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Effects of gestational and breastfeeding caffeine exposure in adenosine A1 agonist-induced antinociception of infant rats

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Abstract

Objectives: Caffeine is extensively consumed as a psychostimulant drug, acting on A_1 and A_{2A} adenosine receptors blockade. Chronic exposure to caffeine during gestation and breast-feeding may be involved in infant rat's behavioral and biochemical alterations. Our goal was to evaluate the effect of chronic caffeine exposure during gestation and breast-feeding in the functionality of adenosine A_1 receptors in infant rats at P14. NTPDase and 5'-nucleotidase activities were also evaluated.

Methods: Mating of adult female Wistar rats was confirmed by presence of sperm in vaginal smears. Rats were divided into three groups on the first day of pregnancy: (1) control: tap water, (2) caffeine: 0.3 g/L until P14, and (3) washout caffeine: caffeine was changed to tap water at P7. Evaluation of nociceptive response was performed at P14 using hot plate (HP) and tail-flick latency (TFL) tests. A_1 receptor involvement was assessed using caffeine agonist (CPA) and antagonist (DPCPX). Enzymatic activities assays were conducted in the spinal cord.

Results: Gestational and breastfeeding exposure to caffeine (caffeine and washout groups) did not induce significant alterations in thermal nociceptive thresholds (HP and TF tests). Both caffeine groups did not show analgesic response induced by CPA when compared to the control group at P14, indicating chronic exposure to caffeine in the aforementioned periods inhibits the antinociceptive effects of the systemic A_1 receptor agonist administration. No effect was observed upon ectonucleotidase activities.

Conclusions: Our results demonstrate that chronic caffeine exposure in gestational and breastfeeding alters A1-mediated analgesic response in rats.

KEYWORDS

caffeine, ectonucleotidases, nociception, pregnancy, Wistar rats

1 | INTRODUCTION

Caffeine is found in a wide variety of beverages, foods, and pharmaceutical compounds. Around 80% of the world's population consumes caffeinated products every day including women of childbearing age (Heckman et al., 2010). Between the caffeine therapeutic uses are the treatment for apnea in premature neonates (Schmidt et al., 2006), headache after spinal anesthesia (Tavares & Sakata, 2012), and in association with analgesic and anti-inflammatory drugs (Derry et al., 2012). Caffeine is a hydrophobic compound permeable to biological membranes, including the placenta (Pemathilaka et al., 2019). Regarding fetal development, previous study highlights important aspects of caffeine metabolization by the fetus (Bakker et al., 2010). Caffeine metabolization is hindered by the absence of its main catalytic enzyme (CYP1A2) in the placenta and fetus, resulting in an increase in caffeine's half-life during gestation (Sasaki et al., 2017).

Caffeine consumption during pregnancy has been associated with increased miscarriage risks, and impaired fetal development (Cnattingius et al., 2000; Weng et al., 2008). On the contrary, some studies found no association between coffee consumption and adverse outcomes for pregnancy (Bech et al., 2007; Morgan et al., 2013). Our previous study showed that chronic of maternal caffeine intake of 0.3 g/L during gestation and breastfeeding impairs neuromotor development, reflected by increased latency of the righting reflex and negative geotaxis behavior accompanied by a reduction in exploratory locomotion activities of the rat offspring (Souza et al., 2015). Also, we showed that high doses of caffeine (1 g/L) induced significant malformations and early death (de Souza et al., 2016). In addition, a study also using maternal caffeine intake of 1 g/L increases the acetylcholinesterase activity (42%) without changes in acetylcholinesterase mRNA transcripts levels in 21-day-old rats (da Silva et al., 2008), whereas an intake of 0.3 g/L impaired the migration of hippocampal GABA neurons and increased the susceptibility to seizures in offspring (Silva et al., 2013). This scenario highlights the importance of further exploring and defining the effects of gestational and breastfeeding caffeine exposure of infants.

On a molecular level, caffeine's mechanism of action involves the blockade of A_1 and A_{2A} adenosine receptors (Fredholm et al., 1999), resulting in phosphodiesterases inhibition (Almosawi et al., 2018). An exposure to caffeine in the neonatal period shows long-lasting alterations in A_1 receptor levels of infant rats, remaining until young adulthood (León et al., 2002). A_1 receptors modulation is caffeine dose-dependent in infant rats after maternal intake of 0.8 g/L of caffeine, increasing A_1 receptor density (Bona et al., 1995). Other studies suggest that different maternal caffeine intakes during gestation and postnatal life have different effects on the development of adenosine A_1 and A_{2A} receptors in the pup rats' brain (Adén et al., 2000; Léon et al., 2002).

