



# Influence of low and high intensity physical exercise on hypernociception threshold and other parameters of rats\*

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## ABSTRACT

Physical exercise practice is an important habit to maintain the physical and emotional well-being of those who regularly adopt it, and can bring great benefits to health. However, depending on the type, intensity, frequency and duration of the exercises, they can also do a certain degree of harm to the organism. As a benefit, the role of physical exercise is described in the specific and non-specific immune function and, in the latter, the inflammatory process stands out. Therefore, the purpose of this study was to show the effects of low and high intensity physical exercises on hypernociception, and to evaluate the body mass and the relative weight of some organs in rats. For the study, male adult Wistar rats were used and submitted (trained group) or not submitted (non-trained group) to physical exercises on an ergometric treadmill. The acute inflammation was induced by an injection of carrageenin-0.5% into the plantar tissue of the left hind paw of each rat and the nociception was measured by the plantar test before and after 1, 2, 3, 4, 6, 8 and 24 hours. At the end of the evaluations, the rats were submitted to deep anesthesia until their euthanasia, to collect and weigh the adrenal glands, heart, spleen and kidneys, and to subsequent histopathological study of those tissues. The statistical analysis of the results showed a significant increase ( $P < 0.05$ ) of the hypernociception threshold in  $H_2$ ,  $H_3$ ,  $H_4$  and  $H_6$  moments in animals trained at high intensity. There was also a significant reduction ( $P < 0.05$ ) in body mass besides hypertrophy of the adrenal glands and heart and an increase in the kidneys relative weight of the rats trained at high intensity, in addition to adrenal hypertrophy of the animals trained at low intensity. There were no statistically significant changes in the other parameters evaluated in this study. We concluded that physical exercises on a treadmill were capable of changing the nociception, body mass and the relative weight of some organs, but in a way that was dependent on the exercises protocol applied to the animals.

## INTRODUCTION

Physical activity is considered an important factor for physical and emotional well-being promotion, besides improving life quality of whom adopt it regularly<sup>(1)</sup>, improving the mood and being the best weapon against obesity, a growing concern in our world nowadays<sup>(2)</sup>. On the other hand, physical inactivity is associated to a considerable increase of the risk development of a series of degenerative and chronic diseases<sup>(1)</sup>.

According to Caspersen *et al.*<sup>(3)</sup>, physical activity is defined as any body movement performed with the skeletal muscles participation involving a bigger energetic use, when compared to resting indices. It is performed through physical exercises, that is, repetitive, structured and planned body movements, resulting in improve-

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ment of one or more physical ability components. Thus, during and after exercises a great number of changes in the neuro endocrinal systems occurs (indices increase of the adrenalin, noradrenalin, cortisol, corticotrophin liberator hormone, adrenocorticotrophic hormone, among other endogenous substances) and immune-system (changes in the concentration and functions of leukocytes, the natural killer cells and the T and B lipocytes, besides alterations in the immunoglobins, cytokines and other soluble factors indices), although the quality and quantity of these alterations and the necessary time for them depend on the intensity and duration of these exercises<sup>(4-5)</sup>.

Regular physical exercises are also able to promote an acceleration of the repairing processes in inflammation<sup>(6)</sup>. Some studies therefore, showed that exercises interfere in several steps of the inflammatory process, promoting leukocytes migration towards the inflammation site (chemiotaxis) and increase of the fagocytosis ability of these cells in humans and animals<sup>(7)</sup>, besides increasing the macrophages anti-tumor activity<sup>(8)</sup>.

Inflammation is a response from the vascularized conjunctiva tissue to a chemical, physical or biological aggression<sup>(9-10)</sup>. The inflammatory response is characterized by redness, heat, edema, pain and function loss. Moreover, it is the expression of functional and structural phenomena in the microcirculation and in the interstitial tissue close to the lesion, with the participation of the local sensitive enervation<sup>(9-11)</sup>. Inflammation is a process commonly limiting which may have different origins and, among them, the traumas (contused or penetrating)<sup>(10)</sup>.

Pain is mainly a body protection mechanism against tissular aggression and may be triggered by several types of stimuli (or agents) which stimulate the pain receptors (nociceptors). In some occasions, the pain fibers excitation becomes progressively higher, especially in the slow pain, as the painful stimulus continues. Such sensibility increase in the nociceptors is named hyperalgesia or hypernociception<sup>(12)</sup>. According to Tribioli<sup>(13)</sup>, pain is characterized by a sensory and emotional experience, of unpleasant characteristic, associated to a tissue injury. The pain perception and the body response to the painful stimuli depend on the nervous system complexity and this process is named nociception.

