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Impact of interaction between chronic variable stress and moderate intensity physical exercise on antibody production in Wistar rats

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Stress in its chronic form has been an important factor in the onset of depression. In addition, it can lead to immunosuppression. On the other hand, physical exercise has a protective action on the immune system and reduces the signs and symptoms of stress. Studies on the effects of chronic variable stress (CVS) and moderate intensity physical exercise (MIPE) on humoral immunity are scarce. Thus, in this study, we investigated the interaction between the effects of CVS and MIPE on antibody production. Wistar rats were divided into four groups: control (C); physical exercise (P); stress (S); and physical exercise and stress (PS). The P and PS groups were trained in MIPE for six weeks. From the fourth week of the study, concomitant with the MIPE, the S and PS groups were subjected to CVS. To evaluate the production of antibodies, all groups were immunized. Regarding antibody production, it was observed that females in the C and S groups presented higher IgM antibody production in relation to males. Furthermore, the production of IgM was potentiated in males of the PS group. On the other hand, no significant differences were observed in relation to the production of IgG1 and IgG2a. We conclude that CVS prevented the increase in IgM production in male rats and MIPE was effective in reversing the effects of stress on the production of IgM in male rats. On the other hand, the production of IgG1 and IgG2a was not affected by MIPE or CVS.

Keywords: Behaviour, Exercise, Chronic variable stress (CVS), Humoral immunity, MIPE, Sex differences

Chronic stress (CS) is an important factor in the onset of depression[1]. In fact, CS can deregulate the hypothalamic-pituitary-adrenal axis (HPA); increasing the reuptake and degradation of serotonin; and decreasing the production of Brain-Derived Neurotrophic Factor (BDNF)[2]. Moreover, CS can promote immune suppression, altering the production of antibodies and cytokines, resulting from an imbalance in the activation of Th1/Th2 cells[3]. A pathway to this imbalance is through deregulation of the HPA axis, in which high levels of circulating cortisol are observed, thus modulating the immune system[4]. The immune system cells have gluco-corticoid receptors, which are related to decreases in the production of inflammatory cytokines such as IL-1β, IL-6, and TNF-α[5] resulting in lower efficacy of the body to fight infectious diseases and increased time taken for wound healing[6]. The imbalance caused by CS on the immune system presents a strong association with the development of depression[7].

Furthermore, the effects of CS on the development of depression and immune dysfunction could be sex dependent[8]. Epidemiological data show that women are twice as likely as men to develop depression during their lives[9]. The occurrence of depression is related to fluctuations in the sex hormones, dysregulation of the HPA and hypothalamic-pituitary-gonadal axis, and inflammatory signaling mediated impacts on synaptic plasticity and neurotransmission[10]. Evidence demonstrates that males and females respond differently to stressors in terms of behavioural outcomes, activation of the HPA axis and the sympathetic nervous system, and research into sex differences in inflammatory and immune processes have revealed nuanced effects of sex on different aspects of immunity, such as wound healing and immunosuppression[11].

In opposition to CS, moderate intensity physical exercise (MIPE), when practiced regularly, has an

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anti-inflammatory and immune stimulatory action, and reduces the signs and symptoms of stress[3]. A reduction in depressive-like behaviour was also observed in mice subjected to 14 days of voluntary running[8]. MIPE was able to reduce serum cortisol levels and stimulate neuroplasticity and serotonin production and to alter the pattern of humoral and cellular immune response in rats[12]. Furthermore, in elderly women, the regular practice of MIPE stimulated the production of specific anti-influenza antibodies (IgM and IgG) compared to sedentary women[13]. Thus, in the present study, we investigated the interaction between the effects of chronic variable stress and moderate intensity physical exercise on antibody production in Wistar rats.

Materials and Methods

Animals and experimental groups

Ninety-day old male and female Wistar rats were purchased from the Central Vivarium at the State University of Londrina and housed in our local vivarium under the following conditions: 3-5 animals per cage, light and dark cycle of 12 h (light on from 7:00 am), food (Nuvital, Colombo, Brazil) and water provided *ad libitum*, and room temperature controlled at 25±1ºC. The animals were separated by sex and randomly assigned into one of four groups: control (C); physical exercise (P); stress (S); and physical exercise and stress (PS). The groups were composed of 9 to 12 rats. All animals were subjected to the immunization procedures for evaluation of antibody production. The project was approved by the Ethics Commission on Animal Use (proc. CEUA-UEL 24606.2012.58).

