometer-Model 1000, Thermo Fisher Scientific).

For the western blot, 40 $\mu$g of protein was used, fractionated by vertical polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were transferred to a nitrocellulose membrane (Amersham – Sigma). After transfer, the membrane was placed in Tween-Tris saline buffer (TTBS; 100 mM Tris-HCL, pH 7.5 including 0.5% Tween 20) with 5% albumin for 1 h at room temperature. Then, the membrane was incubated overnight at 4°C with the primary antibodies MMP-2 (1:500, Santa Cruz Biotechnology – sc13595) and GAPDH (1:100, Invitrogen – ZG003). The next day, the membrane was washed three times with TTBS and incubated for 2 h with the secondary antibodies (1:1000, Invitrogen – A16160). Afterward, the membrane was washed again with TBS. Finally, the immunoreactivity was identified through chemiluminescence modifications (ECL Western Blotting Substrate Kit – Abcam). The percent of density was analyzed by Image J software (National Institutes of Health, USA) and the levels of MPP-2 were standardized with relative levels of their total amount for GAPDH levels.

## 2.8 | Statistical analysis

The normality of data was evaluated using the Shapiro–Wilk test. Results were expressed as mean ± standard error of the mean (SEM). The comparisons between experimental groups were performed using a one-way analysis of variance (ANOVA), followed by the LSD posttest, using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). The significance level was set at $p \leq .05$.

# 3 | RESULTS

## 3.1 | Effects of prenatal stress and maternal physical exercise on inflammatory and HPA axis markers in PND0 mice

### 3.1.1 | Brain

There were no significant differences in the gene expression of GR ($F_{(2,17)} = 0.507$, $p = .61$) and MR ($F_{(2,12)} = 1.508$, $p = .26$) (Figure 1a,b). When inflammatory markers were analyzed, there was a reduction in IL-6 ($F_{(2,16)} = 3.736$, $p = .04$) gene expression in the brain of PNS+EX animals when compared to CONT ($p = .03$) and PNS ($p = .02$) animals (Figure 1c). As for IL-10 ($F_{(2,10)} = 3.924$, $p = .05$), an increase in this anti-inflammatory cytokine was observed in the PNS+EX group when compared to the CONT ($p = .02$) and PNS ($p = .05$) groups (Figure 1d). These results indicate a beneficial effect of maternal physical exercise in reducing the expression of IL-6 and increasing IL-10. There were no significant differences for TNF ($F_{(2,9)} = 1.594$, $p = .25$) (Figure 1e). No other effects of exercise were seen.

### 3.1.2 | Lungs

For the lung analyses, there was a decrease in GR ($F_{(2,14)} = 5.096$, $p = .02$) in the PNS ($p = .01$) and PNS+EX ($p = .01$) groups when compared to the CONT group (Figure 2a). A decrease in MR ($F_{(2,17)} = 13.87$, $p = .0003$) was also observed both in PNS ($p = .0001$) and PNS+EX ($p = .001$) compared to CONT (Figure 2b). A reduction in the IL-6 ($F_{(2,13)} = 6.882$, $p = .009$) gene expression was observed in the PNS ($p = .007$) and PNS+EX ($p = .007$) groups (Figure 2c) compared to the CONT group. The same reduction was found for the TNF ($F_{(2,12)} = 11.94$, $p = .001$) gene expression in PNS ($p = .002$) and PNS+EX ($p = .0005$) groups when compared to CONT (Figure 2e). The PNS group ($p = .006$) showed decreased IL-10 ($F_{(2,11)} = 5.517$, $p = .02$) expression when compared to the CONT group (Figure 2d). There were no significant differences in the protein expression of MMP-2 ($F_{(2.14)} = 0.381$, $p = .69$) (Figure 3).

## 3.2 | Effects of prenatal stress and maternal physical exercise on inflammatory and HPA axis markers in PND10 mice

### 3.2.1 | Female hippocampus

There were no significant differences in the gene expression of GR ($F_{(2,16)} = 0.5261$, $p = .60$) and TNF ($F_{(2,10)} = 1.876$, $p = .20$) (Figure 4a,e). A significant increase in the MR ($F_{(2,14)} = 9.167$, $p = .002$) gene