The results of the majority of the specific studies show that an improvement in pain may occur after physical exercises practice; therefore, some researchers have been referring to this phenomenon as exercise-induced analgesia<sup>(14)</sup>, with several reports in humans<sup>(15-17)</sup> and animals<sup>(18-20)</sup>. However, further research is still needed in order to fully understand the antinociceptor responses after exercise, once the involved mechanisms in this process are rather complex and are not totally clear, despite the studies performed so far<sup>(14)</sup>.

Therefore, there is a lot of evidence about the involvement of the endogenous opioid system modeling pain through the action of the encephalons and  $\beta$ -endorphins, responsible for inducing analgesic effects and influence in the perception of the nociceptor stimulus and in the humor state, due to its action in opioid recep-

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tors<sup>(21-22)</sup>. In some stress situations, such as during physical exercise, the plasmatic index of the  $\beta$ -endorphins increases in 3 to 10 times<sup>(23)</sup>. Moreover, its plasmatic concentration increases in response to extended physical exercises, if the intensity is above 50% of the maximal  $\dot{V}O_2$  and with duration higher than three minutes, while exercises in lower intensities do not cause increase in the plasmatic indices of these peptides, even in long duration exercises<sup>(24)</sup>.

It is also reported that acute physical exercises may lead to responses that involve the activation of the hypothalamus-hypophysis-adrenal axis, reaction similar to the stress one, which induces liberation of ACHT (adrenocorticotrophic hormone) and stimulation of the adrenal glands. Consequently, synthesis and secretion of glucocorticoid hormones are observed, which stimulate the body metabolic adaptations<sup>(25)</sup>; among them the inflammatory response inhibition<sup>(26)</sup>. During light exercises, the cortisol secretion (in humans) is slightly changed, while in exhaustive exercises significant increase of this hormone is seen<sup>(25)</sup>. Actually, several stressing clinical factors, such as surgeries, traumas, burns and infectious processes, induce a model of hormonal and immunological response similar to the one during physical exercise<sup>(27)</sup>.

Once again, according to Parízková and Stanková<sup>(28)</sup> the adrenal glands weight and the final body weight in rats, are reliable indicators in order to verify the stressor effect of physical exercises, especially the ones performed on treadmill. Such weight is directly related to the intensity of the exercises performed by the animals, that is, higher intensities demand bigger energy use and result in lower weight gain<sup>(29)</sup>.

Furthermore, physical exercises produce a state of relative hypox due to the increase of metabolic demand, and induce cardiovascular adaptations<sup>(30)</sup>. As a result, daily physical exercises may lead to a cardiac hypertrophy, observed in many laboratory animals species, in which different physical training protocols were applied<sup>(31)</sup>. However, the heart is not the only organ that may present alterations after physical exercises; the kidneys and the spleen may also suffer alterations, probably due to adaptation processes triggered by exercises<sup>(31)</sup>.

Thus, the literature on analgesia associated to physical exercises is conflicting, once some researchers report alterations in the pain threshold after exercises sessions, in humans and animals, while others do not. In addition, the research on the antinociceptive effect in rats is scarce, using physical exercises protocols on treadmill, once the majority of them uses swimming as protocol. Accordingly, the aim of this work was to study and compare the effects of programmed and controlled physical exercises of low and high intensity on the nociception, besides to evaluate the effects of these exercises on the body mass and the relative weight of rats' adrenal glands, heart, spleen and kidneys.

## METHODS

### Animals

Wistar male rats with approximately 60 days of age and body weight ranging from 180 and 250 grams (at the beginning of the experiments), were used in this work. The animals came from the Central Animal Morgue of the Medicine School of São Paulo (EPM/UNIFESP, São Paulo). These animals were used according to the regulations and procedures relative to the animal use in laboratory, described by the Committee on Care and Use of Laboratory Resources National Research Council (EUA). This study was previously analyzed and approved by the Ethics Committee of the Bandeirante University of São Paulo – UNIBAN, São Paulo (protocol 024/2004).