Moderate intensity physical exercise (MIPE) and Chronic variable stress (CVS)

To perform the MIPE, plastic cylinders (50 cm high and 22 cm diameter) were used, containing 40 cm of water at a controlled temperature of 31±1°C. The animals of the P and PS groups were subjected to swimming exercise for six weeks. Each week the animals performed exercises for five days and rested for two. Fig. 1 shows the chronological organization of the procedures. In the first week, the rats were adapted to the 40 min of swimming (the animals swam 20 min on the first day, 25 min on 2nd day, 30 min on 3rd, 35 min on 4th, and 40 min on the 5th adaptation day). The intensity of the MIPE was controlled by means of loads tied to the thoracic region of the rats. The load was adjusted weekly, respecting the change in body weight of each animal. The use of a load began from the second week (2% of body wt. in the second week, 3% in the third, and 4% in the 4th, 5th and 6th weeks of the study).

On 22nd day, the chronic variable stress protocol (CVS) was applied to the animals in the S and PS groups. CVS was performed for 19 consecutive days. The stressors used in the CVS were: cold, 1 h in the refrigerator at 4°C; wet bedding, 12 h; social isolation, 12 h; box tilted at 45º, 12 h; overcrowded housing (individual cage (30×20×13 cm) with 3 rats), 12 h; light on in the vivarium, 24 h. This procedure was adapted from Carvalho-Netto *et al.*[14]. The sequence of stressors was applied by drawing lots, and the same stressor was not used on consecutive days to avoid familiarity. The stressor stimulus was applied after each MIPE session.

Incremental load test

The incremental load test (ILT) was performed to evaluate the MIPE protocol. For this, lactate and blood glucose levels were evaluated[15]. For measurement of blood lactate and glucose (ILT), 25 µL of blood was collected from the tail vein of the animals and stored in microtubes (1.5 mL) containing 50 µL of 1% sodium fluoride and stored at −20°C until analysis. The blood collection was performed on the 44th day of the experiment, at four time points: before starting the test and 5, 10, and 15 min after the beginning of the test. The test began with no load, after 5 min a load of 4% of body weight was attached to the body, and at the time point 10 min this was replaced by a load of 8% of the body wt.

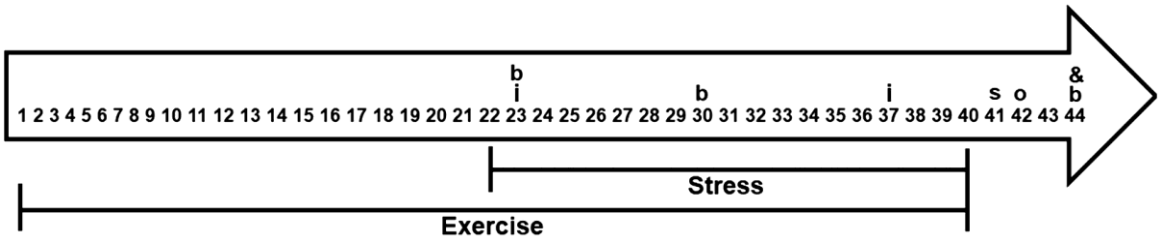

Fig. 1 — Chronological organization of the procedures. [b, Blood collection; i, Immunization; s, Sucrose preference test; o, Open field test; &, Weighing of organs]

The lactate concentrations and blood glucose were measured in a lactate and glucose electro enzymatic analyzer (YSI2300L – Yellow Springs Instruments – Ohio, USA).

Body and organ weight

The weight of the animals was obtained at the beginning of each week of the experiment, to adjust the exercise load. At the end of the experiment (day 44) the animals were euthanized and the left adrenal, heart, and peritoneal fat removed to calculate the relative weight. The difference in body wt. of the rats was calculated by subtracting the final weight (day 44) from the initial weight (day 1).

