ORIGINAL ARTICLE

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Therapeutic Effect of Aquatic Resistance Exercise on Rectus Femoris and Knee in a Model of Rheumatoid Arthritis

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SUMMARY

Background. Rheumatoid Arthritis (RA) is characterized by progressive joint destruction due to an inflammatory, degenerative condition of the joint cartilage and adjacent muscles, impairing the patient's functional status. Resistance exercise is considered a good tool in the management of RA. The objective of this study was to evaluate the effect of resistance exercise, in an aquatic environment, on strength, motor control, and morphometric parameters of the rectus femoris muscle and knee joint of rats submitted to a model of RA.

Methods. Forty male rats divided into four groups were used: CON (Control); RA (Injury Group); EX (Exercise Group) and EXRA (Injury Group treated with resistance exercise in aquatic environment). RA was induced by immunization at the base of the tail and intra-articular injection of Freund's Complete Adjuvant (CFA) in the tibio-femoral joint of the right knee. The exercise protocol was performed three times a week in a water tank with an overload of 50% of the animal's weight for 22 days, progressing the number of sets and repetitions every three treatments. Both pelvic limbs were evaluated for grip, motor control in the inclined plane, joint and muscle morphology, and their respective morphological measurements. Generalized Linear Models with LSD post-test was used for statistical analysis.

Results. There was a reduction in strength of both pelvic limbs of the injured animals and the AR and EXRA groups also had morphological changes in the joint and in the rectus femoris muscle. The resistance exercise protocol in an aquatic environment helped to maintain strength and the quality of histological tissues.

Conclusions. Resistance exercise in aquatic environment promoted functional and muscle tissue improvement in the knee joint of Wistar rats with RA with experimentally induced RA.

KEY WORDS

Autoimmune diseases; rheumatoid arthritis; musculoskeletal; physical exercise; hydrotherapy.

INTRODUCTION

Rheumatoid Arthritis (RA) is a disease characterized by inflammatory and progressive autoimmune activation that manifests itself in connective tissues, markedly in synovial joint (1). The disorders evolve symmetrically, beginning commonly in the extremities with progression to proximal joint region (2). The manifestations occur with the presence of edema, heat, paresthesia, and pain for indeterminate period (3). Chronic inflammation promotes accumulation of cytokines and immune system cells in the synovial membrane, leading to destruction of the articular cartilage

as well as adjacent structures such as the synovial membrane and muscle component (4). RA is estimated to affect 1% of the world's population and about 3 million individuals in the United States alone (5). It also represents a reason for death in 0.03% of the cases in São Paulo, Brazil, and 0.17% in Sweden (6).

In addition to structural involvement, the RA patient also develops functional limitations resulting from symptom exacerbation (7). Thus, the presence of rheumatoid cachexia due to reduced lean body mass and increased muscle catabolism induced by inflammatory cytokines, commonly affects the muscular compartments of the quadriceps femoris, promoting changes in the functional capacity of individual (8).

In general, resistance training provides physical benefits, such as muscle hypertrophy, power, and speed gain, developed through adaptive responses resulting from the training program (9). The elaboration of the treatment consists in the choice of the number of sets, repetitions, rest period and overload (10, 11). The parameters must be determined according to the proposed objectives as well as the individual's functional capacity (12). Muscle strengthening is essential for the treatment of degenerative joints in order to improve neural activation, maintenance of cartilage tissue, muscle strength, joint mobility, and optimize shock absorption (13, 14).

The aquatic environment generates low impact and represents a controlled environment for both therapist and patient. The reduction of gravitational force through buoyancy facilitates postural control (15). In addition, the hydrostatic pressure associated with the viscosity of the water promotes positive sensory and proprioceptive feedback during the execution of movements (16, 17). Exercise in orthostatic position in water is indicated for various populations, including individuals who present joint lesions (18, 19). Although swimming is the most conventional physical practice, it requires specific skills as well as high intensity to perform. Thus, exercises in vertical position in water are suggested for patients to optimize the rehabilitation process (19, 20).

Since resisted physical exercise in water presents benefits to the healthy population in physical training programs due to its benefits to musculoskeletal health, the investigation about the application in chronic rheumatic comorbidities, such as RA, becomes pertinent, even though the literature is controversial regarding the indication of resisted physical exercise for this population (21). Therefore, the present study aims to investigate the effects of resisting aquatic jump exercise on the parameters of grip strength, motor control, and morphometric analysis of the rectus femoris muscle and knee joint of Wistar rats with experimentally induced RA.