The animals were placed in polypropylene plastic boxes measuring 41 x 34 x 16 cm, containing five rats per box and kept in room with temperature and humidity controlled. There was arti-

cial light with photoperiod of 12 hours of light and 12 hours of dark, with the light phase starting at 8:00 hr. The rats were fed with standard food and water *ad libitum*; identified, weighted and randomly divided in groups of trained animals and groups of non-trained animals. These animals were kept in these conditions for an adaptation period of at least seven days prior to the experiments beginning.

### Rats low and high intensity physical training

In order to apply the physical exercises of low and high intensity, the animals of the 2 trained groups (TR, n = 12 in each group) were submitted to experimental protocols performed on programmable and adapted treadmill for rats (10400-Inbramed®), with 8 stainless steel individual lanes, with holes for ventilation and individually covered by transparent acrylic movable lids, with ventilation holes as well. The rats of the 2 non-trained groups (NTR, n = 8 in each group) were only manipulated and placed on the treadmill (without movement), with the aim to expose them to the exercises site (treadmill), which may represent a stress source to these animals.

The physical exercises protocol of low intensity consisted of a 12-week training, 5 times per week. In the two first weeks, the sessions' duration was gradually increased until daily 60 minutes and kept until the end of the training. The velocity was 5 m/min in the two first weeks and was also gradually increased until reaching the 15 m/min velocity. The treadmill inclination was kept at 1% during the entire training.

The physical exercises protocol of high intensity also consisted of a progressive training with 11 weeks duration, 5 times per week. In the first week the animals were placed on the treadmill and trained during 15 minutes, 5 m/min, with 0% of inclination. From the first week until the end of the sixth week, the duration, velocity and treadmill inclination were gradually increased until 75 minutes of duration, 25 m/min velocity and 15% of inclination, being the inclination kept for a period of 5 weeks.

Although they were not directly measured, the low and high physical exercises intensities were based on previous studies, which estimated for the low intensity exercises an oxygen maximal consumption (maximal  $\dot{V}O_2$ ) of approximately 50%<sup>(32-33)</sup>, and for the high intensity ones, a consumption of approximately 70% for the rats<sup>(32-34)</sup>.

### Nociception induction

An acute inflammatory response was provoked in order to induce the nociception. A 0,5% carrageenan injection type Lambda (Sigma®) diluted in Ringer solution (Aster®) was injected in the plantar tissue of the left hind-paw of each rat, in the 0,1 ml volume per animal. The remaining solution was not used in another experiment.

### Measuring of the nociception threshold

The nociception threshold induced by the carrageenan (hypernociception) was evaluated through the Hargreaves method, using the Plantar Test, a device that measures the time for the rat's paw removal (threshold) facing a thermal stimulus (Plantar Test – Ugo Basile®)<sup>(35)</sup>. In order to justify such test, the measurements occurred before and 1, 2, 3, 4, 6, 8 and 24 hours after the carrageenan injection or (not)<sup>(35-36)</sup>. The final results obtained, that is, the latency for the paw's removal from the heat site, was directly expressed in seconds (sec).

### Body weight evaluation

The animals from both groups, TR and NTR, were weighted three times per week, on mechanical scale for rats (Marte®), during the entire training period, with the purpose to verify alterations in the animals' body mass, possibly caused by the low and high intensity physical training.

## Collection and weighting of the adrenal glands, heart, spleen and kidneys

At the end of the experiment, the animals were submitted to euthanasia through general deep anesthesia. Later, the adrenalectomy and the adrenal glands weighting were performed, as well as the removal and weighting of other organs such as heart, spleen and kidneys of the rats from the TR and NTR groups, on analytical scale (Marte®).

The final calculation of the organs relative weight of each rat was performed dividing the weight of each organ (in grams) by the body weight of each animal on the collection day, and multiplying the result by 100. The result was then expressed in grams/100 grams of live weight (g/100 g l.w.).

## Anatomopathological study of the tissues

The collected and weighted adrenal glands, heart, spleen and kidneys were later set and conserved in 10% formaldehyde; fragments of these organs were blocked, cut and stained with Eosin-Hematoxylin (E-H), routine histological technique, for observation of possible microscopic lesions in these tissues.

## Statistical analysis

The obtained results were submitted to the Bartlett test for definition of the test type (parametric or non-parametric). The Vari-

ance Analysis test (ANOVA) was used for analysis of possible differences among the several studied groups, followed by the Tukey-Kramer test of multiple comparisons (parametric data). The Student t-test (parametric data) and the Mann-Whitney test (non-parametric data) were also used for detection of possible differences between the 2 distinct groups (TR and NTR). The results were expressed as average  $\pm$  standard deviation and the statistical significance index adopted was the 5% one ( $P < 0,05$ ).

