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ORIGINAL ARTICLE

Evaluation of nociception induced by whole-body vibration remobilization in Wistar rats

Avaliação da nocicepção induzida pela remobilização com vibração de corpo inteiro em ratos Wistar

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ABSTRACT

BACKGROUND AND OBJECTIVES: The vibrating platform can attenuate the deleterious effects of immobilization related to muscle atrophy. However, there is still a gap regarding the effect of this modality on hyperalgesia related to immobilization. The objective of this study was to analyze the effect of remobilization with whole-body vibration on the nociception of Wistar rats.
METHODS: Sixteen rats were randomly distributed into two groups: the FRG group – immobilization and free remobilization and the VPRG – immobilization and remobilization with the vibrating platform. For remobilization with the vibrating platform, the frequency of 60Hz for 10 minutes, five days a week for two weeks was used. The nociception was evaluated on the right paw by a digital analgesiometer, before and at the end of the immobilization, and after two weeks of remobilization.
RESULTS: There were differences between evaluations but not between groups, indicating that immobilization reduced the nociceptive threshold and free remobilization, and the remobilization associated with vibration improved the nociceptive threshold compared to the post-immobilization moment. However, they were not able to return to the initial parameters.
CONCLUSION: Joint immobilization reduced the nociceptive threshold; however, two weeks of whole-body vibration remobilization were not able to revert the threshold in the immobilized groups.

Keywords: Hyperalgesia, Immobilization, Vibration.

RESUMO

JUSTIFICATIVA E OBJETIVOS: A plataforma vibratória pode atenuar os efeitos deletérios da imobilização relacionados à atrofia muscular, contudo, ainda há uma lacuna com relação ao efeito desta modalidade na hiperalgesia relacionada ao imobilismo. Assim, o objetivo deste estudo foi analisar o efeito da remobilização com plataforma vibratória sobre a nocicepção de ratos Wistar.
MÉTODOS: Dezesseis ratos foram distribuídos aleatoriamente no grupo GRL - imobilização e remobilização livre, e no grupo GRPV - imobilização e remobilização com plataforma vibratória. Para a remobilização com plataforma vibratória foi utilizada a frequência de 60Hz, durante 10 minutos, cinco dias por semana, durante duas semanas. A nocicepção foi avaliada na pata direita, por meio de um analgesímetro digital, antes e ao final da imobilização, e após duas semanas de remobilização.
RESULTADOS: Houve diferenças entre as avaliações, mas não entre os grupos, indicando que a imobilização reduziu o limiar nociceptivo, e a remobilização livre e associada à vibração melhoraram o limiar nociceptivo comparados com o momento pós-imobilização, no entanto, não foram capazes de retornar aos parâmetros iniciais.
CONCLUSÃO: A imobilização articular reduziu o limiar nociceptivo. No entanto, duas semanas de remobilização com a vibração de corpo inteiro não foram capazes de reverter o limiar nos grupos imobilizados.

Descritores: Hiperalgesia, Imobilização, Vibração.

INTRODUCTION

Joint immobilization is used as a treatment for injuries to the musculoskeletal system[1,2]. However, there are risks associated with immobilization, such as joint stiffness, muscle atrophy, cartilage degradation, ligament weakness, reduced muscle strength, and bone resistance[3,4]. Sensory changes, such as hyperalgesia, can occur after immobilization. In addition, there are changes in the neural system associated with motor disuse by the activation of primary nociceptive neurons[5-7].

The longer the inactivity or immobilization, the greater the effects on the body's systems. Also, immobility predisposes to cardiovascular, respiratory, gastrointestinal complications, and can reach the central nervous system (CNS). If the deconditioning severely limits or blocks ambulation, then the reduction in muscle strength and power can become self-perpetuating. These

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muscle deficits are often accompanied by generalized inflammation[8], a condition that can lead to hyperalgesia[9].

Hyperalgesia can be related to the inflammatory process, as the inflammation increases the sensitivity of the A-delta and C fibers at the inflammation site. This increases the excitability of the spinal cord neurons and can increase sensory responses, including the normally harmless tactile stimuli that are transported by the low threshold beta fibers[10]. Also, mechanical hyperalgesia induced by immobilization can be explained by the central sensitization (CS) mechanism, based on changes in the dorsal horn of the spinal cord. Harmful stimuli change the CNS, modifying the mechanisms triggered by afferent stimuli. Persistent stimulation of nociceptors causes spontaneous pain, reduced sensitivity threshold, and hyperalgesia[11,12].

