Histological aspects of whole-body vibration in the knee remobilization of Wistar rats

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ABSTRACT

Introduction and aim. The knee is one of the joints where immobilization is most used, however, it can cause morphological changes in the joint tissues and is a challenge to be overcome during rehabilitation. Whole-body vibration (WBV) is capable of generating repetitive oscillatory movements, which cause mechanical stimuli that interfere with tissue plasticity. The aim of this study was to analyze the knee morphology of Wistar rats submitted to remobilization with WBV.

Material and methods. 32 male rats were used, divided into four groups (n=8): Control Group (G1), Immobilization Group (G2), Immobilized Group and Free Remobilization (G3), Remobilized Group with WBV (G4). For immobilization, a plastered apparatus was used for 15 days. G3 and G4 carried out free remobilization or with WBV, respectively, for 2 weeks. The knee joints were processed for light microscopy.

Results. The WBV led to a reduction in the inflammatory infiltrate in the articular cavity and greater presence of adipocytes in the subintima of the synovial membrane.

Conclusion. Remobilization with WBV induced a better tissue response in the synovial membrane when compared to free remobilization.

Keywords. articular cartilage, knee, synovial membrane, vibration
tion.5–9 The joint capsule is also affected by immobilization, which promotes morphological changes in the intima and subintima synovial, resulting in decreased synovial fluid production and reduced nutritional supply to the cartilage.10

Thus, early rehabilitation is extremely important to recover joint functionality.5–8 There are numerous forms of remobilization, such as treadmill running, resistance and aquatic exercises.10–12 However, the search for new therapies that accelerate the regenerative process in remobilization is of great value. In this sense, studies have shown that whole-body vibration (WBV) can act as an anabolic stimulus for various tissues, including the musculoskeletal system.13–16 Despite their indications, there are still few studies evaluating WBV on joint tissue, as well as in the remobilization process.17,18

**Aim**

Thus, this study analyzed the morphological responses of the knee of immobilized and remobilized Wistar rats on a vibration platform.

**Material and methods**

**Ethical approval**

The study was approved by the Ethics Committee on the Use of Animals of the Universidade Estadual do Oeste do Paraná (UNIOESTE), 2729/2014-GRE.

**Sample and experimental groups**

The experiment was performed using 32 Wistar rats, with a mean age of 10 weeks and weight of 177.20±16.32 g, kept in an environment with a temperature of 23±1°C, with a photoperiod of 12 hours, receiving water and feed ad libitum. The animals were randomly separated into four groups (n=8):

- Control Group (G1): without any kind of intervention;
- Immobilization Group (G2): submitted to the immobilization protocol;
- Group Immobilization/Free Remobilization (G3): submitted to the immobilization and free remobilization protocol;
- Group Immobilization/Remobilization WBV (G4): submitted to the immobilization and remobilization protocol with the use of the vibration platform.

**Immobilization protocol**

To perform the immobilization, the animals were anesthetized (xylazine hydrochloride 15 mg/kg and ketamine hydrochloride 80 mg/kg, intraperitoneally) and immobilized with a plaster bandage. The immobilized groups (G2, G3 and G4) had the orthosis molded from the abdominal region, just below the last ribs, following to the right pelvic limb of each animal, being placed in the whole extension of the limb so that it remained in complete extension of the knee joint as well as, complete plantar ankle flexion. The animals were kept in this position for a period of 15 consecutive days.

**Remobilization protocol**

For the remobilization protocol with the use of the vibration platform. A support was used with the purpose of containing the animal during the vibration and enable the animals to exercise simultaneously, which was made of white MDF wood, and allowed to position 8 animals concomitantly, in stalls with 13 cm wide, 19 cm long and 25 cm high. To minimize a possible bias on the positioning of the animals on different areas of the vibratory platform that received less vibration amplitude, a rotation was performed between the stalls in which each day of treatment the animal was housed in a different place from the previous day.19

For G3, after the 15 days of immobilization and the removal of the cast bandage, the animals remained in the cage for 15 days receiving water and feed ad libitum.