MATERIALS AND METHODS

This study is characterized as experimental, conducted in the Universidade Estadual do Oeste do Paraná (Unioeste). All experimental procedures only began after approval by the Ethics Committee on Animal Use (CEUA) of Unioeste under protocol n°. 19-19, on July 25, 2019.

Animals

The sample consisted of 40 male Wistar rats, three months old and weighing 347.31 ± 25.1, obtained from the Central Animal Facility of Universidade Estadual do Oeste do Paraná, during the experimentation, the animals were kept in the animal cages, obeying a 12-hour light-dark period, temperature of 21 ± 1 °C, and were treated with water and feed *ad libitum*. After one week of adaptation, the animals were randomly distributed into 4 groups, with 10 rats each:

- CON (Control Group): received no intervention;
- RA (Injury Group): induction of RA in the knee joint of the right pelvic limb (RPL);
- EX (Exercise Group): aquatic jumping protocol on alternate days;
- EXRA (Injury + Exercise Group): induction of RA in the knee joint of the RPL and subjected to a water jumping protocol.

RA induction injury protocol

For RA and EXRA, the method described by Gomes *et al*. (22, 23), which consists of Freund's Complete Adjuvant (CFA) with *Mycobacterium butyricum* (0.5 mg/ml, Difco®); isotonic sodium chloride solution (0.9%, Aster®) and iodized alcohol (1%, Rialcool®).

The animals were previously submitted to immunization by intradermal injection at the base of the tail, after trichotomy and asepsis of the injection site with iodized alcohol (1%). Subsequently, a 1 ml syringe and a 13 × 4.5 mm needle were used and inserted approximately 1 centimeter into the subcutaneous region at the base of the tail. The RA and EXRA groups received pre-sensitization with 50 µl (microliters) of CFA (0.5 mg/ml, *Mycobacterium butirycum*). In the RA and EX groups, a saline solution (sodium chloride 0.9%) was injected in the same volume.

Seven days after sensitization, the animals were manually restrained so that the knee region of the RPL was trichotomized and, subsequently, asepsis was performed with iodized alcohol (1%). With the help of a 1 ml syringe and a 13 × 4.5 mm needle, the groups that received the RA induction were submitted to a new application in the tibio-femoral joint of 50 µl (0.5 mg/ml) of CFA *Mycobacterium butirycum*, as well as the other groups, received the application with saline solution (0.9% sodium chloride).

Treatment protocol with aquatic jumping

A 220-liter capacity tank was used, with water at a temperature of 33 °C, where a cylindrical-shaped tube 55 cm high, 30 cm in diameter, and a water level of 45 cm was placed. The EX and EXRA animals received an overload of 50% of body weight, with lead weights positioned in the abdominal region by means of a Velcro strap. Each animal was individually placed in the tube and with the overload, they were submerged to the bottom of the tank, with each impulse to reach the surface counted as a jump (24) (**figure 1**).

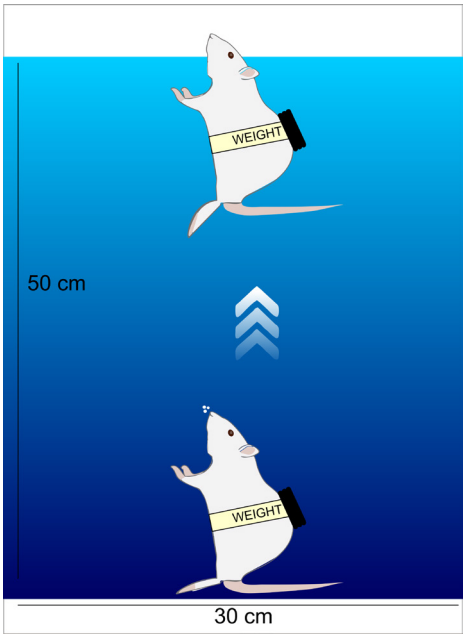

Figure 1. Representation of the jump exercise in aquatic environment, with body overload.

The animals were previously trained, before the induction of RA, with the weight attached to their bodies. The treatment was carried out on alternate days, starting 24 hours after the intra-articular injection, and the last treatment was performed on the 28th day. In the first week, the jumps were made in two series of 10 repetitions; from the second week on, there was a progression in the number of series, being made three series of ten jumps; in the third week, the progression in the number of series followed, being made four series of ten repetitions, with a one-minute interval between series, as can be seen in **figure 1** (24). Prior to the beginning of the experiment, all animals were trained and adapted to the equipment that was used in the evaluations. Seven evaluations were performed during the 30 days of the experiment (**figure 2**).