The endogenous adenosine production is related to physiological pain control at the spinal cord level, and that its release is involved in the nociceptive thresholds' regulation (Bech et al., 2007; Sawynok & Liu, 2003). The association between adenosine receptors and pain is highlighted by the fact that adenosine receptor agonists act in the pre-synapse reducing neuronal excitability (Sperlágh & Vizi, 2011). Adenosine analogs have antinociceptive properties in a wide range of test systems, including neuropathic pain, where pain-signaling mechanisms have been altered (Yamaoka et al., 2013). The production of adenosine is mediated by a set of different enzymes, such as ectonucleotidases, adenosine kinase, and adenosine deaminase (Kowaluk & Jarvis, 2000). Ectonucleotidases (NTPDases and ecto-5'-nucleotidase) are responsible for converting adenine nucleoside 5′-phosphate derivatives into adenosine (Fausther et al., 2012). These enzymes may provide a protective function by maintaining extracellular ATP/ADP and adenosine within physiological concentrations (Burnstock, 2013).

Considering the close relationship between caffeine and adenosine receptors, it is safe to propose caffeine exposure could be responsible for alterations in pain response of infant rats exposed to chronic caffeine during gestation and breastfeeding. To evaluate these effects, the thermal nociceptive threshold and functionality of adenosine A_1 receptors of infant rats was evaluated by nociceptive response assessment at P14. In addition, NTPDase and ecto-5'-nucleotidase activities in synaptosomes from spinal cord of infant rats were evaluated.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult female Wistar rats (weighing around 220 g) of 90 days of age were performed vaginal lavage to verify the estrous

cycle. In their fertile phase, females were housed at the evening with knowingly a male fertile. In the subsequent morning, males were removed, and the vaginal smears collected. Mating was confirmed by sperm presence in vaginal smears. Females were housed individually in polypropylene home cages (49 cm × 34 cm × 16 cm) with sawdust-covered floors. All animals were kept in standard condition of bioterium. At birth, litters were standardized to contain up to eight pups per dam, and only males were assessed. The Ethics Institutional Committee approved all experiments and procedures for Animal Care and Use (GPPG-HCPA protocol N°. 110034).

2.2 | Experimental design

On the first day of pregnancy (G0), dams were divided into three groups: (1) control group, which received only tap water; (2) caffeine group, which received only of 0.3 g/L caffeine diluted in tap water, and (3) washout group, which receives the same solution of 0.3 g/L caffeine diluted in tap water until the seventh day after birth (P7) and after replaced by tap water. Dams' treatment was conducted during gestation and lactation until 14 days of age of pups (P14), when tests were conducted. Rats at postnatal day 8 (P8) exhibit similar neurological development to a human newborn, and rats aged 2–3 weeks are similar to human 1-year-old infants (Fitzgerald, 2005). Considering that, the purpose of the washout group was to mimic an intervention up to the stage at which the rats exhibit a similar neurological development to that of a human neonate. Rats were killed at P14, and for ectonucleotidases activity assays the rats were not subjected to nociceptive tests.

2.3 | Hot plate test

The hot plate test was carried out to assess the thermal nociceptive threshold (Woolf & Mcdonald, 1944). The hot plate was preheated and kept at a temperature of 55 ± 0.5°C. All rats were acclimated to the hot plate for 5 min, 24 hr prior to testing, to avoid the novelty of the apparatus. Rats were placed in glass funnels on the heated surface and the nociceptive threshold was assessed recording to the time taken to first response (foot licking, jumping, or rapidly removing paws). Response was recorded in seconds (s) and a cutoff time of 20 s was used.

2.4 | Tail-flick latency test

Rats were tested for antinociception using the tail-flick test (TFL) (Castilho et al., 2002). Each animal was placed on the apparatus and its tail was laid across a nichrome wire coil that was then heated using an electric current. The equipment was calibrated to obtain three consecutive baseline tail-flick latencies between 3 s and 5 s (a cutoff time of 10 s was used to avoid tissue damage). The animals were exposed to the tail-flick apparatus to acclimate to the procedure 24 hr prior to the test session. Tail-flick latencies were measured before (baseline), immediately, 15, 30, and 60 min after the drug injections at P14.