**Morphological analysis**

The animals of the control group (G1) and the immobilization group (G2) were euthanasiated soon after the 15 days of intervention, while the animals of the free remobilization group (G3) and the platform remobilization group (G4) were euthanasiated after 2 weeks of remobilization. All the animals were weighed, duly anesthetized by intramuscular injection of 80 mg/kg ketamine and 8 mg/kg xylamine and decapitated in a guillotine. Then the right pelvic limb was dissected and the knee joints followed for histological processing, being fixed in a 10% formaldehyde solution for 24 hours, washed in running water for 1 hour and remaining immersed in 5% trichloroacetic acid for approximately 20 days, following the routine steps for inclusion in paraffin, where they were dehydrated in increasing series of alcohol and diaphanized in xylol. The histological paraffin embedding was performed, longitudinally sectioned in 7 μm thickness in a microtome (Olympus CUT 4055) and stained with hematoxylin and eosin.

The slides were photomicrographed under a light microscope (Olympus), with 200x magnification for morphology analysis. The morphological characteristics of the articular cartilage were observed, such as the distribution of chondrocytes, appearance of the articular surface and the presence of cracks in the extracellular matrix, flocculation and cellular clones, as well as the subchondral bone and the synovial membrane, and the tissue aspects of the intima and subintima were observed.
Results
Morphological analysis of the synovial membrane of the control group (G1) revealed normal characteristics, that is, from two to three layers of synoviocytes (types A and B) in the intima synovialis, and subintima with a predominance of adipose cells and blood vessels within the normal range (Figure 1A). In the immobilized group (G2), the synovial membrane presented altered, with thickened and disorganized intima as to the distribution of the synoviocytes and subintima with substitution of adipocytes by connective tissue rich in collagen fibers, inflammatory infiltration and intense angiogenesis, with an increase in the number and caliber of blood vessels (Figure 1B). In the animals of the free remobilization group (G3) (Fig. 1C) and trained with WBV (G4) (Figure 1D) the morphological aspects were similar to the control (G1), with the intima organized in its characteristic layers, while the subintima presented tissue reorganization, with a small amount of connective tissue and inflammatory cells and vascularization within the normal range, when compared to G2, but with inflammatory infiltrate in the joint cavity in G3 (Fig. 1C) and larger amount of adipocytes in the subintima in G4 (Fig. 1D).

Fig. 1. Photomicrographs of the synovial membrane of the knee joint of Wistar rats, sagittal section, staining in hematoxylin and eosin. In A, control group (G1), with thin synovial intima (black arrow) and subintima (S), with predominance of adipose cells (Ad) and blood vessels within normal range (thin arrow). In B, immobilization group (G2), thick synovial intima and disorganization of synoviocytes, in subintima replacement of fat tissue by connective rich in collagen fibers (white arrow), presence of intense inflammatory infiltrate (▼) and large amount of blood vessels. In C, free remobilization group (G3) and D, WBV group (G4), intima and subintima returning to reorganization, with presence of inflammatory cells in the joint cavity of G3. Joint cavity (star)
In the morphological analysis of the knee joint, the cartilage of the femur and tibia, in G1, showed normal characteristics, where the surface was smooth and the chondrocytes organized in the four cell layers, and in the surface area, they were in horizontal clusters with a flattened appearance, in the intermediate more rounded, being isolated or in isogenic groups, in the deepest organized in gaps, being separated from the calcified area by a basophilic line, tidemark. Deep in the calcified zone, the subchondral bone had a normal aspect (Fig. 2A).

In G2, it was found that immobilization caused severe morphological changes, such as disorganization and flocculations on the joint cartilage surface, cracks in the extracellular matrix, increase in the number of chondrocytes that, lost the pattern of organization in areas with increased total thickness of the joint cartilage, absence of tidemark, besides invaginations in the subchondral bone with the presence of vascular connective tissue filling the medullar space immediately adjacent to the cartilage, where vascular tunnels appeared and pen-

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**Fig. 2.** Photomicrographs of femoral joint cartilage in the patellofemoral joint of Wistar rats, sagittal section, staining in hematoxylin and eosin. In A, control group (G1), normal joint cartilage organization (Ca), smooth surface, chondrocytes distributed in the characteristic zones (Black arrow), presence of tidemark (thin arrow), normal looking subchondral bone (OS) In B, immobilization group (G2), flocculations (Fl) on the surface of the cartilage, fissure (Fi) in the extracellular matrix, increase in the number of chondrocytes and disorganization of the zones, invaginations in the subchondral bone with presence of vascular connective tissue (asterisk) and absence of tidemark. In C, free remobilization group (G3) and D, platform remobilization group (G4), surface flocculation, with large number of isogenic groups (Gi) in the various layers, vascular connective tissue. Joint cavity (star)
Histological aspects of whole-body vibration in the knee remobilization of Wistar rats

In animals of groups G3 (freely remobilized) and G4 (remobilized by WBV), the morphological results were similar. The cartilage still showed flocculation, but absence of fissures in the extracellular matrix, being possible to observe the presence of groups of cellular clones or isogenic groups in the superficial layer in great quantity, with beginning of the organization of the cellular zones and beginning of tidemark organization in some points. In the subchondral bone remained areas of invagination, but with a decrease in the vasculonnective tissue that filled the medullary space (Fig. 2C and 2D).