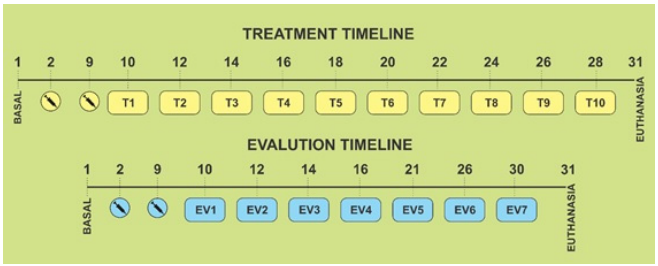

Figure 2. Timeline of treatments and assessments.

Day 1 (Basal), day 2 (Sensitization at the base of the CFA tail), day 9 (Intra-articular CFA Injection), T1-T12 (Treatment Day with jumping in an aquatic environment), EV1-EV7 (Functional Evaluation Day) and day 31 (Euthanasia).

Functional assessments

Muscle strength assessment – Grip strength

To assess the muscle strength of both pelvic limbs, a grip strength device (Insight®) was used, in which the animal was immobilized by the evaluator, leaving only the limb to be evaluated free. The animal was positioned so that its plantar region could grip the transducer grid and then the evaluator pulled the animal with increasing force until it was released. Three measurements were made for each pelvic limb and the mean value was considered (25).

Motor function evaluation

In the evaluation of motor function, the animal's ability to maintain itself at different angles according to its joint proprioception and motor activation was tested. We used an inclined plane equipment (Insight®) consisting of an acrylic ramp with a manual mechanism for angular variation (0°-90°) and non-slip surface. The animal was positioned in two directions at the top of the ramp: the first with the cephalic region up and the second with the cephalic region to the side (vertical position), and the RPL under evaluation was positioned at the lowest point of the ramp. The evaluation started at 45°, and if the animal remained there for 5 seconds, the ramp was raised another 5° until it showed any reaction of instability or imbalance. Three measurements of the maximum angle were made, and the average of the values was considered (26).

Analysis of the rectus femoris muscle

On the twenty-second day, the animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (95 mg/Kg) and xylazine (12 mg/Kg) and subjected to euthanasia by anesthetic overdose, the rectus femoris muscle of both pelvic limbs was collected and fixed in methacarn (70% methanol, 20% chloroform, 10% glacial

acetic acid) for 24 hours and stored in 70% alcohol. The muscle tissues were then subjected to an alcoholic increasing series dehydration process, diaphanization in xylol, and paraffin embedding. Cross sections of 5 µm thickness were obtained with a microtome (Olympus CUT 4055) and stained with Hematoxylin and Eosin (HE) for general analysis of the muscle tissue.

Measurements of cross-sectional area and the smallest diameter of muscle fiber were performed, as well as evaluation of muscle fiber morphology. The slides were analyzed under a light microscope (Olympus BX60) and for the measurement of the cross-sectional area and smallest diameter of the muscle fiber, 10 images were obtained using a 40x objective. We considered 10 fibers from each image to perform the measurements using the Image-Pro-Plus 6.0 program (Media Cybernetics), totaling 100 measurements per animal (27).

Morphological analysis of the knee joint

After euthanasia, the right and left knee joints were collected and dissected, and then fixed in Metacarn (70% methanol, 20% chloroform, 10% glacial acetic acid) for 48 hours, and after that period the material was fixed in 70% alcohol for 15 days. After washing for 24 hours in running water, the material started the decalcification process in 5% trichloroacetic acid where it remained for 7 days. Afterwards, the material was washed again for 24 hours, following the routine histological processing for paraffin embedding. To make the slides, the joints were cleaved using a microtome (Olympus CUT 4055) at a thickness of 7 µm. In the staining protocol, Hematoxylin and Eosin was used to identify the structures in the morphological analysis. The slides were analyzed using a light microscope and photomicrographed in a photomicroscope (Olympus DP71).

The measurement of cartilage thickness and the counting of the number of chondrocytes were performed in three distinct areas of the femur and tibia: P1, anterior articular extremity; P2, middle region of the joint; and P3, posterior articular extremity. These areas were photomicrographed at 200x magnification and later analyzed using Image Pro Plus 6.0 software. The thickness was measured from the end of the cartilage to the osteochondral junction. For the chondrocyte count, a rectangle 100 µm deep by 200 µm long was used, superimposed on the three photomicrographed points (P1, P2, and P3), disregarding the upper margin and the deep margin closest to the subchondral bone region. The average of the three regions was considered for both cartilage thickness and the number of chondrocytes in the femur and tibia (28).