In this context, both physical exercise and electrostimulation are excellent resources for restoring physical valences after immobilization, being beneficial for muscle recovery[13]. Studies confirm that exercise can restore muscle trophism, in addition to increasing bone strength[14,15]. Whole body vibration (WBV) reproduces the effects of exercise, positively influencing muscle function and coordination, increasing muscle strength and power, improving blood supply and muscle nutrition, in addition to effects on muscle flexibility, reducing the onset of fatigue, increased bone density, and reduced pain[16,17].

WBV can promote adaptive biological phenomena when the individual is in direct contact with the vibration, consisting of mechanical oscillation capable of generating force, acceleration, and displacement over time[16]. Vibration induces the transmission of the stimulus through the skin to the other body segments. Therefore, the skin and tendon receptors can also be activated and provide sensory signals to the somatosensory cortical areas of the brain[16,18,19].

Physical exercise seems to be an important pain modulator. Exercises release beta-endorphin from the hypothalamic axons. Beta-endorphin is secreted by the hypothalamus efferent to PAG and activates the opioid receptor of GABA neurons and the modulating system of the descending pain. Thus, hyperalgesia can be reduced because of the systemic exercise, even if after immobilization, the affected limb was not functioning normally[6,19].

The mechanisms of action proposed for the WBV analgesia are linked to the gate control theory, in which the activation of mechanoreceptors and Aβ fibers compete with the peripheral and central nociceptive activity in the dorsal horn of the spinal cord, which promotes the reduction of second-order nociceptive activity with a subsequent decrease in pain perception. In addition, it can lead to pain reduction by presynaptic inhibition of nociceptive and motor neurons[19]. Study[20] points out that WBV can reduce the level of pain and increase functionality in individuals with osteoarthritis. Authors[21] using WBV in older adults with osteoarthritis found that vibration improves self-perception of pain, balance, gait quality, and inflammatory markers.

It is known that the vibrating platform can attenuate the deleterious effects of immobilization related to muscle atrophy and improve vascular capacity[22,23]. However, the effect of this treatment modality on hyperalgesia related to immobilization is still poorly understood. Thus, this study aimed to analyze the effect of remobilization with a vibrating platform on the nociception of Wistar rats.

METHODS

An experimental and quantitative study with 16 *Wistar* rats, aged eight weeks and an average weight of 267.3±13.8g, kept in standard polypropylene boxes, in an environment with a temperature of 22±1°C, with a 12-hour photoperiod, receiving water and food *ad libitum*.

The animals were randomly assigned to two independent groups (n=8 in each group): immobilization and free remobilization (FRG) group, in which animals submitted to immobilization for 15 days were free remobilized and euthanized on the 30th day of the experiment, without any type of intervention; immobilization and free remobilization group associated with the vibrating platform (VPRG), in which animals submitted to immobilization were remobilized with WBV, in addition to being loose in the box, like the FRG group (Figure 1).

To perform the immobilization, the animals were anesthetized (15mg.kg^{-1} xylazine hydrochloride and 80mg.kg^{-1} ketamine hydrochloride, intraperitoneal), and immobilized with a cast bandage. The immobilized experimental groups had the orthosis molded from the abdominal region, just below the last ribs, going to the right pelvic limb of each animal; the knee joint remained in extension and the ankle in plantar flexion. The animals were kept in this position for 15 consecutive days (Figure 2)[3].

For the mechanical vibration treatment, the platform used was the professional triplanar Vibro Oscillatory platform, Arktus® brand (Santa Tereza do Oeste-PR, Brazil). The frequency used was 60Hz, with alternating vibrations with an amplitude of 2 millimeters, for 10 minutes[21]. The procedure was carried out for two weeks, five days a week, with a two-day break at the end of the week. A wooden support (MDF) was used, which allowed positioning eight animals simultaneously in stalls with 13cm (width), 19cm (length), and 25cm (height). There was a rotation of the stalls during the treatment[24,25] to minimize a possible bias on different points of acceleration and amplitude on the vibrating platform (Figure 3).