## RESULTS

Table 1 presents the effects of low intensity physical exercises on the hypernociception threshold. Similar response is observed between the animals from the TR and NTR groups during the entire evaluation period, that is, statistically significant differences ( $P > 0,05$ ) of this threshold in the two groups of animals were not detected at any of the analyzed moments. The hypernociception peak (low intensity training) was observed at the  $H_4$  moment in both groups. Table 2 presents the effects of high intensity physical exercises on the hypernociception threshold and, differently from the low intensity physical training, the statistical analysis revealed an increase of the hypernociception threshold at the  $H_2$ ,  $H_3$ ,  $H_4$  ( $P < 0,05$ ) and  $H_6$  ( $P < 0,01$ ) moments in the animals of the TR group in relation to the ones from the NTR group. The hypernociception

**TABLE 1**  
Hypernociception threshold in Wistar rats submitted or not to low intensity physical exercises, on treadmill, and measured at different moments (from 0 to 24 hours)

Animal groups (n = 8-12) <sup>a</sup>	Average and standard-deviation of the latency for paw removal (in seconds)							
	H <sub>0</sub>	H <sub>1</sub>	H <sub>2</sub>	H <sub>3</sub>	H <sub>4</sub>	H <sub>6</sub>	H <sub>8</sub>	H <sub>24</sub>
TR	10,5 $\pm$ 3,0	6,3 $\pm$ 2,8 <sup>A</sup>	3,2 $\pm$ 1,4 <sup>AB</sup>	2,3 $\pm$ 0,5 <sup>AB</sup>	1,9 $\pm$ 0,6 <sup>AB</sup>	2,7 $\pm$ 1,0 <sup>AB</sup>	4,0 $\pm$ 2,0 <sup>AB</sup>	8,8 $\pm$ 3,4 <sup>D</sup>
NTR	11,0 $\pm$ 2,4	7,2 $\pm$ 3,9 <sup>A</sup>	3,3 $\pm$ 1,9 <sup>AB</sup>	2,0 $\pm$ 0,6 <sup>AB</sup>	1,8 $\pm$ 0,4 <sup>AB</sup>	2,9 $\pm$ 1,2 <sup>AB</sup>	2,7 $\pm$ 1,0 <sup>AB</sup>	8,8 $\pm$ 2,0 <sup>C</sup>

<sup>a</sup> number of animals per group; <sup>A</sup>  $P < 0,05$  in relation to the H<sub>0</sub> moment (ANOVA followed by Tukey-Kramer); <sup>B</sup>  $P < 0,05$  in relation to the H<sub>1</sub> moment (ANOVA followed by Tukey-Kramer); <sup>C</sup>  $P < 0,05$  in relation to the H<sub>1</sub> moment until H<sub>8</sub> (ANOVA followed by Tukey-Kramer); <sup>D</sup>  $P < 0,05$  in relation to the H<sub>2</sub> moment until H<sub>8</sub> (ANOVA followed by Tukey-Kramer).

Ps.: There were not statistically significant differences between the TR and NTR groups (not paired "t" Student test).

**TABLE 2**  
Hypernociception threshold in Wistar rats submitted or not to high intensity physical exercises, on treadmill, and measured at different moments (from 0 to 24 hours)

Animal groups (n = 8-12) <sup>a</sup>	Average and standard-deviation of the latency for paw removal (in seconds)							
	H <sub>0</sub>	H <sub>1</sub>	H <sub>2</sub>	H <sub>3</sub>	H <sub>4</sub>	H <sub>6</sub>	H <sub>8</sub>	H <sub>24</sub>
TR	9,6 $\pm$ 0,6	5,9 $\pm$ 1,1	2,8 $\pm$ 1,1 <sup>AB*</sup>	3,1 $\pm$ 1,0 <sup>AB*</sup>	2,7 $\pm$ 0,6 <sup>AB*</sup>	3,9 $\pm$ 1,2 <sup>AB**</sup>	5,3 $\pm$ 1,9 <sup>AC</sup>	10,1 $\pm$ 2,7 <sup>E</sup>
NTR	9,2 $\pm$ 0,8	5,3 $\pm$ 1,0 <sup>A</sup>	1,9 $\pm$ 0,4 <sup>AB</sup>	2,2 $\pm$ 0,9 <sup>AB</sup>	2,2 $\pm$ 0,6 <sup>AB</sup>	2,4 $\pm$ 0,7 <sup>AB</sup>	4,8 $\pm$ 1,4 <sup>AD</sup>	9,1 $\pm$ 2,8 <sup>E</sup>