Statistical analysis

The SPSS 20.0 program was used for statistical analysis. The data were presented as mean and standard deviation by means of illustrated figures in the GraphPad Prism 8.0.2 program. The inferential analysis was performed with Generalized Mixed Linear Models for the functional data, for comparison of total leukocytes, Generalized Linear Models was used, in all cases, the post-test was LSD, with an accepted significance level of 5%.

RESULTS

In the assessment of muscle strength for RPL, there was a statistical difference between the groups (WaldX2(3;254) = 61.931, $p < 0.001$) and in the interaction between the groups and assessments (WaldX2(21;254) = 2.252, $p < 0.05$). A statistical difference was also observed for the left pelvic limb (LPL) between the groups (WaldX2(3;253) = 8.951, $p < 0.001$).

With the induction of injury, the RA and EXRA groups showed a reduction in the grip strength of the RLP ($p < 0.05$), however the group treated with exercise obtained a restoration of strength from the AV5, and in the AV7 it was similar to the group EX. In the intragroup analysis, RA and EXRA showed a difference ($p < 0.05$) in the reduction of strength from AV2, but an increase in values was observed in AV7, where the means returned to baseline values (**table I**).

In LPL, there was a statistical difference for RA and EXRA with reduced grip strength in relation to CON and EX ($p < 0.05$) (**table II**).

In the evaluation of motor function on the inclined plane, there was a statistical difference between groups in the vertical position (WaldX2(3;256) = 6.560, $p < 0.001$) and interaction between groups and evaluations (WaldX2(21;256) = 1.654, $p < 0.005$). In the horizontal layout, there was a statistical difference only for the groups (WaldX2(3;256) = 11.017, $p < 0.001$).

In the vertical plane, there was no difference between the groups in the Baseline evaluation. There was a statistical difference ($p < 0.05$) in EV1 compared to the Baseline evaluation for the RA and EXRA groups for CON and EX, with a reduction in the inclination angles. Throughout the evaluations, both injured groups showed recovery of the averages, similar to the CON group. The same was observed in the intragroup evaluation, where there was a statistical difference ($p < 0.05$) from baseline to EV1 in the RA and EXRA groups, with a reduction in the mean. Throughout the evaluations, values similar to baseline were obtained in the groups with RA induction (**table III**).

In the horizontal arrangement, there was a statistical difference ($p < 0.05$) in the CON group, which obtained a higher mean compared to the other groups (**table IV**).

Table I. Grip analysis of right pelvic limb (RPL).

MUSCLE STRENGTH PREHENSION - RPL

	BASAL	EV1	EV2	EV3	EV4	EV5	EV6	EV7
CON	68.525 ± 4.72^{Aa}	95.92 ± 18.02^{Ab}	104.60 ± 22.99^{Ab}	97 ± 16.86^{Ab}	96.11 ± 12.87^{Ab}	95.1 ± 17.99^{Ab}	99.76 ± 16.44^{Ab}	95.72 ± 15.69^{Ab}
RA	74.53 ± 12.80^{Aa}	68.93 ± 19.86^{Bac}	40.06 ± 23.57^{Bbd}	57.28 ± 28.45^{Bade}	46.55 ± 20.45^{Bbef}	55.96 ± 33.06^{Bbceg}	61.56 ± 28.72^{Bafg}	61.12 ± 30.36^{Bafg}
EX	80.03 ± 5.67^{Aa}	94.63 ± 21.50^{Aa}	95.95 ± 17.81^{Aa}	89.83 ± 9.70^{Aa}	92.14 ± 17.89^{Aa}	87.58 ± 11.42^{Aa}	94.88 ± 12.32^{Aa}	84.25 ± 13.69^{ACa}
EXRA	76.37 ± 5.28^{Aa}	65.76 ± 20.56^{Bac}	54.22 ± 30.58^{Bbcd}	55.96 ± 23.17^{Bbce}	61.58 ± 16.77^{Badef}	52.34 ± 23.34^{Bbcfg}	58.69 ± 18.16^{Bbcfh}	68.15 ± 19.26^{BCadegh}

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

Table II. Grip analysis of left pelvic limb (LPL).