Biochemical analysis

To evaluate the levels of creatine kinase, aspartate aminotransferase, alanine aminotransferase, cholesterol, triglycerides, creatinine, urea, and total protein, the blood of the rats was collected through cardiac puncture in anticoagulant-free tubes on the 44th day of the experiment (Fig. 1). After coagulation, the samples were centrifuged at 1000 ×*g* for 5 min. The serum was recovered, aliquoted, and stored at –20°C until analysis. Biochemical analyzes were performed in the biochemical analyzer Dimension® Clinical Chemistry System (SIEMENS – Munich, Germany) using commercial kits according to the manufacturer's specifications.

Behavioural analysis

Two tests were used for the behavioural analyzes: the sucrose preference test and open field test. The sucrose preference test was performed on the 41st day of the experiment (Fig. 1). In this test the animals were placed individually in cages with two similar bottles, one containing water and the other a 1% sucrose solution. After 24 h, the volume of water or 1% sucrose was determined and used to calculate the sucrose preference using the following equation: SP = SC×100/SWC, being SP, sucrose preference; SC, sucrose 1% volume consumed within 24 h; and SWC, volume of 1% sucrose solution and water consumed in the same period of time.

In the open field test, each animal was individually placed in a 54×54 cm arena for 15 min and filmed by a camera (JVC, GZ-MG750BU model, São Paulo, Brazil) attached to the ceiling of the test room. The parameters evaluated were time spent in the center and squares crossed. This procedure was performed on day 42, after evaluation of sucrose preference (Fig. 1).

Immunization and enzyme-linked immunosorbent assay (ELISA) for the detection of immunoglobulins

To evaluate the humoral immunity, the animals were inoculated subcutaneously with 50 µL of phosphate buffered saline (PBS, pH 7.4) containing 500 µg of aluminum hydroxide and 50 µg of chicken IgY as antigen. The two immunizations were performed on the 23rd and 37th days of the experiment.

To evaluate the production of IgM, IgG1, and IgG2a anti-chicken IgY antibodies, three samples of peripheral blood were obtained through cardiac puncture: prior to the first immunization (day 23), seven days after the first immunization (day 30), and seven days after the second immunization (day 44). The blood was collected in tubes containing 5% EDTA and the plasma was separated by centrifugation at 1000 ×*g* for 5 min and frozen at –4°C prior to use.

The levels of anti-IgY antibodies were determined by ELISA as described by Fernandes *et al*.[16] with the following modifications: plates were coated with 100 µL of chicken IgY solution at a concentration of 1.0 µg/mL in sodium carbonate-bicarbonate buffer, pH 9.6. The plasma was diluted 1:100 in PBS (PH 7.4) containing 1% skimmed milk powder (Molico®, Nestle, Araçatuba, Brazil). The dilutions of the peroxidase conjugated antibodies; anti-IgM (03-9820, Zymed, Carlsbad, USA), anti-IgG1 (A110-106P, Bethyl, Montgomery, USA), and anti-IgG2a (03-9620, Zymed, Carlsbad, USA) were 1:5,000, 1:50,000, and 1:5,000, respectively in the same buffer used for the dilution of the plasma.

Hematological analysis

To carry out the hematological analysis, blood was collected through cardiac puncture and stored in tubes containing 5% EDTA (day 44). Blood samples were analyzed on the same day as the blood collection in an automated hematology analyzer (BC-2800 VET, Mindray, Shenzhen, China).

Statistical analysis

The data were submitted to homogeneity (Levene's) and normality (Kolmogorov-Smirnov) tests. According to these tests, the ANOVA or Kruskal-Wallis test was applied, followed by a post hoc Bonferroni or Dunn's, for parametric or nonparametric data, respectively. Three-way ANOVAs were performed to evaluate the effects of three independent variables (sex X stress X exercise). Four-way repeated measures ANOVAs were run when time was included as an additional variable.

Parametric data are presented as mean and standard deviation while nonparametric data are expressed as median and interquartile range. The significance level was set at P <0.05.