<sup>a</sup> number of animals per group; \*  $P < 0,05$  in relation to the NTR group (not paired "t" Student test); \*\*  $P < 0,01$  in relation to the NTR group (not paired "t" Student test); <sup>A</sup>  $P < 0,05$  in relation to the H<sub>0</sub> moment (ANOVA followed by Tukey-Kramer); <sup>B</sup>  $P < 0,05$  in relation to the H<sub>1</sub> moment (ANOVA followed by Tukey-Kramer); <sup>C</sup>  $P < 0,05$  in relation to the H<sub>2</sub> moment until H<sub>4</sub> (ANOVA followed by Tukey-Kramer); <sup>D</sup>  $P < 0,05$  in relation to the H<sub>2</sub> moment until H<sub>8</sub> (ANOVA followed by Tukey-Kramer); <sup>E</sup>  $P < 0,05$  in relation to the H<sub>2</sub> moment until H<sub>8</sub> (ANOVA followed by Tukey-Kramer).

peak (high intensity training) occurred between the H<sub>2</sub> and H<sub>6</sub> moments in both groups.

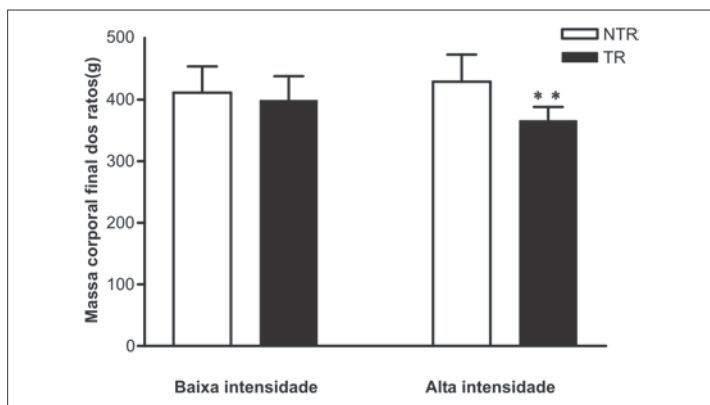
Concerning the final body weight of the trained animals under low intensity protocol, there was not statistically significant difference between the animals from TR group in relation to the NTR group ones ( $P > 0,05$ ). However, a lower final body weight of rats from TR group under high intensity protocol in relation to those animals from NTR group ( $P < 0,01$ ) was detected (figure 1).

Moreover, the statistical analysis of the results did not demonstrate significant differences ( $P > 0,05$ ) in the relative weight of heart, spleen and kidneys of animals from TR group in low intensity (TR-LI) in relation to those animals from NTR group (NTR-LI). Nonetheless, after high intensity exercises, heart hypertrophy ( $P < 0,05$ ) and relative kidneys weight increase ( $P < 0,01$ ) were detect-

ed in the organs weighting and anatomopathological study in the rats from the TR group TR (TR-HI) in relation to those from the NTR group (NTR-HI). No statistically significant differences ( $P > 0,05$ ) in the relative weight of spleen of animals from the TR group in high intensity (TR-HI) occurred in relation to those in the NTR group (NTR-HI) (figure 2). Likewise, adrenal glands hypertrophy in the rats from the TR groups in low intensity (TR-LI) ( $P < 0,05$ ) and in high intensity (TR-HI) ( $P < 0,001$ ) was observed in relation to those animals from the NTR group (NTR-HI) (figure 3).