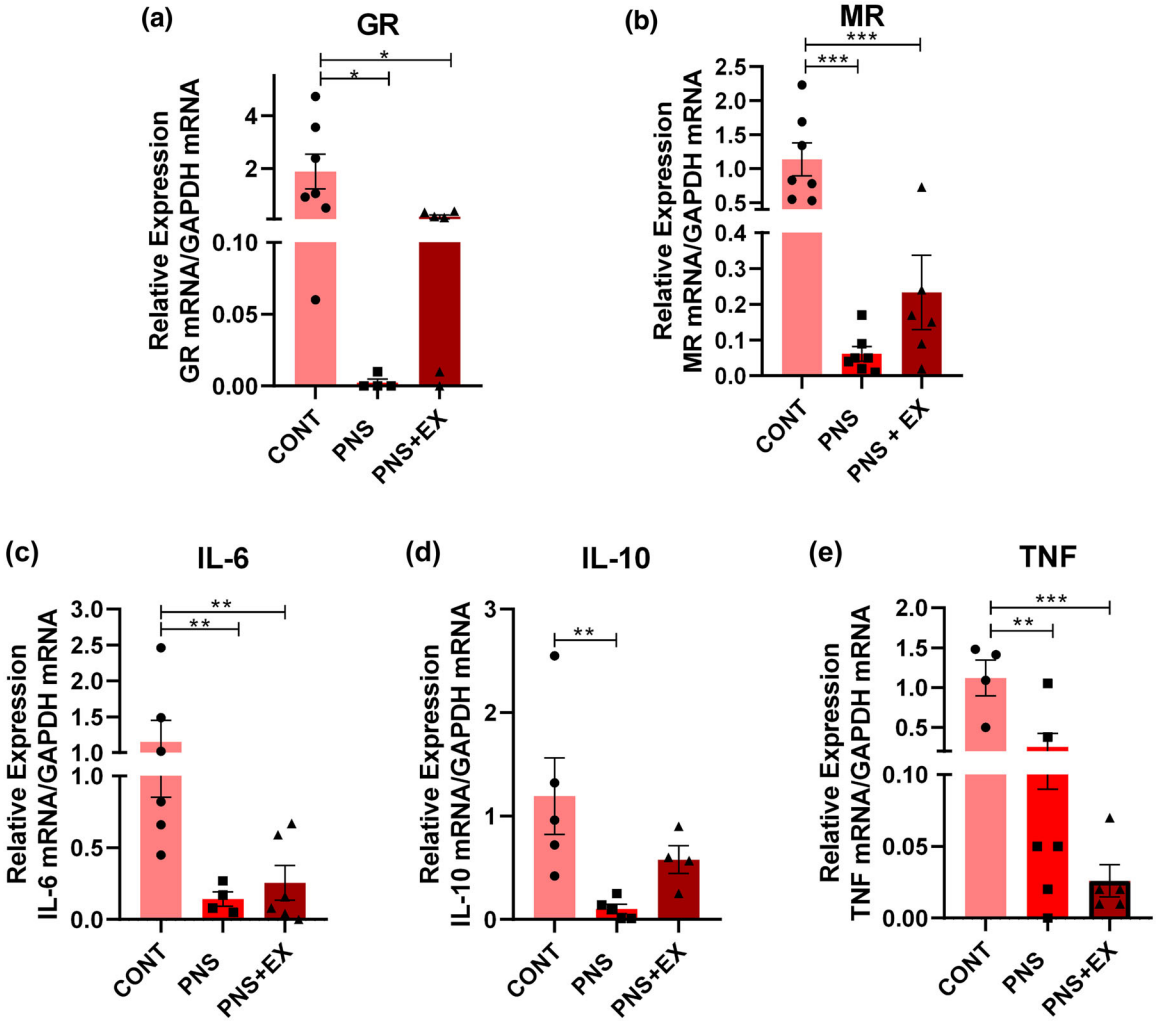


**FIGURE 2** Effects of prenatal stress and maternal physical exercise on the lung of animals on day 0 (PND0). Gene expression of GR (a), MR (b), IL-6 (c), IL-10 (d), and TNF (e) is shown. The number of animals is presented in each bar graph. Results were evaluated by one-way ANOVA followed by the LSD posttest. Data are presented as mean and standard error of the mean. Single asterisk (*) indicates significant difference with $p \leq .05$. Double asterisks (**) indicate significant difference with $p \leq .01$. Triple asterisks (***) indicate significant difference with $p \leq .001$ in comparisons between groups

expression in the PNS group when compared to both CONT ($p = .003$) and PNS+EX ($p = .001$) groups was identified (Figure 4b). A similar effect of prenatal stress was seen for both IL-6 ($F_{(2,10)} = 15.62$, $p = .0008$) and IL-10 ($F_{(2,9)} = 5.254$, $p = .03$) gene expression when compared to CONT ($p = .004$ and $p = .01$, respectively) and PNS+EX ($p = .0003$ and $p = .04$, respectively) groups (Figure 4c,d). Maternal exercise was able to reverse the effects of prenatal stress on both MR (Figure 4b) and IL-6 (Figure 4c) gene expression, evidenced by the significant reduction seen in the PNS+EX group when compared to the PNS group ($p = .003$ and $p = .001$, respectively).

### 3.2.2 | Male hippocampus

There was a significant reduction in the GR ($F_{(2,14)} = 6.313$, $p = .01$) gene expression in PNS animals when compared to CONT ($p < .01$). Maternal exercise failed to reverse the stress-induced effects (Figure 4f). No other significant differences were found (Figure 4g–j).

### 3.2.3 | Female lung

Measurements in lung tissue showed that physical exercise before and during the gestational period may reverse the effects of stress on TNF ($F_{(2,11)} = 232.7$, $p = .0001$) gene expression, as shown by the difference between PNS+EX ($p = .03$) and PNS groups (Figure 5e). Regarding IL-6 ($F_{(2,11)} = 2.766$, $p = .10$) and IL-10 ($F_{(2,12)} = 1.454$, $p = .27$) gene expression, no significant differences were observed (Figure 5c,d). As for GR ($F_{(2,9)} = 6.031$, $p = .02$), an increased expression was observed in the PNS+EX group ($p = .02$) compared to the PNS group (Figure 5a). In addition, there was an increase in the gene expression of MR ($F_{(2,10)} = 5.504$, $p = .02$) in PNS animals compared to both