Results

Effects of MIPE and CVS on parameters related to training

Fig. 2 presents the lactate and glucose values from the peripheral blood of male and female rats during the ILT. It was observed that the MIPE, regardless of sex, promoted adaptations in the animals by preventing the rapid increase in lactate (P <0.01, Fig. 2 A and B) and maintaining blood glucose levels (P <0.01, Fig. 2 C and D) during the test. In relation to lactate, an effect of exercise (F [1, 84] = 6.362, P <0.05), time (F [1, 84] = 144.814, P <0.001), and an interaction between time and exercise (F [3, 252] = 10.585, P <0.001) were observed. In relation to glucose, effects of time (F [3, 252] = 20.950, P <0.001) and interactions between time and sex (F [3, 252] = 4.884, P <0.01), time and exercise (F [3, 252] = 4.816, P <0.01), and time, sex, and stress (F [3, 252] = 3.614, P <0.02) were observed.

In relation to body weight, there were effects of sex (F [1, 84] = 75.500, P <0.001), stress (F [1, 84] = 5.738, P <0.02), and exercise (F [1, 84] = 11.997, P <0.001). The post hoc analysis showed female and male differences for the C, P and S groups. In males, the PS group presented lower body wt. variation (P <0.01, *vs*. Control Group), data not shown.

Regarding fat, effects of stress (F [1, 84] = 7.796, P <0.01), exercise (F [1, 84] = 23.895, P <0.001), and an interaction between sex and stress (F [1, 84] = 4.987, P <0.05) were observed. The post hoc showed that MIPE reduced the peritoneal fat in females belonging to the P and PS groups (P <0.05 *vs*. Group C) while, in males, MIPE did not change the peritoneal fat (P >0.05), data not shown.

In relation to the heart, there were effects for sex (F [1, 84] = 17.540, P <0.001) and exercise (F [1, 84] = 37.780, P <0.001). The post hoc showed that MIPE increased the heart weight in the females and males belonging to the P and PS groups (P <0.05 *vs*. Group C), data not shown.

Regarding the biochemical parameters, no differences were observed between the animals of the same sex or between sexes (P >0.05), data not shown.