## DISCUSSION

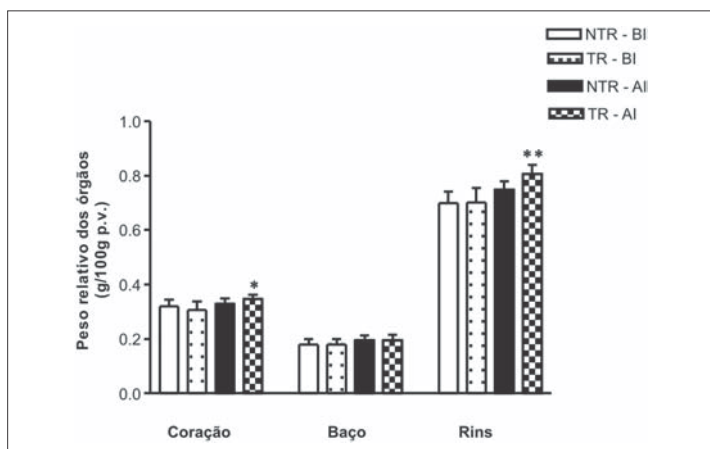
Physical exercises regularly practiced lead to physiological and morphological adaptations important to the body's homeostasis



**Figure 1** – Final body mass (in grams) of rats submitted or not to low and high intensities physical exercises

\*\* P < 0,01 (in relation to the NTR group).

Results expressed as average and standard-deviation, n = 8 to 12 per group.



**Figure 2** – Relative weight of organs (g/100 g l.w.) of rats submitted or not to low (LI) and high (HI) intensities

\* P < 0,05 (in relation to the NTR group).

\*\* P < 0,01 (in relation to the NTR group).

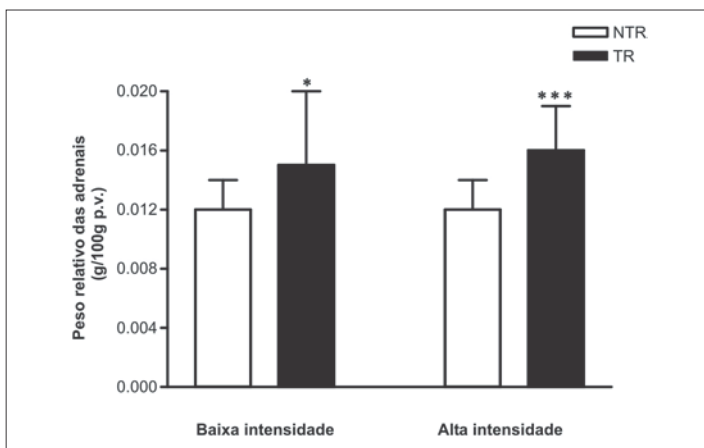
Results expressed as average and standard-deviation, n = 8 to 12 per group.

maintenance and these adaptations are also important to the control of several diseases, especially the ones with cardiovascular and endocrine-metabolic nature<sup>(37)</sup>.

Thus, several studies have shown that physical exercises are able to promote changes in many functions of the human and rats' bodies. However, the research published so far mentions several results discrepancies for many reasons, such as the use of different forced or voluntary physical training protocols, the variation in the duration and intensity of the physical exercises and the multiplicity of used tests for evaluation of the influence of exercises in the investigated parameters<sup>(38)</sup>. Therefore, the comparison of the effects of different intensities of physical exercises, such as the ones used here, in the modulation of the nociception and other biological parameters, is important in order to try to contribute to the clarification of issues still obscure concerning this subject.

Hence, this paper evaluated the influence of low and high intensity exercises on treadmill on the hypernociception modulation, the body mass and the relative weight of the adrenal glands, heart, kidneys and spleen of rats, considering the physical exercises as a means of stressing stimulus capable of inducing changes in the homeostasis state and reorganize responses of several systems, among them the neuroendocrinal system<sup>(39)</sup>. Nonetheless, although the experimental models used here are standardized and the evaluations quantified, the research was conducted in animals, which may limit its extrapolation to humans.

The study showed that low intensity physical exercises did not alter the hypernociception threshold at any of the evaluation mo-



**Figure 3** – Relative weight of the adrenal glands (g/100 g l.w.) of rats submitted or not to low and high intensities physical exercises.

\* P < 0,05 (in relation to the NTR group).

\*\*\* P < 0,001 (in relation to the NTR group).

Results expressed as average and standard-deviation, n = 8 to 12 per group.

ments, once there were not statistically significant differences in this parameter, between the rats in the trained groups (TR) and non-trained ones (NTR). It is known that acute exercises may trigger several body's responses, among them the stress reactions involving the hypothalamus-hypophysis-adrenal axis, which induces the CRH liberation (corticotrophin release hormone) and consequently stimulates the ACTH secretion and increases synthesis and secretion of glucocorticoid hormones by the adrenal glands. Moreover, these facts generate metabolic adaptations to physical exercise<sup>(25)</sup>. In addition, the animals used in this work were trained from a protocol whose training intensity corresponds to 50% of the maximal  $\dot{V}O_2$ <sup>(32-33)</sup> and thereby, the obtained results in this exercise intensity corroborate those by Pertovaara and Kempainen<sup>(40)</sup> and by Droste *et al.*<sup>(16)</sup>, from studies with humans which questioned whether the low intensity of physical exercise would be sufficient in order to cause analgesic effect.