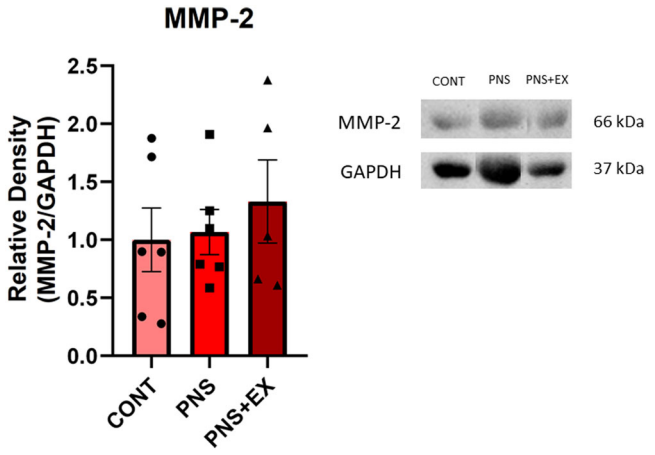


**FIGURE 3** Effects of prenatal stress and maternal physical exercise on MMP-2 protein expression in the lungs of animals on day 0 (PND0). Relative density and molecular weights of MMP-2 and GAPDH are shown. The number of animals is presented in each bar graph. Results were evaluated by one-way ANOVA followed by the LSD posttest. Data are presented as mean and standard error of the mean. There were no significant differences between groups

CONT ($p = .02$) and PNS+EX ($p = .01$) (Figure 5b). There were no differences between groups in the MMP-2 ($F_{(2.15)} = 0.696, p = .51$) protein analysis (Figure 6a).

### 3.2.4 | Male lung

In the analysis of male lung tissue, there was an effect of stress with a reduction of GR ($F_{(2,15)} = 7.915$, $p = .004$) expression in the PNS ($p < .01$) and PNS+EX ($p = .01$) groups compared to the CONT (Figure 5f). Regarding MR ($F_{(2,15)} = 5.014$, $p = .02$), there was an exercise effect demonstrated by a decrease in the PNS+EX group ($p < .01$) when compared to the CONT (Figure 5g). There were no differences between groups on markers of the inflammatory process (Figure 5h–j), as well as on the protein expression of MMP-2 ($F_{(2.13)} = 0.301, p = .74$) (Figure 6b).

## 4 | DISCUSSION

The results obtained in this study indicate that prenatal stress generated a series of alterations, including a reduction in HPA axis markers and cytokines in the lungs of animals on day 0, as well as an increase in the hippocampus of females on day 10. Furthermore, physical exercise before and during the gestational period was able to reverse some of these effects, particularly in the hippocampus and lungs of females at day 10 of life. Available evidence indicates that prenatal stress can generate several deleterious effects, including an increase in inflammatory cytokines, hyperactivation of the HPA axis, an increase in glucocorticoid exposure, and reduction in GR and MR receptors, in addition to behavioral effects leading to the development of anxiety and depression (Burgueno et al., 2020; Harris & Glucocorticoids, 2011; Rakers et al., 2020). On the other hand, the effects of prenatal stress are still poorly explored in peripheral organs, such as the lungs, and at early ages, especially during the neonatal period. Likewise, the effects of physical exercise during pregnancy as an alternative for the prevention of stress-induced changes and diseases are also poorly studied. Our data demonstrated different responses promoted by exercise and stress on inflammatory and HPA axis markers, in an effect that appears to be tissue, age, and sex dependent.

At first, we have analyzed the brain and lung tissues of animals on day 0 (PND0), in which it was possible to see a beneficial effect of exercise on inflammatory cytokines in the brain. PNS+EX group showed a reduction on IL-6 and an increase on IL-10 gene expression. On the other hand, in the lungs, stress induced different effects, including a reduction of GR, MR, and IL-10, which may induce long-lasting alteration for the fetus. Surprisingly, prenatal stress reduced the levels of IL-6 and TNF in the lungs of animals on day 0. IL-6 and TNF are known markers of the inflammatory process and have been associated with inflammatory diseases in the respiratory system (Tutkun et al., 2019). IL-6 is a multifunctional pro-inflammatory cytokine that causes an increase in airway resistance, depending on its levels, which may lead to greater mechanical effort, playing a role in diseases such as asthma and chronic obstructive pulmonary disease (Rubini, 2013). However, during muscle contraction generated by physical exercise, there is a higher production of IL-6 that acts by activating glycogenolysis and generating extra energy to the muscles, which demonstrates that this cytokine does not exclusively acts promoting inflammation (Metsios et al., 2020). On the other hand, TNF has the function of organizing some cytokines, increasing the inflammatory response, and contributing to the process of fibrosis and terminal lung diseases (Zhang et al., 1997). A previous study from our group showed that prenatal stress may reduce the impact of asthma in adult mice (Vargas et al., 2016). On the other hand, evidence in the literature demonstrates that gestational stress is related to an increased risk of developing asthma and other diseases, including mental disorders and morphological and structural alterations in the hippocampus (Burgueno et al., 2020; Turcotte-Tremblay et al., 2014). Nevertheless, the results presented here indicate beneficial effects of maternal treadmill exercise on inflammatory markers in the brain of prenatally stressed mice, corroborating a previous study in which exercise has been shown beneficial by helping to prevent the effects caused by stress in adult mice (Luft et al., 2020). The impacts of prenatal stress and the possible effects of physical exercise on lung tissue still need to be further investigated. Indeed, although we are not able to fully explain the differences found between brain and lung tissue, there is evidence showing that cytokines react in different ways depending on the analyzed tissue, such as an increase in peripheral muscles and a reduction in the heart (Metsios et al., 2020).