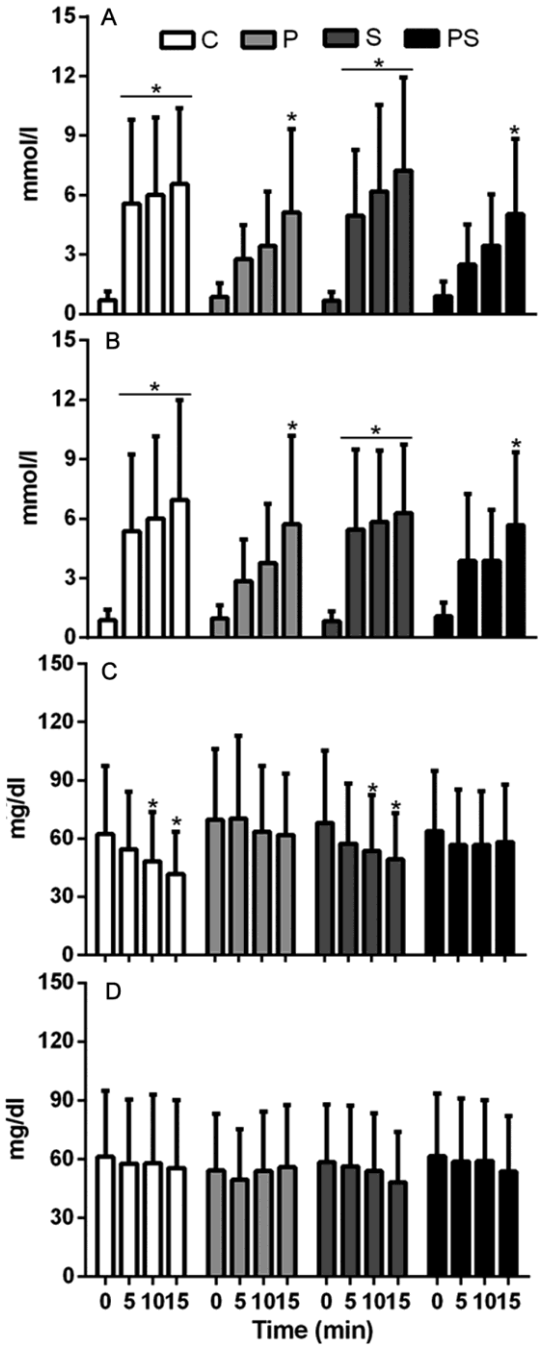

Fig. 2 — Variation in the serum levels of glucose and lactate during the incremental load test. (A) Females - Lactate; (B) Males - Lactate; (C) Females - Glucose; and (D) Males - Glucose. [Females: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=11); Males: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=9). 0, before starting the test; 5, after 5 min of MIPE without load; 10, after 5 min of MIPE with 4% of body wt. attached to the body; 15, after 5 min of MIPE with 8% of body wt. attached to the body. Analyses performed through four-way ANOVA for repeated measures followed by the Bonferroni post hoc. Data presented as mean ± SD. *, different from time point 0, in the same treatment. P <0.05]

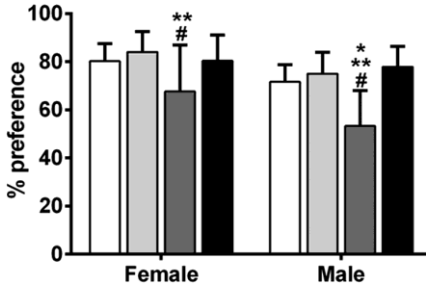

Fig. 3 — Effect of CVS and MIPE on sucrose preference. [Females: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=11); Males: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=9). Analysis performed through the three-way ANOVA test followed by the Bonferroni post hoc. Data presented as mean ± SD. *, different from C group of the same sex; **, different from P group of the same sex; #, different from PS group of the same sex. P <0.05]

Effects of MIPE and CVS on behaviour and adrenal weight

Fig. 3 shows the results of the sucrose preference test in female and male rats. Effects of sex (F [1, 84] = 19.278, P <0.001), stress (F [1, 84] = 10.623, P <0.01), exercise (F [1, 84] = 19.229, P <0.001), and a stress and exercise interaction (F [1, 84] = 8.682, P <0.01) were evidenced. The post hoc test showed a reduction in sucrose consumption in females and males of group S (P <0.05).

In the results of the open field test (Fig. 4), time spent in the center and squares crossed parameters were evaluated. The ANOVA test showed an interaction between sex and exercise (F [1, 84] = 4.345, P <0.05) in relation to the time spent in the center. In this parameter, the post hoc analysis showed that males of group S reduced the time in the center in relation to males of groups P and PS (P <0.05, Fig.4 A). In relation to squares crossed, there was an effect of sex (F [1, 84] = 36.966, P <0.001). The post hoc showed differences between females and males in relation to the groups C, P, and S (P <0.05, Fig.4 B).

Fig. 5 shows the weight of the adrenal glands of the male and female rats, a sex effect being observed (F [1, 84] = 12.909, P <0.001). The post hoc test showed that control females presented higher adrenal weight than control males (P <0.01).

Effect of MIPE and CVS on immunological parameters

Fig. 6 shows the levels of IgM, IgG1 and IgG2a anti-IgY in the peripheral blood of female and male rats. In relation to the IgM (Fig. 6A), effects of sex (F [1, 84] = 26.411, P <0.001), time (F [2, 168] =

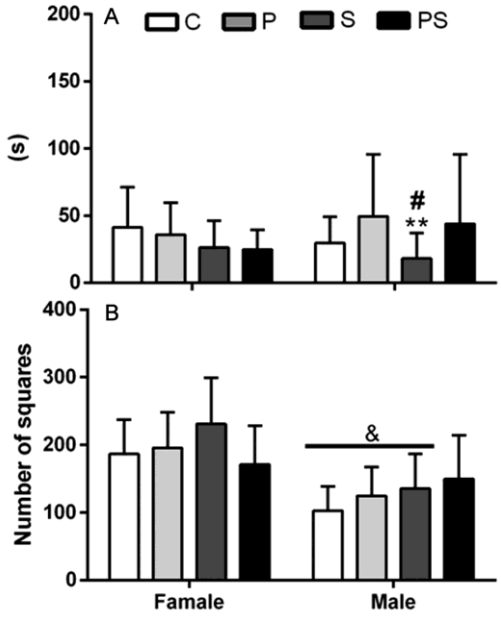

Fig. 4 — Time in the center and locomotion (squares crossed) in the open field test. (A) Time spent in the center; and (B) Squares crossed. [Females: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=11); Males: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=9). Analysis performed through the three-way ANOVA test followed by the Bonferroni post hoc. Data presented as mean ± SD. **, different from exercise animals of the same sex; #, different from stress+exercise animals of the same sex; &, different from females of the same group. P <0.05]