Discussion

Morphological observations of the knee joint in the control group showed normality in the cellular organization of the synovial membrane and joint cartilage, expected in synovial joints.20,21 On the other hand, the control group showed normality in the cellular organization in some points. In the subchondral bone remained areas of invagination, but with a decrease in the vascular connective tissue that filled the medullary space (Fig. 2C and 2D).

Regarding the thickening of the synovial membrane, Del Carlo et al., found that immobilization degenerates the synoviocytes, affecting the production of synovial fluid and the nutritional supply of the cartilage, making it rigid and causing its thickening, which is in line with what was observed in the present study, since the synovial membrane of the immobilization group (G2) proved to be totally disorganized in terms of its tissue structure, as well as the articular cartilage.22

The synovial membrane of G3 and G4 animals presented repair characteristics, with reorganization of the synoviotic layers in the intima and subintima, reduction of fibrous tissue, reappearance of adipocytes and reduction of angiogenesis. Besides these changes, a decrease in the amount of inflammatory infiltrate in the articular cavity of the remobilization group with vibratory platform was noted, while in the free remobilization (G3) and WBV (G4) groups, there was absence of cracks in the extracellular matrix, presence of cellular clone and organization principle of the zones and tidemark, not differing between the forms of remobilization.10

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in the case of hormonal deprivation, may have caused the divergences in the results found with this study, and no degenerative effect was observed in the animals treated with the platform, but a subtle improvement in the inflammatory process. Seeing studies that point to a reduction in the level of pain with the use of WBV, and being that this is a basis for neurogenic inflammation, it can be inferred as one of the means by which there was the tissue alteration found.35,36

Qin et al., and McCann et al., submitted rats and mice, respectively, to experimental procedures of osteoarthritis, using WBV at lower frequencies of 35 Hz with a wave amplitude of 0.3 millimeters, for 20 minutes, for 5 days a week, with total duration of 6, 12 and 18 weeks according to the groups, while in McCann’s study the frequency used was 45 Hz, with a wave amplitude of 0.3 millimeters, for 30 minutes a day, for 5 days a week, with a total duration of 4 and 8 weeks and 4 weeks with another 4 weeks of recovery according to the group, obtaining in both studies the degradation of cartilage as a tissue response.37,38

In the present study, the tissue reorganization in the subintima and the decrease of inflammatory infiltrate in the articular cavity and the presence of cellular clones of the group treated with platform vibration, showed an initiation of more effective tissue recovery response in the platform group when compared to the free. Thus, it can be inferred that the application of the platform with other treatment parameters, could accelerate the recovery process, thus, new comparative research is needed regarding the increase in treatment time and the diversification of vibratory parameters to be used.

Conclusion
Use of WBV as a therapeutic method for remobilization, in the parameters used, showed better results in the tissue recovery of the synovial membrane of the knee joint of Wistar rats.

Declarations
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This research received no external funding.

Author contributions
Conceptualization, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Methodology, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Formal Analysis, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Investigation, J.C.R.G., A.L.F.T., M.L.S.W., C.D.T.B. and D.F.S.R.; Resources, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Writing – Original Draft Preparation, J.C.R.G.; Writing – Review & Editing, A.L.F.T., G.R.F.B., M.L.S.W., C.D.T.B., D.F.S.R., R.M.C. and L.F.C.R.; Supervision, G.R.F.B., R.M.C. and L.F.C.R.; Project Administration, G.R.F.B., R.M.C. and L.F.C.R.; Funding Acquisition, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.

Conflicts of interest
All authors declare that they have no conflicts of interest.

Data availability
The data have not been made public, but are kept with the authors, if necessary.

Ethics approval
Study was approved by the Unioeste Animal Use Ethics Committee (2729/2014-GRE).

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Histological aspects of whole-body vibration in the knee remobilization of Wistar rats

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