Considering that the effects of prenatal stress can generate different responses throughout development, we have performed another experiment to analyze the hippocampus of males and females at PND10. In females, we have observed that maternal exercise was able to prevent the effects of prenatal stress, promoting a decrease in

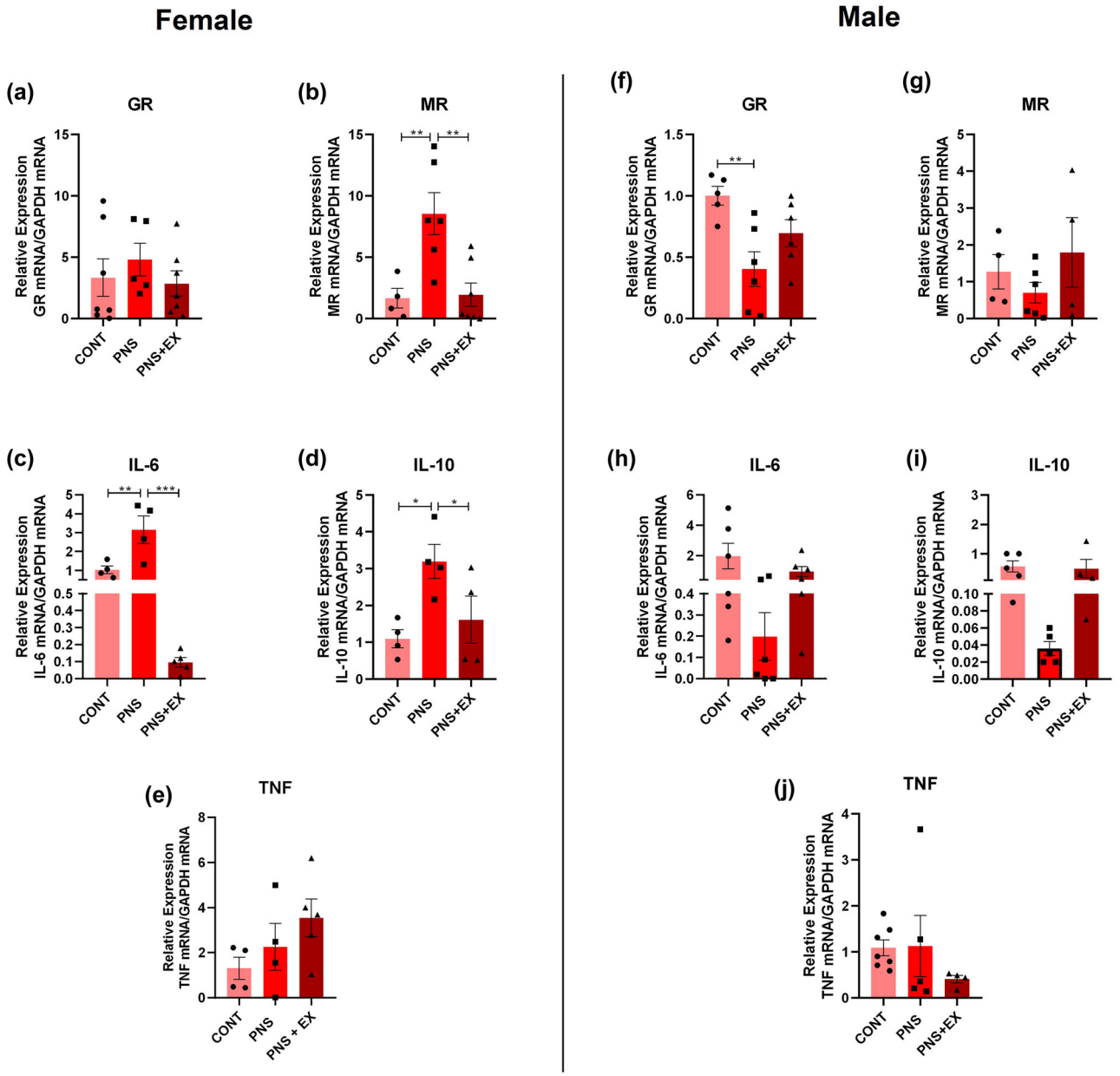


**FIGURE 4** Effects of prenatal stress and maternal physical exercise on the hippocampus of animals on day 10 (PND10). Gene expression of GR (a), MR (b), IL-6 (c), IL-10 (d), and TNF (e) in females, and GR (f), MR (g), IL-6 (h), IL-10 (i), and TNF (j) in males, is shown. The number of animals is presented in each bar graph. Results were evaluated by one-way ANOVA followed by the LSD posttest. Data are presented as mean and standard error of the mean. Single asterisk (*) indicates significant difference with $p \leq .05$. Double asterisks (**) indicate significant difference with $p \leq .01$. Triple asterisks (***) indicate significant difference with $p \leq .001$ in comparisons between groups