2.5 | Drugs administration

Drugs used were N6-Cyclopentyladenosine (CPA, a selective adenosine A_1 receptor agonist, 3.35 mg/Kg) and 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX, an adenosine A1 receptor-selective antagonist, 0.8 mg/Kg) were purchased from Sigma-Aldrich, USA. CPA was dissolved in 0.9% saline, DPCPX in 5% dimethyl sulfoxide + 1.25% NaOH 1 M, pH 4.0, administered i.p. in a volume of 1.0 ml/kg. Caffeine 0.3 g/L was diluted in tap water via the drinking water. The dose of caffeine (0.3 g/L) was chosen because it produces blood levels in the dams comparable to those obtained in humans after consumption of ~3 cups of coffee by day (Bona et al., 1995).

2.6 | Synaptosomal preparation

Rats were killed by decapitation at P14 and the spinal cord was rapidly removed and gently homogenized in 10 vol of ice-cold medium consisting of 320 mM sucrose, 0.1 mM EDTA, and 5 mM HEPES, pH 7.5, with a motor driven Teflon-glass homogenizer. The synaptosomes were isolated as described and adapted previously (Torres et al., 2002).

2.7 | Ectonucleotidases activity

The reaction medium used to assay ATPase–ADPase activities was described previously (Battastini et al., 1991). All samples were run in duplicate. Protein was measured by the Coomassie Blue method (Bradford, 1976), using bovine serum albumin as standard. The specific activities were expressed as nmol Pi/min/mg protein.

2.8 | Statistical analysis

Data were expressed as means ± standard error of the mean (S.E.M.) or median and interquartile range after testing for normality distribution using Kolmogorov–Smirnov. For behavioral results, the data were analyzed using Kruskal–Wallis or Friedman test followed by Mann–Whitney or Wilcoxon

for multiple comparisons, respectively, and adjusted with Dunn–Sidak, when indicated. For enzymatic activities, we used one-way ANOVA followed by Tukey if it is necessary Differences were considered statistically significant if $p < .05$.

3 | RESULTS

3.1 | Effect of maternal chronic caffeine intake on nociceptive response of infant rats measured by the hot plate test (HPT)

We did not observe any significant difference in the latency of paw withdrawal between the groups (Kruskal–Wallis, $p > .05$; Figure 1).

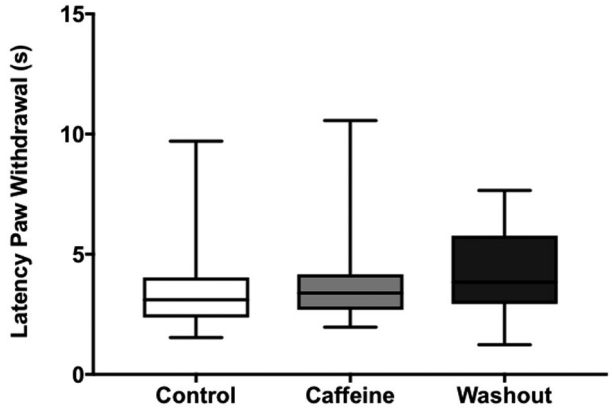

FIGURE 1 Effect of prenatal caffeine exposure on Paw Withdrawal Latency assessed by HPT at P14. Data presented as the median and interquartile range of the licking paws or jumping as latency of response in seconds (s) (Kruskal–Wallis, $p > .05$; $n = 12–34$)

3.2 | Effect of maternal chronic caffeine intake on selective adenosine A1 agonist (CPA) and adenosine A1 receptor-selective antagonist (DPCPX) in the nociceptive response of infant rats

At baseline assessment, we observed that there was no difference in the tail withdrawal latency between groups at P14 (Kruskal–Wallis, $p > .05$; Table 1). The control group presented the classic CPA-induced analgesia at 15 and 30 min in relation to baseline (Friedman test/Wilcoxon, $p < .05$; Table 1). However, chronic caffeine exposure in the gestational and breastfeed periods (caffeine and washout groups) inhibits/reduces the antinociceptive effect of CPA (Friedman test, $p > .05$; Table 1).

3.3 | Effect of maternal chronic caffeine intake on ectonucleotidases activity in synaptosomes from spinal cord of infant rats

At P14, there was not significant difference between groups in NTPDases and 5'-nucleotidase activities in synaptosomes from spinal cord of infant rats (one-way ANOVA, $p > .05$, $F_{(2,16)} = 0.23$, 0.02, and 0.09 for ATP, ADP, and AMP, respectively, $n = 4–6$ for all; Figure 2).