The assessment of the nociceptive threshold was performed using a digital analgesiometer, Von Frey filament-like, from Insight® (Ribeirão Preto, SP, Brazil). The animal was kept in a raised box with a mesh floor, and the polypropylene tip of the filament was applied perpendicularly to the plantar region of the right pel-

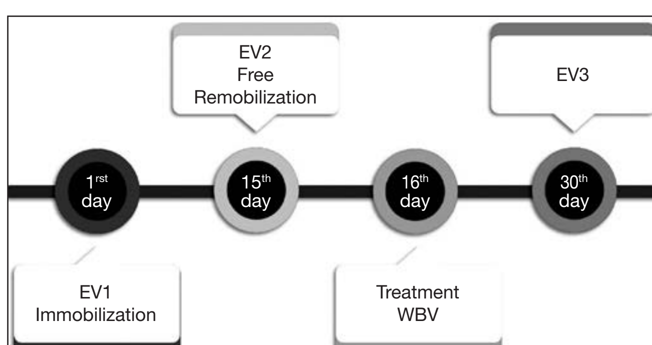

Figure 1. Experiment timeline
EV=evaluation; WBV=whole-body vibration.

Figure 2. Cast immobilization from the abdominal region to the right pelvic limb

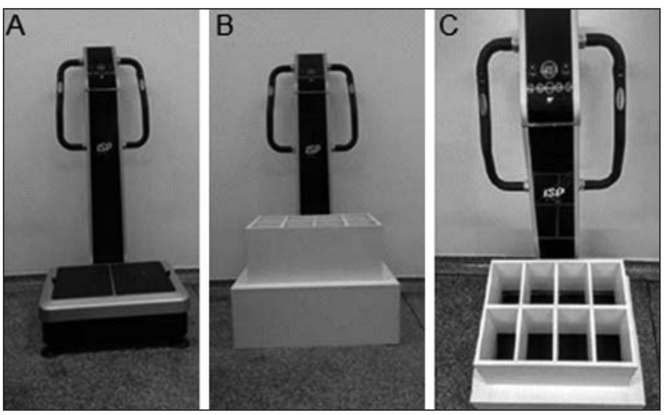

Figure 3. A: Vibrating platform used. B: front view of the support. C: top view of the support

Figure 4. A: Von Frey filament type digital analgesiometer, Insight® (Ribeirão Preto, São Paulo). B: Animal in the raised box with a mesh floor

vic member, with increasing pressure until the animal removed the paw. In each evaluation, the test was repeated three times, and the average value was used, always performed by the same evaluator. The procedure was carried in all groups before immobilization (EV1), after 15 days of immobilization (EV2), and at the end of the vibration protocol (EV3).

The Committee on Animal Research and Ethics (CARE) of UNIOESTE approved this project, and the national and international guidelines applicable to the care and use of animals were followed. This project followed the International Ethics Standards for Animal Experiments.

Statistical analysis

Data analysis was performed with Generalized Linear Models with LSD post-test. In both cases, the level of significance was 5%, for which SPSS 20.0 was used. The effect size was also assessed by Cohen's d, based on the first assessment for a given group, and rated as:<0.2: trivial; 0.2-0.5: small; 0.5-0.8: moderate; > 0.8: large.

RESULTS

There were differences between the evaluations (F=225.51, p<0.001), but there was no difference between the groups (F=0.26, p=0.614), nor interaction (F=0.491, p=0.616), indicating that the immobilization reduced the nociceptive threshold, and free and vibration-associated remobilization improve the nociceptive threshold compared to EV2. However, they were unable to return to the initial parameters. It was possible to observe, in both groups, that the effect sizes were significant (Figure 5).

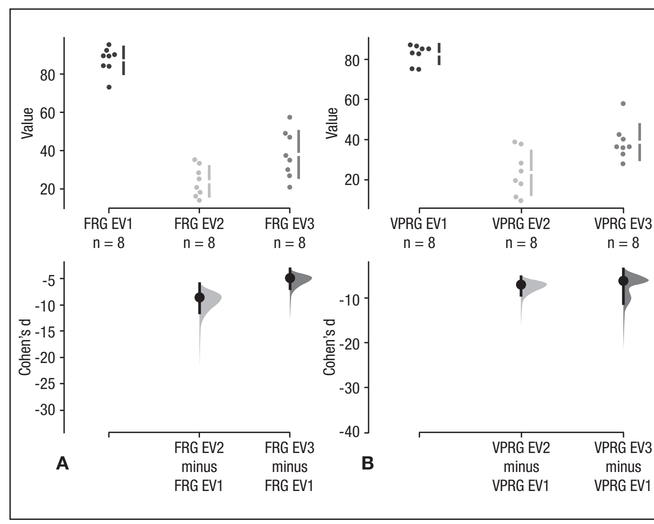

Figure 5. Presentation of observed data for the free remobilization group (FRG) and for the group remobilization group with vibrating platform (VPRG) was associated, with the distribution and effect sizes EV = evaluation. FRG = free remobilization group; VPRG = remobilization group with vibrating platform.