IL-6 gene expression. This cytokine is closely related to the adaptations induced by physical exercise, since muscle contraction is one of the stimuli for its regulation (Shephard, 2002). Despite having important effects on peripheral metabolism, a sustained increase on its levels can trigger an inflammatory process (Metsios et al., 2020). In females, our data demonstrate that prenatal stress induced an increase in markers such as MR and IL-10. MR and GR in the hippocampus have the role of regulating the HPA axis by controlling the levels of glucocorticoid secretion. Studies have shown that changes in the levels of these receptors in the hippocampus may promote hyperactivation of the axis (Zhe et al., 2008). IL-10 is an anti-inflammatory cytokine with several functions in the brain, including the survival of neurons and glial cells (Strle et al., 2001). It also plays a protective role against the action of pro-inflammatory cytokines, a mechanism that occurs in the progression of several diseases in the central nervous system (Strle et al., 2001). Regarding the effects on males, our data showed that stress caused a decrease in GR expression, confirming evidence from the literature (Stephens & Wand, 2012). In addition, a study (Weinstock, 2007) reported that when prolonged restraint stress was applied to mothers, there was a decrease in GR and MR in the

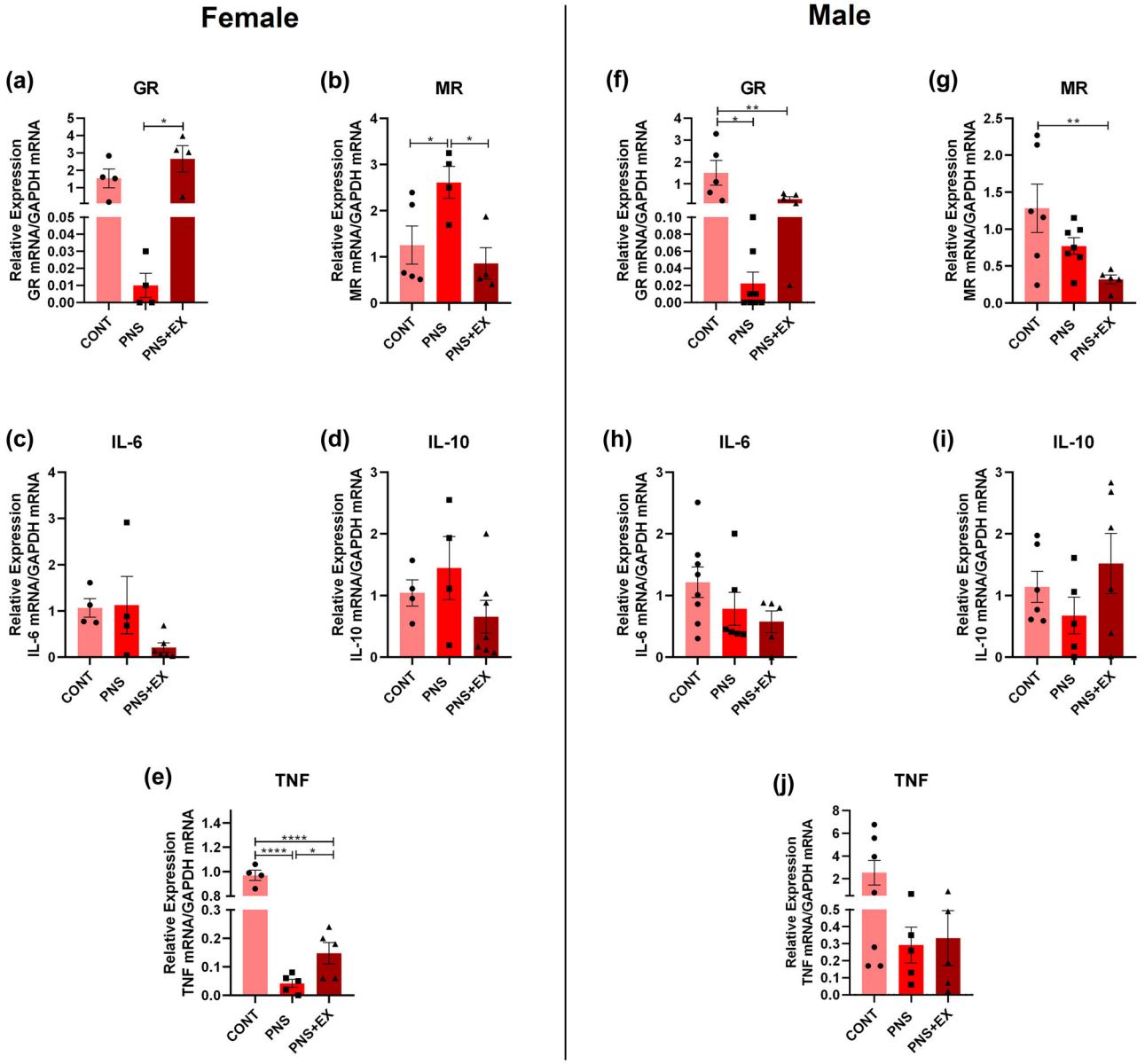


**FIGURE 5** Effects of prenatal stress and maternal physical exercise on the lungs of animals on day 10 (PND10). Gene expression of GR (a), MR (b), IL-6 (c), IL-10 (d), and TNF (e) in females, and GR (f), MR (g), IL-6 (h), IL-10 (i), and TNF (j) in males, is shown. The number of animals is presented in each bar graph. Results were evaluated by one-way ANOVA followed by the LSD posttest. Data are presented as mean and standard error of the mean. Double asterisks (**) indicate significant difference with $p \leq .01$. Quadruple asterisks (****) indicate significant difference with $p \leq .001$ in comparisons between groups

hippocampus of both sexes, impairing the regulation of the feedback of the HPA axis. On the other hand, restraining once a day only generated responses in female offspring, showing a lower sensitivity for males (Weinstock, 2007). Comparatively, the development of male fetuses is more directed to growth, becoming less adaptable to environmental challenges in utero (Sutherland & Brunwasser, 2018). Although few studies present comparisons between sexes, it is well-known that there are numerous sexual differences, including clinical data demonstrating that men have slower cortical development, increasing the risk for schizophrenia and the susceptibility to long-term developmental alterations (Damiani et al., 2005).