Characteristically, the induced analgesia by stress may occur through one of the two systems, that is, the opioid and the non-opioid systems<sup>(41)</sup>. It is mentioned in the literature then, that during inflammatory pain, the CRH may stimulate the opioid peptides release (especially  $\beta$ -endorphins) of several types of immune-cells, producing antinociception in inflamed sites, due to the activation of opioid receptors present in peripheral sensory nervous terminations<sup>(42)</sup>.

In this work, the results of hypernociception after low intensity physical training suggest that the neuroendocrine axis (hypothalamus-hypophysis-adrenal) stimulation was not sufficient so that the circulating CRH indices could promote such effects. Moreover, the non-alteration of the nociception threshold in this exercise intensity may be indicative that such threshold may have balanced if this endogenous opioid system has been activated, once these animals were tested 16 hours after the last physical exercises session. In research conducted by Shyu *et al.*<sup>(18)</sup> with spontaneous training of rats in metal wheel, increase in the nociception threshold after the end of the exercises was observed, which gradually decreased in the six first hours of inactivity followed by the physical exercise.

Besides that, according to Terman *et al.*<sup>(19)</sup> and Gamaro *et al.*<sup>(43)</sup>, repeated exposition to the same aversive stimulus may lead to an adaptation process to this stimulus. In this work, the rats trained under a low intensity protocol during 12 weeks, may have developed an adaptation to the not very severe exercises and consequently, a lower activation of the neuroendocrine axis as well, what, at a certain extent, would explain the fact that an increase in the nociception threshold in this training intensity did not occur.



On the other hand, Kemppainen *et al.*<sup>(44)</sup> reported a significant increase in the dental pain threshold only after physical exercises using 74% of the individuals' maximal aerobic ability. Yet, Koltyn<sup>(17)</sup> reported significant increase in the pain threshold when a single exercise session is used in bicycle ergometric test respecting a protocol with around 75% of the individuals' maximal aerobic ability. The results of this study show that high intensity physical exercises increase the hypernociception threshold in the H<sub>2</sub>, H<sub>3</sub>, H<sub>4</sub> and H<sub>6</sub> moments, reinforcing the data already published.

Concerning this topic, maybe high intensity physical exercises here used, differently from the low intensity ones, are a stressor stimulus more intense to animals, and, hence, are able to trigger more evident neuroendocrine responses in the body, with increase in the seric indices of CRH, ACTH and glucocorticoid hormones. Such hypothesis can be corroborated by the bigger hypertrophy of the adrenal glands of the rats under high intensity training than in the ones under low intensity training. Therefore, it is possible that a rats' body adaptation to the high intensity exercises would be more difficult in comparison to low intensity ones. According to this hypothesis, the results of other research in rats, are conclusive in the demonstration that exercises promote hypertrophy of the adrenal glands<sup>(31-45)</sup> and that the physical training intensity influences the intensity-dependent response of the hypothalamus-hypophysis-adrenal axis activation<sup>(46)</sup>. Therefore, the results obtained here suggest the participation of these hormones in the antinociceptor effects, even if the rats have been evaluated after 16 hours of the end of physical training.

Another justification to be considered for the results obtained in this work about hypernociception after high intensity exercises, is the activation of the peripheral opioid system (activation of opioid receptors present in peripheral sensory nervous terminations) through the release of opioid peptides of inflamed tissues, especially  $\beta$ -endorphins, stimulated by the plasmatic high indices of CRH and IL-1 (Interleukin 1), triggered by such exercises. These findings support the ideas by Schäfer *et al.*<sup>(47)</sup> and Mousa *et al.*<sup>(42)</sup>, who reported that, in the inflammatory pain, the endogenous and exogenous CRH and IL-1 are able to release opioid peptides of inflamed tissues and that these immune derivate opioids would be released by conditions that involve environmental stress, resulting in nociception inhibition.