4 | DISCUSSION

The present study showed that chronic caffeine exposure in the gestational and breastfeeding periods reduces the antinociceptive effects of a selective adenosine A_1 receptor agonist (CPA) in infant rats. That is, the caffeine exposure in early

TABLE 1 Effect of maternal chronic caffeine intake upon drugs active at A1 adenosine receptor at P14

	Baseline	Immediately	15 min	30 min	60 min
Control					
Vehicle	4.48 (3.87–5.22)	5.52 (4.41–6.05)	4.78 (4.35–6.23)	5.30 (4.45–5.93)	5.30 (3.89–5.66)
CPA	4.70 (3.90–4.96)	4.99 (4.27–6.60)	6.64 (4.73–7.94)*	6.77 (4.92–7.41)*	4.70 (4.55–6.16)
DPCPX	4.38 (3.68–5.82)	4.96 (4.13–5.62)	5.28 (4.18–6.60)	4.64 (3.88–5.73)	4.25 (3.14–6.15)
Caffeine					
Vehicle	3.64 (3.31–4.44)	3.93 (3.29–4.74)	3.93 (3.10–4.83)	4.59 (4.10–5.30)	4.31 (3.68–4.80)
CPA	4.36 (3.62–5.32)	4.35 (3.73–5.06)	5.84 (4.16–6.70)	5.14 (3.86–5.95)	4.34 (3.37–6.12)
DPCPX	4.89 (4.13–5.53)	4.46 (4.00–5.03)	4.73 (3.30–5.47)	5.07 (4.35–5.65)	4.45 (3.57–5.36)
Washout					
Vehicle	5.28 (5.03–5.49)	5.01 (4.07–5.75)	4.68 (3.27–6.35)	4.72 (3.06–5.70)	5.07 (4.34–5.66)
CPA	4.95 (3.99–5.65)	5.25 (4.71–6.80)	6.04 (5.57–6.56)	5.73 (5.43–6.27)	5.30 (3.78–6.76)
DPCPX	4.65 (4.02–5.23)	4.61 (3.52–4.93)	3.53 (2.73–4.28)	4.25 (3.38–5.18)	4.89 (3.91–6.37)

Note: Data presented as median and interquartile range of the tail withdrawal latency in seconds (s).

*Significant difference in relation to baseline in the control group (Friedman test/Wilcoxon, $p < .05$, $n = 13–28$).

