

# USE OF RESISTANCE EXERCISE AS A FACTOR ANTAGONIZED BY NALOXONE OF ANALGESIA IN ACUTE KNEE SYNOVITIS IN WISTAR RATS



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## ABSTRACT

Analgesia arising from exercising can occur via release of endogenous opioids in the central nervous system and periphery. However, the literature remains controversial about exercise ways and actions in pain. Thus, the aim of this study was to evaluate whether resistance exercise produces changes on the nociception and suffers interference by applying an opioid inhibitor. 18 rats divided into three groups were used: G1 –hyperalgesia on right knee and untreated; G2 –hyperalgesia and treated with jump sin water; G3–hyperalgesia with previous injection of naloxone and subsequent jumps. To produce hyperalgesia, 100µl of 5% formalin was injected in the tibio femoral joint space. Pain was assessed using a digital von Frey filament on the right medial tibiofemoral joint. The evaluation periods were: pre-injury (EV1) after 15 minutes (EV2) and 30 minutes (EV3) and one hour (EV4). The applied exercise was jumping in water and it occurred after EV2. The animal performed 4 sets of 5 jumps, with an interval of 3 minutes and overload of 50% of body weight. In G1, nociceptive increase was observed, with significant decrease and return to initial baseline values in AV4; G2 showed threshold restoration after exercise and return to baseline; G3 reduced thresholds, without restoration or significant increase in them. We concluded that there was analgesia with use of exercise and that it was altered by blocking beta-endorphin.

**Keywords:** pain measurement, beta-endorphin, knee joint.

## INTRODUCTION

Knee osteoarthritis (OA) is considered the main articular disease and the majority of patients reports pain and incapacity which generate devastating effects on the quality of life. Muscular weakness is a well-established functional limitation which can precede the development of OA. The articular injury affects the receptors which provide afferent information for muscles and fuses, decreasing the motor activity and proprioception. Additionally, the aerobic deconditioning can also increase incapacity<sup>1,2</sup>.

Therefore, physical exercise is recommended for such cases and presents important results in pain and functional incapacity. Resistance training improves many of the factors which lead to functional incapacity, such as muscular strength, reflex inhibition, proprioception, range of motion (ROM), cardiovascular aptitude and pain, besides comorbidities such as diabetes, hypertension and obesity. Similarly, resistance exercise also presents positive results, being even considered more beneficial<sup>1-3</sup>.

Regular physical exercise plays an important role in prevention, or as palliative care for functional limitations, associated with articular disease and can be performed as programs of water recreational activity and according to Wang *et al.*<sup>4</sup>, the program improves flexibility, muscular strength and aerobic capacity in adults with hip or knee OA.

One of the explanations for the analgesia derived from physical exercise is the opioid, which can be produced in the central nervous system and in the periphery, via activation of receptors by exogenous or endogenous opioid in painful inflammatory conditions. In the inflammation initial phases, central and peripheral opioid recep-

tors are involved in the antinociceptive effects, and receptors located in peripheral nervous terminals may be activated by exogenous and endogenous opioids, expressed in immune cells to produce significant antinociception<sup>5,6</sup>.

Although physical exercise is frequently recommended for patients with chronic pain<sup>7</sup> and exercises in water be commonly prescribed for those who should avoid activities of weight discharge, the literature is still controversial<sup>8,9</sup>. Thus, the aim of this research was to evaluate whether resistance exercise produces alterations on the nociceptive episodes and if it suffers interferences by the application of an opioid inhibitor.

## METHODS

### Sample and experimental groups

18 Wistar rats, mass of 407.00 ± 25.26g, obtained in the Central Animal Facility of the Unioeste and kept in polypropylene cages with free access to water and food *ad libitum*, with controlled light/dark cycle of 12 hours and controlled room temperature (24 ± 1°C) were used. The study was conducted according to the international ethics guidelines in animal experimentation, being approved by the Ethics in Animal Experimentation Committee and Practical Classes of Unioeste under number 6,011.