In this research, no alteration in the final body mass of the animals submitted to low intensity physical exercises was observed, although the TR group animals were visually smaller in relation to the control group (NTR). The fact that no statistically significant differences were observed in the end of the training may be indication of a substitution of adipose tissue (fat) for muscular mass in the TR group animals, according to indication by Mayer *et al.*<sup>(48)</sup>.

On the other hand, it was observed that the final body mass of rats trained with high intensity exercises was smaller after 11 weeks period of physical training, suggesting increase in the energy use in these animals. These results corroborate the literature with trained rats on treadmill under high intensity protocols, which reduce the food intake with consequent weight loss<sup>(31-48)</sup>. As mentioned before and reinforcing such idea, physical exercises represent a type of stress and promote CRH release by the hypothalamus due to the dependence of the stressor agent intensity. Actually, Rivest and Richard<sup>(49)</sup> demonstrated anorexigenic effects for the CRH, simulating hence, the effects triggered by exercises. In this context, the high intensity physical exercises applied in this study seem to actually represent a stressor stimulus sufficiently capable to activate the hypothalamus-hypophysis-adrenal axis, consequently increasing the seric indices of CRH.

Likewise, Stevenson *et al.*<sup>(50)</sup> mentioned that the degree of appetite decrease may be related to the stress degree provoked by the physical exercise intensity in animals and that such anorexia

may be mediated by the high indices of catecholamines derived from this stress. Accordingly, the histopathological finding of this work showing hypertrophy of the medullar and cortical areas of the adrenal glands is indicative of a stress situation in these animals.

Furthermore, countless variables may induce cardiac hypertrophy in rats submitted to physical exercises, such as the frequency, intensity and duration of the physical training sessions, besides the training program duration. Other factors include the age at the beginning of the training, sex and type of animal used in the physical training<sup>(51)</sup>. Thus, the results found in this research related to the heart's relative weight after low and high intensities training, agree with the authors mentioned above. In addition, low intensity exercises did not cause increase in the heart weight of trained rats, in relation to the non-trained ones. However, there was increase in the relative weight of these organs and myocytes hypertrophy, detected through the histopathological analysis of the rats trained at high intensity.

Despite everything, the heart is not the only organ that presents adaptation alterations after physical exercises; the spleen's relative weight may also present alteration, but the results are rather conflicting<sup>(31)</sup>. Bloor *et al.*<sup>(45)</sup> did not detect significant alterations in the relative weight of spleen in adult rats trained with swimming exercises, 1 daily hour for 10 weeks. Likewise, decrease in the spleen weight in exercised rats has been reported, possibly due to a higher blood expelling stored in the organ<sup>(52)</sup> or still, due to the fact that physical exercises promote an increase in the synthesis and secretion of corticosterone (glucocorticoid hormone), which would lead to a decrease of the splenic tissue<sup>(31)</sup>. The anatomopathological findings of this study did not reveal alterations in the spleen's relative weight and, therefore, do not agree with the results in the literature.

Furthermore, the histopathological analyses showed increase in the relative weight and moderate congestion of the kidneys of trained animals at high intensity and moderate renal congestion, without alteration in the kidney weight of rats trained at low intensity. According to Shizuru *et al.*<sup>(53)</sup>, the renal responses to physical exercises are related to their intensity, therefore, exercises performed at low intensities increase the urinary flow and the sodium excretion, while at high intensities, these two parameters considerably decrease. Maybe such decrease is due to the high plasmatic indices of aldosterone, hormone which progressively increases, reaching up to six times more than the indices observed in resting bodies, as means of keeping the body liquids and the homeostasia. Actually, Oliveira *et al.*<sup>(54)</sup> reported that the primary function of the kidneys is to regulate the volume and composition of the extra cellular liquid, and hence, these alterations that occur during the performance of physical exercises may generate hemodynamic changes and changes in the sodium and water excretion. The findings of this study concerning the kidneys weight of the trained animals do not confirm the findings of the literature<sup>(45-55)</sup>, showing decrease in this organ's weight, maybe by the decrease of the number of glomerulus caused by the hypoxia conditions imposed by the exercises. One of the possible causes of this results divergence may be the fact that the research was performed with rats too young for the physical training, differently from the age of the rats from this study.

As a result, one may suggest that the type, intensity, frequency and duration of the physical exercises, besides other factors, may affect the nociception and other biological variables in rats, depending on the adopted exercises protocol.

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