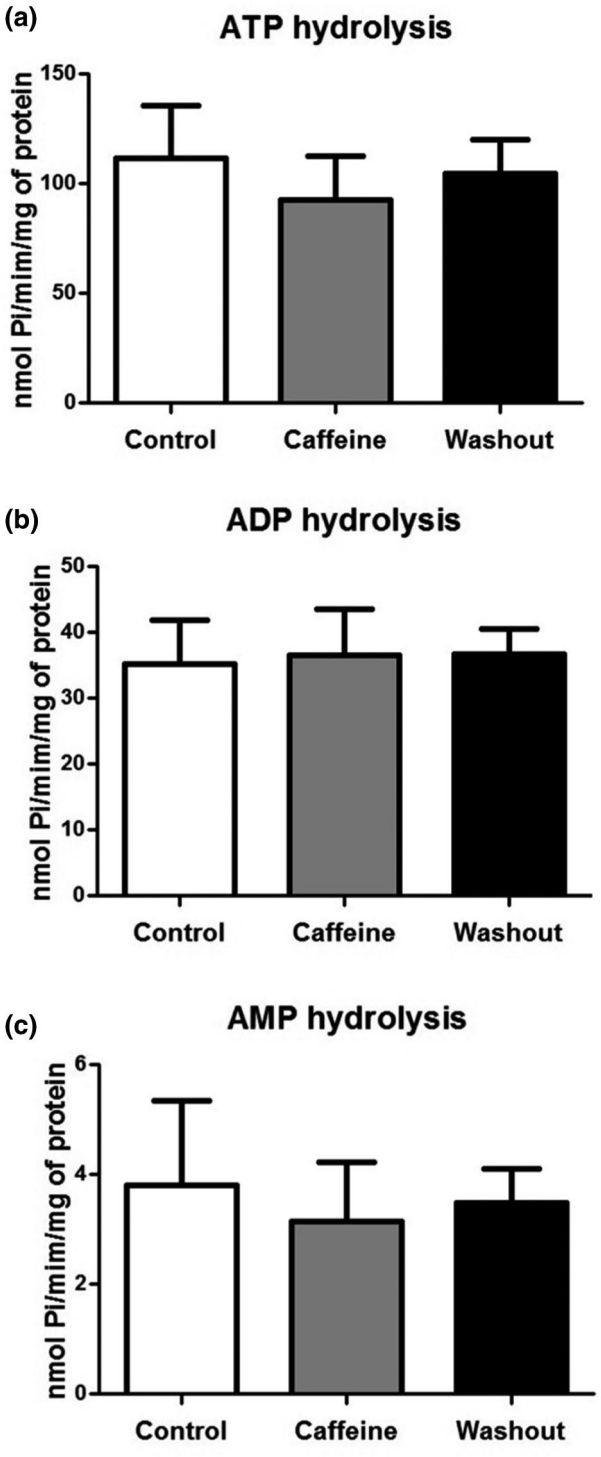

FIGURE 2 Effect of maternal chronic caffeine exposure upon the enzymatic activity of ectonucleotidases in the spinal cord of rats at P14. Bars represent the mean ± SEM. No differences were found (one-way ANOVA, $p > .05$; $n = 4–6$)

life changes the characteristic adenosinergic antinociceptive response as observed in both caffeine and washout groups. However, caffeine exposure in the gestational and breast-feeding periods did not modify the basal thermal nociceptive threshold. Interestingly, there were no alterations in the nucleotidase activities in synaptosomes from the spinal cord of these animals.

It is interesting to note that the rats submitted to chronic caffeine exposure (caffeine and washout groups) did not show any difference in the baseline thermal nociceptive response indexed by paw and tail withdrawal latencies. These responses were evaluated, in the HPT and TFL tests, respectively. HPT triggers a nociceptive response mainly involving C-fibers. The observed responses include characteristic behaviors as "paw licking" and "jumping," which are considered integrated supraspinal responses (Fan et al., 2014). While, TFL involves Aδ-fibers stimulation; and its nociceptive responses are related to the spinal cord reflex with some degree of supraspinal control evoked by the stimulation of myelinated fibers (Le Bars et al., 2001). The Aδ-fibers are present at birth (P0) and C-type fibers increase in the spinal cord in the first 3 weeks of life and the corresponding decrease of Aδ-fiber does not occur immediately. Thus, in the first 3 weeks of life, both fibers occupy the same space within the spinal cord (Fitzgerald, 2005). Thus, we could suggest that after chronic caffeine administration possible changes may occur in the type Aδ-fibers, as a possible downregulation of A_1 receptors. And, the chronic caffeine exposure at a stage of maturation promotes alteration in the thermal nociceptive response in relation to A_1 agonist, which can be resulting from an imbalance of nociceptive fibers.

Our hypothesis is corroborated by alteration in the adenosinergic receptor functionality in response to CPA administration, which was observed after caffeine exposure in early life. Where, only the naive rats showed a classical CPA antinociceptive effect (Torres et al., 2003), suggesting the caffeine and washout rats present the tolerance phenomenon. Adenosine analogs have antinociceptive properties in experimental and clinical settings (Zylka, 2011), in pain conditions in absence or presence of spinal cord sensitization (Curros-Criado & Herrero, 2005). However, previous study showed that repeated administration of A_1 or μ-opioid agonists develop tolerance to the peripheral antinociceptive effects (Aley et al., 1995). It is important to note that the mRNA A_1 expression receptor in the brain and in the ventral horn of the spinal cord was first detected on gestation day 14 (G_{14}); and at gestational age 17 (G_{17}), the patterns of A_1 receptor expression in the brain were similar to the adults (Burnstock, 2007).

Previous research, corroborating our results, showed that chronic caffeine exposure induced to a decrease in the number of adenosine A_1 receptors during gestation (80%), lactation (76%), and gestation plus lactation (80%) in male neonates, without variation in the level of mRNA coding adenosine A_1 receptor (Lorenzo et al., 2010). In addition, another study showed downregulation in the fetal brain after caffeine or theophylline treatment in pregnant rats (León et al., 2002). Caffeine modulates the ontogeny of the adenosine A_1 receptor, since the exposure to caffeine during postnatal period

induced a transient increase (only at postnatal day 6) in the number of immunopositive neurons in brainstem areas (Gaytan et al., 2006). It is important to highlight that in the current study, we exposed dams to caffeine (0.3 g/L) in their drinking water inducing blood levels comparable to those in humans after the intake of about three cups of coffee (Bona et al., 1995). This makes our results more relevant because they showed an altered long-term response induced by usual coffee consumption to antinociceptive drugs.