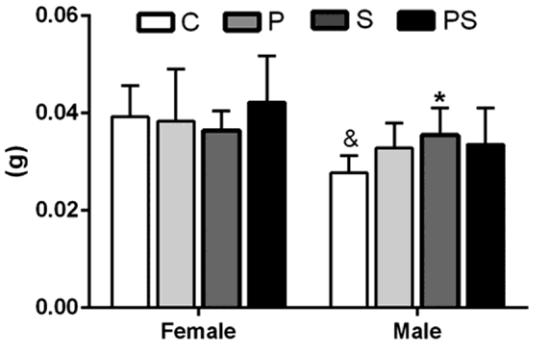

Fig. 5 — Total adrenal weight. [Females: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=11); Males: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=9). Analysis performed through three-way ANOVA followed by the Bonferroni post hoc. Data presented as mean ± SD. *, different from C group of the same sex; &, different from the female C group. P <0.05

98.447, P <0.001), and an interaction between time and sex (F [2, 168] = 10.586, P <0.001) were found. The post hoc test showed that females of groups C and S presented increased IgM production on day 30 (P <0.05, *vs*. groups C and S on day 23, respectively) and on day 44 all groups demonstrated increased IgM

production (*P* <0.001, *vs*. groups C, P, S, and PS on day 23, respectively). In males, an increase in IgM production was observed only in the PS group on day 44 (*P* <0.01, *vs*. group PS on day 23). In the comparison between the sexes, it was observed that the females of groups C and S, on day 44, presented higher IgM production when compared to males of groups C and S on day 44 (*P* <0.01).