DISCUSSION

The present study evaluated the nociception of rats submitted to joint immobilization, and the effect of WBV on remobilization. Joint immobilization affected the nociceptive threshold, as there was a reduction in the withdrawal threshold. It is known that immobilization is used to treat injuries and musculoskeletal changes[1,2]. However, due to disuse, it is possible to have complications, that is, undesirable effects during this period.

According to the study[26], immobilization promotes local inflammatory changes and pain in the immobilized limb. They point out that two weeks of immobilization resulted in skin changes, such as flushing, edema, and changes in the temperature of the immobilized limb, characteristics found during an inflammatory process.

Another mechanism of hypersensitivity induced by immobilization is CS, which is caused by increased membrane excitability, altered synaptic efficacy, or reduced inhibition of this system[27]. Evidence points out that harmful stimuli cause changes in the CNS, modifying the mechanisms triggered by afferent stimuli. Thus, homeostasis changes can alter the properties of neurons and increase the responses to nociceptive afferences[11,12,27].

Study[7] points out that CS may occur due to neuronal plasticity. When analyzing the expression of the calcitonin gene-related peptide (CGRP) in the spinal cord and the dorsal root ganglion (DRG), they found an increased expression of the CGRP in the DRG in animals submitted to immobilization. Furthermore, CS is present as an abnormal response to the nociceptive stimuli, and there is a dispersion of sensitivity beyond the place where the pain is generated[27], confirmed by another study[26] that points out that mechanical hyperalgesia goes beyond the immobilized limb, also occurring in the contralateral pelvic limb and tail.

Study[28] observed that there are inflammatory changes during immobilization, and there is an association with CS. These data corroborate the hypothesis that cast immobilization for four weeks favors an exaggerated neuropeptide signaling in the immobilized limb, which contributes to the development of pain behavior and vascular inflammatory changes. In addition, they found a proliferation of keratinocytes and expression of the inflammatory mediator, increased expression of nerve growth factor (NGF) in the lumbar spinal cord, nociceptive sensitization of the posterior limbs, increased temperature and edema.

WBV appears in rehabilitation as a proposal to accelerate recovery during and after disuse. However, it can be harmful and evoke pain in animals. Study[29] states that seven days of WBV can change the withdrawal threshold of the pelvic limb. It is known that high-intensity vibrations are responsible for muscle injuries, pain in the back, and joints[17]. Conversely, in a study[24] with oophorectomized rats, WBV for four and eight weeks did not change the nociceptive threshold. Still, studies in humans pointed out that WBV was able to reduce low back pain, osteoarthritis, joint and muscle pain[19]. However, there was no report on the effect of the vibrating platform on nociception after immobilization.

In this study, the assumption was that vibration increases the nociceptive threshold, based on its activation of spinal and supraspinal neurophysiological mechanisms during WBV[19,30]. Other possible explanations include decreased inflammation with repeated WBV, suggested by the reduction of inflammation biomarkers such as cortisol[19]. A difference was observed between the evaluations. Thus, the vibrating platform and free remobilization reduced hyperalgesia. However, two weeks of treatment were unable to return to the initial parameters. Also, that mechanical hyperalgesia can persist for up to ten weeks of remobilization[26].

In study[6], local vibration was performed during the immobilization period, for 15 minutes, once a day, five days a week, for eight weeks. The authors observed that the group that received vibration therapy during the immobilization period had primary prevention and inhibition of hypersensitivity. The use of vibration therapy as a secondary prevention after four weeks of immobilization was not effective in the treatment of hypersensitivity induced by immobilization.

No effects on restoring the nociceptive threshold were observed nor deleterious effects either. It is necessary to carry out studies that verify the effect of WBV in the initial phase of immobilization, even with longer WBV time, which is another limitation of the present study. Future studies should explore other ways of assessing the pain signal, such as cold and heat pain tests.

CONCLUSION

Joint immobilization reduced the nociceptive threshold. However, two weeks of remobilization with WBV, at 60Hz, 10 minutes daily, were not able to reverse the threshold in the immobilized groups.

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