The animals were randomly divided in three groups:

Group 1 (G1, n = 6) – composed of animals submitted to hyperalgesia on the right knee and untreated;

Group 2 (G2, n = 6) – submitted to hyperalgesia on the right knee and treated with jumps in water; and

Group 3 (G3, n = 6) – submitted to hyperalgesia on the right knee, with previous naloxone injection and subsequent jumps in water.

## Experimental model of hyperalgesia induction

The animals were manually held and injected 100µl of 5% formalin solution in the tibiofemoral articular space for hyperalgesia induction<sup>10</sup>.

## Naloxone application

1µg of naloxone chlorhydrate (Narcan 0.4mg/ml, Cristália<sup>®</sup>)<sup>11</sup> was injected in the right tibiofemoral articular space of the G3 animals, 15 minutes before the hyperalgesia induction. G1 and G2 had 9% sterile saline solution injected.

## Pain assessment

Pain was assessed through the use of the digital Von Frey filament (Insight<sup>®</sup>), which is used to evaluate the nociceptive sensitivity to the mechanical stimulus in animals. The test was performed with the animal manually restrained and the Von Frey filament was applied on the medial part of the tibiofemoral joint of the right posterior limb. The polypropylene tip of the filament was perpendicularly applied to the area, with gradual pressure increase, and, as soon as the animal removed the paw, the test was interrupted for record of the removal threshold. The animals were trained about the evaluation manner during five days. On the day following the last training, the pressure values at the pre-injury moment were collected (AV1); after 15 (AV2) and 30 (AV3) minutes from the hyperalgesia induction, and reevaluations were performed one hour after as well (AV4).

## Exercise protocol

Concerning G2 and G3, the treatment protocol occurred after the evaluation of the AV2 moment. The animals were submitted to jumps in water, using a PVC tube with 20cm of diameter, with overload of 50% of their body weight, which was performed with lead weight attached to the animals' thorax with a Velcro strap, trying not to harm movements. The animal performed four sets of five jumps each, with three-minute interval between each set, inside the PVC tube. The repetitions count occurred each time the animal went to the water surface to breathe. The site used was a plastic oval 200-liter water reservoir with 60cm depth and the water temperature was kept between 30-32°C. G1 did not perform exercises, it was only placed in water (for less than 30s) to receive similar stress. After the last evaluation (one hour after injury), all animals were decapitated in guillotine.

## STATISTICAL ANALYSIS

Data normality was verified with the Kolmogorov-Smirnov test with intragroup analysis by the ANOVA test for repeated measures and unidirectional for comparison between groups, with Tukey post-test. In all cases significance level accepted was of 5%.

## RESULTS

Significant decrease of the removal threshold in AV2 and AV3 compared to AV1 ( $p < 0.05$ ) was observed for G1, but there was restoration of the values when compared to AV4 ( $p > 0.05$ ). Concerning AV2, both AV3 and AV4 presented significant thresholds increase ( $p < 0.05$ ) (figure 1). G2 presented significant threshold reduction when comparing AV1 and AV2 ( $p < 0.05$ ); however, after physical exercise, the basal values were restored already in AV3, and remained in AV4. In the comparison with AV2, both AV3 and AV4 were significantly higher ( $p < 0.05$ ) (figure 2).

Finally, for G3 there was significant thresholds reduction for all moments after AV1 ( $p < 0.05$ ), with no significant threshold increase when comparing with AV2 ( $p > 0.05$ ) (figure 3), showing hence that exercise did not produce effect when associated with naloxone. In the comparisons between groups, significant difference was only found in AV1 when compared G2 with G1 and G3 ( $p < 0.05$ ).

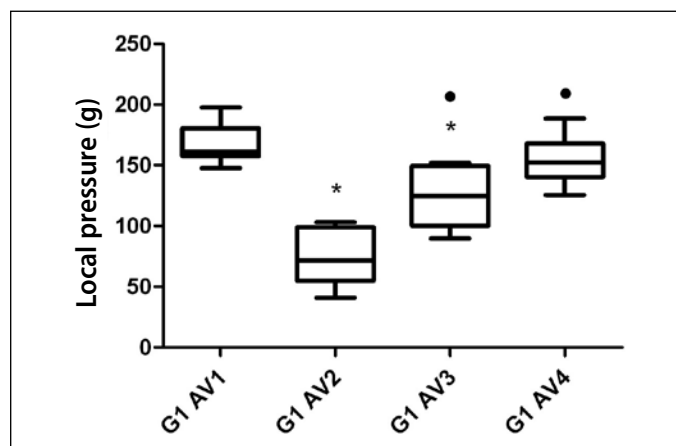


Figure 1. Graphic representation of the values obtained in the removal threshold for G1, according to the different evaluation moments.