Previous study showed a significant loss of A1 receptors in the maternal whole brain and in that of their fetuses after chronic caffeine intake during pregnancy (León et al., 2002). The first 2 weeks after birth is a critical period to development and synaptogenesis of rats, with intense outgrowth and maturation of neurons (Semple et al., 2013). Therefore, the caffeine effect on neonates may be in part caused by a blockade in adenosine A_1 receptor or an indirect effect via dopaminergic (DA) interaction; once adenosine can inhibit several effects of dopamine in the cerebral cortex and basal ganglia (Ciruela et al., 2011). Previous study has suggested that dopaminergic neurotransmission, through an A_1–D1 interaction, might also be involved in the behavioral effects of adenosine agonists and antagonists not related to motor activity (Ferré et al., 1996). In this way, we cannot discard the influence of caffeine intake during gestational or breastfeeding periods upon this interaction (A_1/D1 and A_1/D2), once it is known the importance of the dopaminergic system in the outgrowth and maturation of neurons (Money & Stanwood, 2013). In the same sense, no recent studies were found regarding the investigation between these interactions during the development neonatal period, mainly investigating nociceptive pathways modulation. Furthermore, it is known that all receptor complexes, such as GABAergic, dopaminergic, and others, may be influenced by the caffeine intake, however, further studies are needed to carry all these investigations.

On the contrary, it is interesting to point out the interaction between A_1 and A_2 receptors, which caffeine acts preferably; with a cross-talking between these receptors (Cunha, 2001). Other mechanisms of caffeine action are the inhibition of phosphodiesterase and mobilization of intracellular calcium, but these effects require higher concentrations of caffeine (Lorist & Tops, 2003). In addition, we did not observe differences between groups in NTPDases and 5'-nucleotidase activities in synaptosomes from spinal cord, suggesting that the CPA-induced analgesia is independent of adenosine spinal cord levels. But, we highlighted that caffeine can also act as an analgesic in both peripheral and central systems (Baratloo et al., 2016).

Concerning the limitation of the preclinical studies, we used only male rats, making it difficult to translate the results to both genders in humans, particularly because the nociceptive process is altered by modulations in hormone state (Ribeiro et al., 2005). And, there is a lack of assessment of the binding and expression of adenosine receptors in the spinal cord.

In conclusion, the present study shows that the rats submitted to chronic caffeine administration in gestational and breastfeeding (caffeine and washout groups) did not present alterations in the thermal nociceptive responses. However, this exposure triggered a noncharacteristic adenosinergic antinociceptive response after CPA administration, and no alterations of ectonucleotidases activity in synaptosomes from spinal cord of infant rats. Our results demonstrate the importance of extending studies related to chronic caffeine administration in gestational and breast-feeding in formation and central nervous system maturation period, where different neurotransmitter systems are being developed or matured. Further studies are necessary to elucidate the real mechanisms involved in the pharmacological alterations found in this study.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

IraciL.S.Torres:Supervision,Conceptualization,Methodology, Writing-Original draft preparation; José A. F. Assumpção: Writing-Reviewing and Editing; Joanna R. Rozisky: Conceptualization and Methodology; Andressa de Souza: Methodology, Data curation; Carla de Oliveira: Methodology, Data curation; Vanessa Scarabelot: Methodology, Data curation; Stefania Giotti Cioato: Methodology, Data curation; Wolnei Caumo: Conceptualization and Methodology; Rosane S. Silva: Writing-Reviewing and Editing; Ana Maria de Oliveira Battastini: Writing-Reviewing and Editing; Liciane Fernandes Medeiros: Supervision, Writing-Reviewing and Editing.

ETHICS APPROVAL STATEMENT

The Ethics Institutional Committee of the Hospital de Clínicas de Porto Alegre (HCPA) approved all experiments and procedures for Animal Care and Use (GPPG-HCPA protocol N^o. 110034).

PATIENT CONSENT STATEMENT

Not applicable.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

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