Furthermore, we have evaluated the lung tissue of these animals. In females, we found a reduction on TNF and an increase on MR in response to prenatal stress. On the other hand, it was also possible to observe a beneficial effect of physical exercise in the effects of prenatal stress on GR gene expression. Moreover, we have also evaluated MMP-2 protein expression. MMP-2 plays an important role in lung development, being associated with tissue remodeling, regulation of the inflammatory process, and repair of tissue injuries, in addition to playing a crucial role in maintaining oxygenation (Hendrix & Kheradmand, 2017; Ling et al., 2019). Despite this, our results did not show significant effects of prenatal stress and/or exercise on

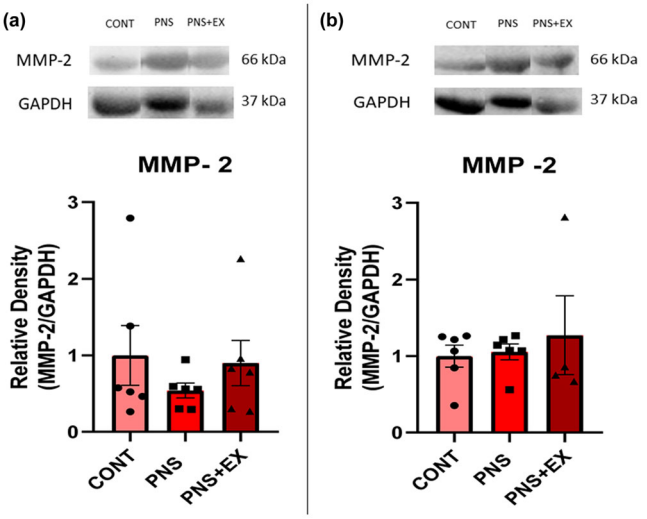


**FIGURE 6** Effects of prenatal stress and maternal physical exercise on MMP-2 protein expression in the lungs on day 10 (PND10). Relative density and molecular weights of MMP-2 and GAPDH are shown for females (a) and males (b). The number of animals is presented in each bar graph. Results were evaluated by one-way ANOVA followed by LSD posttest. Data are presented as mean and standard error of the mean. There were no significant differences between groups

MMP-2 protein expression in the lungs of any of the experimental groups evaluated. As for the western blotting analysis, it should be noted that some bands have different intensities, although all samples were previously quantified and both the target protein (MMP-2) and the charge control (GAPDH) from the same sample were run on the same gel. In addition, previous studies with prenatal stress used GAPDH as a load control, demonstrating that early life stress does not promote effects on the expression of this protein (Zhou, 2020). Taken together, females seem to be more vulnerable to the effects of prenatal stress on markers of both HPA axis and inflammatory response. These effects may be related to an interaction between different programming mechanisms and hormonal factors (Brunton et al., 2015; Moisiadis & Matthews, 2014).

This study also presents limitations, including the impossibility to adequately separate brain structures and sexes on day 0 (PND0). Therefore, considering the age of the animals studied and the respective size of the structures, results were presented for the whole brain and without separation between males and females at this age. In addition, the small sample size for some of the analysis may also be considered as a limitation of present study.

## 5 | CONCLUSION

The results of the present study confirm the effects induced by prenatal stress and demonstrate that physical exercise before and during the gestational period may have a protective role on inflammatory changes. Our findings seem to be specific and different depending on the tissue, age, and sex studied.

## AUTHOR CONTRIBUTIONS

Mariana Severo da Costa conceived the work, acquired data, drafted the paper, performed data analysis, and approved the final version. Carolina Luft and Mariana Sbruzzi acquired data, revised the article, and approved the final version. Carolina Luft, Jarbas Rodrigues de Oliveira, and Márcio Vinícius Fagundes Donadio conceived the work, revised the paper, and approved the final version. Márcio Vinícius Fagundes Donadio acquired funding and performed data analysis.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## 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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## REFERENCES

Akbaba, N., Annagur, B. B., Annagur, A., Akbulut, H., Akyurek, F., & Celik, C. (2018). Neurotrophins and neuroinflammation in fetuses exposed to maternal depression and anxiety disorders during pregnancy: A comparative study on cord blood. *Archives of Women's Mental Health*, *21*(1), 105–111. https://doi.org/10.1007/s00737-017-0774-1

Bangasser, D. A., & Valentino, R. J. (2014). Sex differences in stress-related psychiatric disorders: Neurobiological perspectives. *Frontiers in Neuroendocrinology*, *35*(3), 303–319. https://doi.org/10.1016/j.yfrne.2014.03.008

Boersma, G. J., & Tamashiro, K. L. (2015). Individual differences in the effects of prenatal stress exposure in rodents. *Neurobiology of Stress*, *1*, 100–108. https://doi.org/10.1016/j.ynstr.2014.10.006

Brunton, P. J., Donadio, M. V., Yao, S. T., Greenwood, M., Seckl, J. R., Murphy, D., & Russell, J. A. (2015). 5alpha-Reduced neurosteroids sex-dependently reverse central prenatal programming of neuroendocrine stress responses in rats. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, *35*(2), 666–677. https://doi.org/10.1523/JNEUROSCI.5104-13.2015

Burgueno, A. L., Juarez, Y. R., Genaro, A. M., & Tellechea, M. L. (2020). Prenatal stress and later metabolic consequences: Systematic review and meta-analysis in rodents. *Psychoneuroendocrinology*, *113*, 104560. https://doi.org/10.1016/j.psyneuen.2019.104560

Buss, C., Entringer, S., & Wadhwa, P. D. (2012). Fetal programming of brain development: Intrauterine stress and susceptibility to psychopathology. *Science Signaling*, *5*(245), pt7.