\* significant difference when comparing with AV1. • significant difference when comparing with AV2.

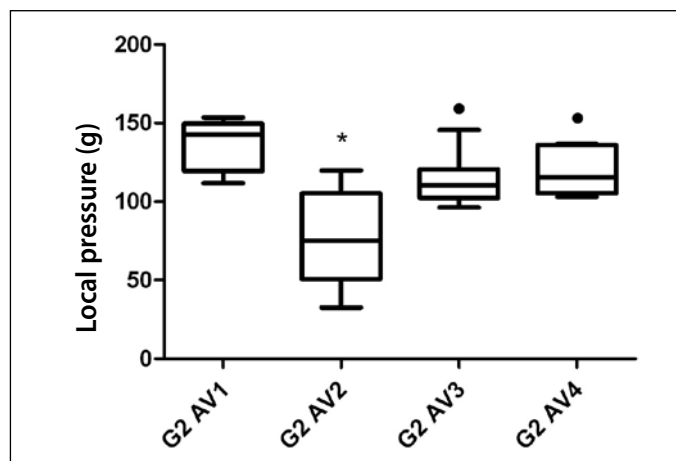


Figure 2. Graphic representation of the values obtained for the withdrawal threshold G2, according to the different evaluation times.

\* significant difference when comparing with AV1. • significant difference when comparing with AV2.

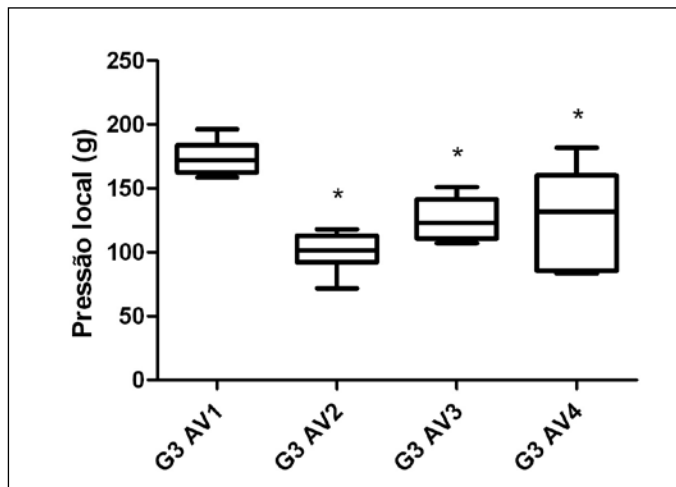


Figure 3. Graphic representation of the values obtained in the removal threshold for G3, according to the different evaluation moments.

\* significant difference when comparing to AV1.

## DISCUSSION

Resistance physical exercise presents benefic effects on the musculoskeletal function and body composition in the cardiovascular system, in the insulin action, bone repair, energetic metabolism, psychological health and functionality. These resistance training adaptations are potentially relevant to the knee AO, being reported that its progression and development are potentially changeable by training<sup>3</sup>.

Picard *et al.*<sup>12</sup> reported that there is no evidence of the role of opioids in the peripheral analgesia for acute pain; however, it is worth mentioning that they did not evaluate the role of opioid drugs of intra-articular action, being different from the present study which tried to evaluate the antinociceptive effect of exercise and whether it could occur via peripherally and intra-articular opioid, since the naloxone injection followed different dose and via recommended for systemic action<sup>13-15</sup>.