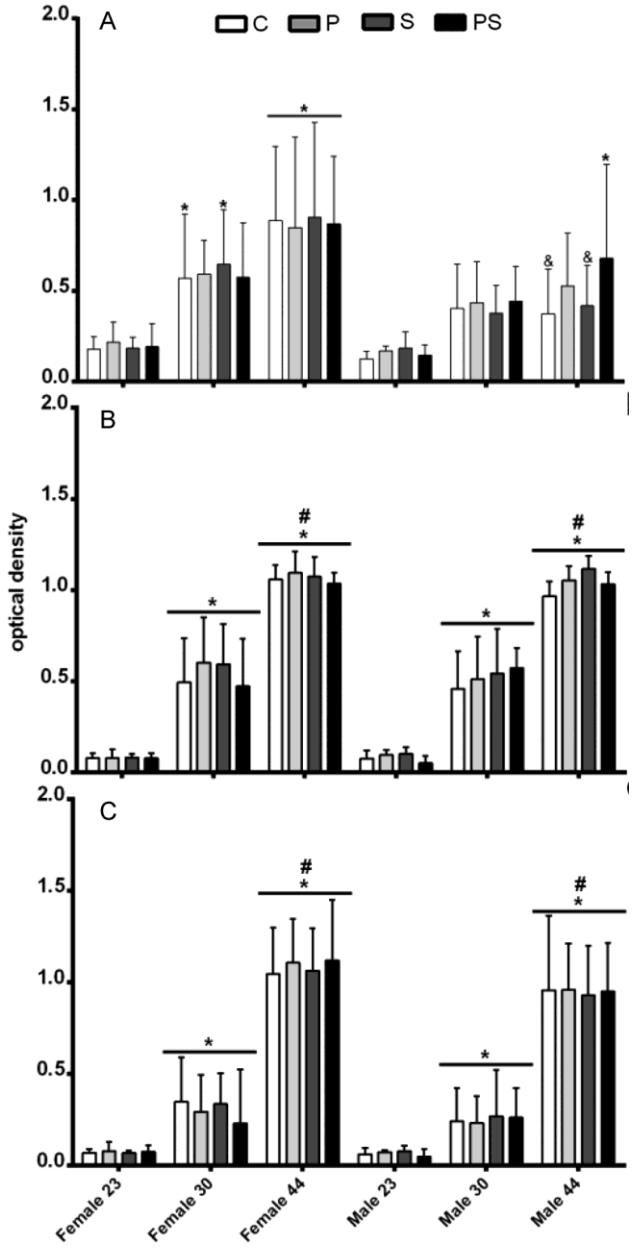

Fig. 6 — The influence of MIPE and CVS on the production of anti-IgY antibodies. (A) IgM; (B) IgG1; and (C) IgG2a. [Females: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=11); Males: (C, control, n=12; P, physical exercise, n=12; S, stress, n=12; PS, physical exercise and stress, n=9). Numbers in the horizontal axis identify the day of experiment. *, different from day 23 in the same treatment; #, different from day 30 in the same treatment; &, different from females in the same treatment. The four-way ANOVA test followed by the Bonferroni post hoc. Data presented as mean ± sd. *P* <0.05]

Fig. 6B shows the production of IgG1. There was an interaction between stress and exercise (F [1, 84] = 6.375, *P* <0.05) and a time effect (F [2, 168] = 1210.976, *P* <0.001). The post hoc showed increased IgG1 production in females and males on days 30 and 44 (*P* <0.001, *vs*. groups C, P, S and PS on day 23, respectively). The production of IgG2a is shown in Fig.6 C. Sex (F [1, 84] = 4.597, *P* <0.05) and time effects were found (F [2, 168] = 688.351, *P* <0.001). The post hoc showed increased IgG2a production in both females and males on days 30 and 44 (*P* <0.001, *vs*. groups C, P, S and PS on day 23, respectively).

In the results of the blood count and differential leukocyte count in the peripheral blood of female and male rats, no differences were observed between the groups (*P* >0.05) (data not shown).

Discussion

The present study investigated the influence of chronic variable stress (CVS) and moderate intensity physical exercise (MIPE) on antibody production in Wistar rats. Several studies have shown that physical exercise when performed at moderate intensity promotes positive adaptations such as body weight control[17], improvement in the cardiovascular system[18], reduction in body fat[19], and greater ability to remove blood lactate[20]. On the other hand, chronic stress negatively influences behaviour, causing, for example, some signs of anhedonia, such as decreased exploratory activity and reduced preference for sweetened solution, which may be accompanied by an increase in the weight of the adrenal glands[1,21].

Thus, in the present study, we observed a similar effect of MIPE in relation to the aforementioned studies, in which, regardless of sex, MIPE resulted in benefits to the animals, such as maintenance of body weight, cardiac hypertrophy, and a greater capacity to remove blood lactate. In addition, in females, exercise reduced peritoneal fat. These effects demonstrate that the training protocol was effective in promoting improvements in animal health. Control of body wt. through MIPE occurs as the energy used during its performance originates, mainly, from fats[22]. These adaptations also reflect in lactate production, where the muscular system removes more lactate in exercises performed at the same intensity[20]. In

accordance with the literature, the data from our study reinforce the role of MIPE in promoting physiological improvements.

Regarding stress, a reduction in the preference for sucrose was observed in females and males. In addition, stress reduced exploratory activity and increased adrenal weight in male rats. In relation to sex, we can emphasize that CVS affected males more than females. This can be explained by the fact that females produce estrogen, a hormone known to inhibit the HPA axis, regulating, through negative feedback, the production of the hormone cortisol by the adrenal glands[8]. As female rats present this protective factor, it is understandable that male rats are more susceptible to CVS[23]. Another observation in the study is that females presented higher exploratory activity than males, an effect also seen in the study by Cavigelli *et al.*[24]. This is relevant since, depending on the behavioural model, the use of females or males may be a decisive factor in the results.