Damiani, D., Damiani, D., Ribeiro, T. M., & Setian, N. (2005). Brain sex: Just beginning to pave the way. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *49*, 37–45.

Delhaye-Bouchaud, N. (2001). [Development of the central nervous system in mammals]. *Neurophysiologie Clinique = Clinical Neurophysiology*, *31*(2), 63–82. https://doi.org/10.1016/S0987-7053(01)00249-0

Denney, J. M., Nelson, E., Wadhwa, P., Waters, T., Mathew, L., Goldenberg, R. L., & Culhane, J. F. (2021). Cytokine profiling: Variation in immune modulation with preterm birth vs. uncomplicated term birth identifies pivotal signals in pathogenesis of preterm birth. *Journal of Perinatal Medicine*, *49*(3), 299–309. https://doi.org/10.1515/jpm-2020-0025

Donovan, S. L., & Dyer, M. A. (2005). Regulation of proliferation during central nervous system development. *Seminars in Cell & Developmental Biology*, *16*(3), 407–421.

Du, S. F., Yu, Q., Chuan, K., Ye, C. L., He, Z. J., Liu, S. J., Zhu, X. - Y., & Liu, Y. - J. (2017). In obese mice, exercise training increases 11beta-HSD1 expression, contributing to glucocorticoid activation and suppression of pulmonary inflammation. *Journal of Applied Physiology*, *123*(4), 717–727. https://doi.org/10.1152/japplphysiol.00652.2016

Harris, A., & Glucocorticoids, S. J. (2011). prenatal stress and the programming of disease. *Hormones and behavior*, *59*(3), 279–289. https://doi.org/10.1016/j.yhbeh.2010.06.007

Hendrix, A. Y., & Kheradmand, F. (2017). The role of matrix metalloproteinases in development, repair, and destruction of the lungs. *Progress in Molecular Biology and Translational Science*, *148*, 1–29. https://doi.org/10.1016/bs.pmbts.2017.04.004

Iwasaki-Sekino, A., Mano-Otagiri, A., Ohata, H., Yamauchi, N., & Shibasaki, T. (2009). Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. *Psychoneuroendocrinology*, *34*(2), 226–237.

Khan, J., Wang, Q., Ren, Y., Eliav, R., Korczeniewska, O. A., Benoliel, R., & Eliav, E. (2021). Exercise induced hypoalgesia profile in rats is associated with IL-10 and IL-1 beta levels and pain severity following nerve injury. *Cytokine*, *143*, 155540. https://doi.org/10.1016/j.cyto.2021.155540

Kim, Y. K., Amidfar, M., & Won, E. (2019). A review on inflammatory cytokine-induced alterations of the brain as potential neural biomarkers in post-traumatic stress disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *91*, 103–112.

Lee, S. J., Kim, T. W., Park, H. K., Yoon, S., You, A. H., Moon, E. J., Shin, D. H., & Cho, H. (2016). Postnatal treadmill exercise alleviates prenatal stress-induced anxiety in offspring rats by enhancing cell proliferation through 5-hydroxytryptamine 1A receptor activation. *International Neurourology Journal*, *20*(1), S57–S64. https://doi.org/10.5213/inj.1632600.309

Ling, L., Li, Y., Li, H., Li, W., & Zhang, H. B. (2019). MMP-2 and MMP-9 gene polymorphisms act as biological indicators for ulinastatin efficacy in patients with severe acute pancreatitis. *Medicine*, *98*(24), e15831. https://doi.org/10.1097/MD.0000000000015831

Luft, C., Levices, I. P., da Costa, M. S., Haute, G. V., Grassi-Oliveira, R., de Oliveira, J. R., & Fagundes Donadio, M. V. (2020). Exercise before pregnancy attenuates the effects of prenatal stress in adult mice in a sex-dependent manner. *International Journal of Developmental Neuroscience*, *80*(2), 86–95. https://doi.org/10.1002/jdn.10001

Marcelino, T. B., de Lemos Rodrigues, P. I., Miguel, P. M., Netto, C. A., Pereira Silva, L. O., & Matte, C. (2015). Effect of maternal exercise on biochemical parameters in rats submitted to neonatal hypoxia-ischemia. *Brain Research*, *1622*, 91–101. https://doi.org/10.1016/j.brainres.2015.06.024

Metsios, G. S., Moe, R. H., & Kitas, G. D. (2020). Exercise and inflammation. *Best practice & research Clinical rheumatology*, *34*(2), 101504.