Sensitization of articular primary afferent nociceptors (peripheral sensitization) and of neurons of the spinal cord (central sensitization) are basic neuronal processes in pain and mechanical hyperalgesia. The two models of sensitization are generated by interaction of countless neuronal mediators and receptors. Evidence suggests that pro-inflammatory cytokines not only mediate inflammation and articular destruction, but also contribute to pain production and maintenance<sup>16</sup>. However, samples of synovial membrane present immune cells with abundance of  $\beta$ -endorphin and enkephalin. Such cells are recruited, and due to the secretion of opioids, they reduce pain<sup>6</sup>.

Some studies present positive effects of resistance exercise on the nociception in rats, such as Kuphal *et al.*<sup>8</sup>, who observed that nine days of swimming in water at 37°C, for 90 minutes a day, decreased the pain response by chemical stimulus and nervous injury. Such fact was also observed by Bement and Sluka<sup>7</sup>, who performed light walking protocol on treadmill, for five days, after application of the model of non-inflammatory muscular pain. The authors highlight that the analgesic effect was reverted with systemic administration of naloxone. Mazzardo-Martins *et al.*<sup>17</sup> evaluated the use of swimming in mice during 30 minutes for five days, with decrease of the number of abdominal contortion caused by acetic acid. Since there was reversion of the effect with the use of naloxone, chlorophenilalanine and bilateral adrenalectomy, swimming produced hyponociception via opioid and serotonergic systems. Thus, the results presented here are in concordance with the previous studies, since there was significant reduction of nociception with physical exercise antagonized by the use of naloxone; however, we remind that the exercise used was not resistance one.

Nevertheless, there are controversial reports such as by Vierck *et al.*<sup>18</sup>, who mention that physical exercise produces increase of the painful threshold in normal individuals; however, for fibromyalgia patients, extenuating exercise increases the time of pain. And Quintero *et al.*<sup>9</sup>, who observed that swimming subchronic stress produced increase of hypernociception both thermal and chemical in rats, and pointed out a possible interference in the central mechanisms of serotonin as a cause factor.

When resistance exercise was evaluated in three sets of 10 repetitions, with load of 75% of a repetition maximal in humans, Koltyn and Arbogast<sup>19</sup> observed increase of pain threshold. However, they stress that the analgesic effect after resistance exercise was short, that is, five minutes. Such fact was similar to what was presented here, since the evaluation subsequent to the exercise occurred less than five minutes after its end, and the last evaluation which occurred after 30 minutes (despite still pointing to restoration of the pre-injury values) was not different from what was observed for the control group, but rather for the naloxone group, which did not present analgesic effect as in the group only exercised.

We should consider that stress activates neural systems which inhibit the pain sensation. Such adaptation response, named stress-induced analgesia, depends on the recruiting of brain ways which project from the amygdala to the periaqueductal grey substance and go down the dorsal horn of the bone marrow<sup>20</sup>. In other words, the analgesic effect observed by exercise may have occurred by the animal's stress in the water medium, and not completely by the resistance exercise.

The opioids attenuate the excitability of nociceptors, the propagation of potential action and release of pro-inflammatory neuropeptides<sup>21</sup>. The activity of opioid receptors in primary afferents is increased under inflammatory conditions. Nevertheless, inside the inflamed tissues the opioids are exposed to hydrolysis by enzymes, in which, by local acidosis, occurs the proteins and peptides denaturation. Particularly, endogenously released opioids are very prone to the proteolytic action, resulting in short peripheral and central antinociceptive action<sup>5</sup>. Thus, it is imagined that even the control group, in which physical exercise has not been used, produced some kind of analgesic effect, observed by the significant increase of the mechanical threshold in the third and fourth evaluations compared with the second, also explained via endogenous opioids, since its behavior was not followed by the naloxone group.

We should highlight that the pressure evaluation is more commonly found using Von Frey filaments<sup>22-24</sup>, but the digital filament occurs as an alternative of easy use and visualization for nociception evaluation, with increasing application in research<sup>25-27</sup>. However, the lack of biochemical evaluation and the difficulty in establishing whether analgesia was caused by stress or by the exercise are limitations of the present study, being these indications for further studies.

## CONCLUSION

Decrease in nociception mediated by endogenous opioids was observed with the methods used.

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