Although CVS is effective in causing behavioural changes in rats, a key issue is the role of MIPE in preventing the effects of CVS. In this sense, all parameters affected by CVS (preference for sucrose, open field activity, and adrenal weight) demonstrated a positive influence from MIPE. The effectiveness of MIPE in stress resilience is comparable to that of pharmacological treatment and psychological interventions[25].Although the ideal amount and type of exercise to promote behavioural improvements are not yet fully elucidated, physically active individuals present fewer health problems, especially when faced with stress situations[26] which, in excess, may manifest in behavioural signs (e.g., anxiety, insomnia, and hyperactivity) and lead to depression[27].

MIPE adaptations also extend to the immune system (IS)[28], as can be observed by the greater protection against infections, increased efficiency of vaccines, and increased proportion of differentiated T cells in the circulation[29]. An example of the action of MIPE on the IS was observed in mice exercised and infected with *S. pneumoniae*. These animals were more effective in preventing the increase in the number of bacteria and lung inflammatory process compared to the non-exercised animals[29]. It is probable this result is due to MIPE normalizing inflammatory cytokines (TNF-α and IL-6) and presenting an antioxidant effect[29].

Another important point is the fact that MIPE can contribute to hematological homeostasis[30]. A similar effect was found in the present study, the rats did not present changes in blood count or differential leukocyte count in relation to control animals.

In humoral immunity, MIPE is known to potentiate the production of specific antibodies[31,32]. As an example, older women vaccinated against influenza presented higher production of specific IgM and IgG antibodies when submitted to a MIPE program[29]. In another study it was observed that 15 minutes of MIPE resulted in higher production of antibodies in the individuals immunized with the pneumococcal vaccine[33]. In the present study, it was observed that both females and males of the P group produced levels of specific IgG1 and IgG2a antibodies similar to the animals of group C. A difference between the sexes was observed for the IgM class, in which females produced more IgM antibodies than males. These observations have also been reported earlier by Hatzakis & Hadziyannis[34]. On the other hand, MIPE increased IgM production in the males, eliminating differences between the sexes. Antibodies of the IgM class are the first to be produced in response to an antigen, being potent activators of the complement system (important effect or of innate and adaptive immunity), and thus, increases in production favor the host against pathogens[35]. The deficiency in IgM production is related to the onset of pathologies such as pneumonia, sepsis, allergic diseases, and meningitis[36]. Thus, the influence of MIPE on increasing the production of IgM in males is clinically important, especially for individuals with IgM deficiency.

Stress is also known to modulate the immune system[36]. However, the effects of stress on antibody production vary according to the stress protocol, antibody class, and sex[16,37]. As an example, mice immunized with *keyhole limpet hemocyanin* (KLH) immediately after an inescapable shock session, presented lower production of anti-KLH antibodies (IgM and IgG2a) 28 days after immunization[8]. Another study showed that forced swimming stress did not interfere with the production of *Sheep Red Blood Cells* (SRBC) specific antibodies (IgM and IgG2a) but inhibited the production of anti-SRBC IgG[17]. Regarding sex, it was observed that chronic stress from restraint enhanced the production of anti-SRBC antibodies only in female rats[38]. In the present study, it was observed that CVS did not affect the production of antibodies in females, however, in males, CVS affected the production of specific IgM

class antibodies, a condition reversed by MIPE. The role of CVS in suppressing antibody production can be explained by the fact that stress alters the immune response pattern from Th2 to Th1[5], a condition that explains the lower production of IgM antibodies in stressed animals. Thus, stimulation in the production of IgM in males of the PS group can be explained by the fact that MIPE favors the Th2 response pattern through an increase in the production of anti-inflammatory cytokines[12].

Conclusion

It is concluded from the present investigation that moderate intensity physical exercise (MIPE) is effective in promoting morphological and behavioural improvements in animals. Regarding behaviour, MIPE was highlighted by preventing all alterations caused by chronic variable stress (CVS). With respect to the production of antibodies, IgG1 and IgG2a were not affected by stress. For IgM, it was observed that females, in addition to being unaffected by CVS, naturally produce more antibodies than males. This male deficiency was reversed through MIPE. In this sense, the ability of moderate intensity physical exercise to reverse and/or prevent the effects of chronic stress and favour the production of antibodies is evidenced.

Conflict of interest

Authors declare no competing interests.

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