Moisiadis, V. G., & Matthews, S. G. (2014). Glucocorticoids and fetal programming part 2: Mechanisms. *Nature reviews Endocrinology*, *10*(7), 403–411. https://doi.org/10.1038/nrendo.2014.74

Mok, P. L., Anandasayanam, A. N. K., Oscar David, H. M., Tong, J., Farhana, A., Khan, M. S. A., Sivaprakasam, G., Ee-Hwan Koh, A., & Alzahrani, B. (2021). Lung development, repair and cancer: A study on the role of MMP20 gene in adenocarcinoma. *PLoS ONE*, *16*(4), e0250552. https://doi.org/10.1371/journal.pone.0250552

Munck, A., Guyre, P. M., & Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews*, *5*(1), 25–44. https://doi.org/10.1210/edrv-5-1-25

Rakers, F., Rupprecht, S., Dreiling, M., Bergmeier, C., Witte, O. W., & Schwab, M. (2020). Transfer of maternal psychosocial stress to the fetus. *Neuroscience and Biobehavioral Reviews*, *117*, 185–197.

Rodier, P. M. (1994). Vulnerable periods and processes during central nervous system development. *Environmental Health Perspectives*, *102*(2), 121–124.

Rothe, N., Steffen, J., Penz, M., Kirschbaum, C., & Walther, A. (2020). Examination of peripheral basal and reactive cortisol levels in major depressive disorder and the burnout syndrome: A systematic review. *Neuroscience and Biobehavioral Reviews*, *114*, 232–270. https://doi.org/10.1016/j.neubiorev.2020.02.024

Rubini, A. (2013). Interleukin-6 and lung inflammation: Evidence for a causative role in inducing respiratory system resistance increments. *Inflammation & allergy drug targets*, *12*(5), 315–321.

Shephard, R. J. (2002). Cytokine responses to physical activity, with particular reference to IL-6: Sources, actions, and clinical implications. *Critical reviews in immunology*, *22*(3), 165–182.

Spijker, S. (2011). Dissection of rodent brain regions. In K. Li (Ed.), *Neuroproteomics* (Vol. 57, pp. 13–26). Humana Press.

Stephens, M. A., & Wand, G. (2012). Stress and the HPA axis: Role of glucocorticoids in alcohol dependence. *Alcohol research : current reviews*, *34*(4), 468–483.

Stranahan, A. M., Lee, K., & Mattson, M. P. (2008). Central mechanisms of HPA axis regulation by voluntary exercise. *Neuromolecular Medicine*, *10*(2), 118–127. https://doi.org/10.1007/s12017-008-8027-0

Strle, K., Zhou, J. H., Shen, W. H., Broussard, S. R., Johnson, R. W., Freund, G. G., Dantzer, R., & Kelley, K. W. (2001). Interleukin-10 in the brain. *Critical reviews in immunology*, *21*(5), 427–449.

Sutherland, S., & Brunwasser, S. M. (2018). Sex differences in vulnerability to prenatal stress: A review of the recent literature. *Current psychiatry reports*, *20*(11), 1–12. https://doi.org/10.1007/s11920-018-0961-4

Sze, Y., & Brunton, P. J. (2020). Sex, stress and steroids. *The European Journal of Neuroscience*, *52*(1), 2487–2515. https://doi.org/10.1111/ejn.14615

Turcotte-Tremblay, A. M., Lim, R., Laplante, D. P., Kobzik, L., Brunet, A., & King, S. (2014). Prenatal maternal stress predicts childhood asthma in girls: Project ice storm. *BioMed Research International*, *2014*, 201717. https://doi.org/10.1155/2014/201717

Tutkun, L., Iritas, S. B., Deniz, S., Oztan, O., Abusoglu, S., Unlu, A., Türksoy, V. A., & Çetintepe, S. P. (2019). TNF-alpha and IL-6 as biomarkers of impaired lung functions in dimethylacetamide exposure. *Journal of medical biochemistry*, *38*(3), 276–283. https://doi.org/10.2478/jomb-2018-0040

Vargas, M. H., Campos, N. E., de Souza, R. G., da Cunha, A. A., Nunez, N. K., Pitrez, P. M., & Fagundes Donadio, M. V. (2016). Protective effect of early prenatal stress on the induction of asthma in adult mice: Sex-specific differences. *Physiology & behavior*, *165*, 358–364.

Weinstock, M. (2007). Gender differences in the effects of prenatal stress on brain development and behaviour. *Neurochemical research*, *32*(10), 1730–1740. https://doi.org/10.1007/s11064-007-9339-4

Won, E., & Kim, Y. K. (2020). Neuroinflammation-associated alterations of the brain as potential neural biomarkers in anxiety disorders. *International Journal of Molecular Sciences*, *21*(18), 6546. https://doi.org/10.3390/ijms21186546

Wood, C. E., & Walker, C. D. (2015). Fetal and neonatal HPA axis. *Comprehensive Physiology*, *6*(1), 33–62. https://doi.org/10.1002/cphy.c150005

Zhang, K., Gharaee-Kermani, M., McGarry, B., Remick, D., & Phan, S. H. (1997). TNF-alpha-mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis. *Journal of immunology*, *158*(2), 954–959.

Zhe, D., Fang, H., & Yuxiu, S. (2008). Expressions of hippocampal mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in the single-prolonged stress-rats. *Acta histochemica et cytochemica*, *41*(4), 89–95. https://doi.org/10.1267/ahc.08013

Zhou, Q. (2020). Effects of maternal chewing on prenatal stress-induced cognitive impairments in the offspring via multiple molecular pathways. *International Journal of Molecular Sciences*, *21*(16), 5627. https://doi.org/10.3390/ijms21165627

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