MUSCLE STRENGTH PREHENSION - LPL

	BASAL	EV1	EV2	EV3	EV4	EV5	EV6	EV7
CON^{A}	86.04 ± 13.39	87.60 ± 12.88	96.41 ± 12.63	89.48 ± 6.67	90.85 ± 9.84	84.60 ± 15.83	87.83 ± 9.38	83.45 ± 9.38
RA^{B}	92.67 ± 14.93^{Aa}	85.75 ± 16.24	74.82 ± 9.22	79.41 ± 7.46	81.04 ± 8.93	74.73 ± 9.85	80.29 ± 11.41	79.08 ± 11.50
EX^{A}	73.92 ± 11.11^{Aa}	87.14 ± 11.24	81.78 ± 8.98	79.72 ± 7.42	85.87 ± 8.64	86.03 ± 10.18	88.97 ± 6.74	82.81 ± 15.84
EXRA^{B}	76.50 ± 3.86^{Aa}	75.06 ± 12.01	70.03 ± 16.83	74.03 ± 18.37	83.62 ± 14.08	78.26 ± 11.27	79.28 ± 18.73	73.76 ± 14.01

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

Table III. Motor funcion analysis of right pelvic limb (RPL).

MOTOR FUNCTION - VERTICAL POSITION

	BASAL	EV1	EV2	EV3	EV4	EV5	EV6	EV7
CON^{A}	67.46 ± 2.16^{Aa}	63.51 ± 1.08	64.56 ± 2.65	64.15 ± 4.28	62.67 ± 3.21	64.16 ± 1.78	62.46 ± 3.57	62.07 ± 4.93
RA^{B}	66.07 ± 5.07^{Aa}	59.77 ± 3.66	60.88 ± 6.68	60.34 ± 4.49	60.53 ± 4.57	62.18 ± 3.12	60.51 ± 3.53	58.86 ± 3.02
EX^{B}	63.85 ± 1.67^{Aa}	64.41 ± 2.67	63.64 ± 2.74	63.11 ± 3.27	61.82 ± 4.42	63.12 ± 3.04	61.84 ± 3.18	61.45 ± 4.87
EXRA^{A}	63.14 ± 1.66^{Aa}	62.3 ± 3.16	59.96 ± 3.50	58.97 ± 3.17	61.13 ± 4.16	59.64 ± 3.58	60.48 ± 3.86	61.47 ± 4.04

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

Table IV. Motor funcion analysis of left pelvic limb (LPL).

Motor Function - Horizontal Position								
	BASAL	**EV1**	**EV2**	**EV3**	**EV4**	**EV5**	**EV6**	**EV7**
CON	71.95 ± 2.04^{Aa}	70.32 ± 1.83^{ABa}	70.25 ± 1.75^{Aa}	70.96 ± 2.06^{Aa}	70.93 ± 1.96^{Aa}	71.92 ± 1.42^{Aa}	71.76 ± 3.42^{ABa}	72.22 ± 1.28^{Aa}
RA	72.75 ± 2.05^{Aa}	68.87 ± 3.10^{Ab}	71.46 ± 3.17^{Aa}	70.53 ± 3.83^{Aa}	70.16 ± 3.86^{Aa}	71.83 ± 3.48^{Aa}	70.54 ± 2.76^{Aa}	70.32 ± 3.36^{Aa}
EX	71.45 ± 2.28^{Aa}	72.01 ± 2.61^{ABa}	70.68 ± 3.61^{Ba}	70.9 ± 2.22^{Aa}	69.24 ± 3.24^{Aa}	71.44 ± 3.17^{Aa}	70.92 ± 1.88^{ABa}	69.77 ± 2.95^{Ba}
EXRA	71.64 ± 2.23^{Aa}	69.81 ± 2.15^{ABa}	68.15 ± 4.21^{Bb}	70.15 ± 3.09^{Aa}	71.64 ± 2.94^{Aa}	70.47 ± 3.43^{Ba}	73.31 ± 2.09^{Bc}	72.48 ± 3.07^{Aac}

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

In the analysis of the morphology of the rectus femoris muscle, the CON showed normal tissue appearance, with polygonal, multinucleated fibers, with myonuclear in subsarcolemmal position, loose joint tissue with a characteristic arrangement involving the fibers and fascicles composing the endomysium and perimysium (**figure 3 A**). On the other hand, the RA the RPL muscle demonstrated discrete fascicular disorganization, some amorphous fibers with reduced area, congested blood vessels with the presence of mononuclear and polymorphonuclear cells both in the intravascular region and in the interstitium between the muscle fibers (**figure 3 B**). The rectus femoris of the LPL also exhibited inflammatory cells in its morphology, but with better tissue appearance than the lesion side.

The EX showed muscle fibers with morphology like the CON without changes (**figure 3 C, G**). The EXRA, on the other hand, showed a morphological aspect with good organization of the muscle fibers, presence of connective tissue for the formation of the endomysium and perimysium, medium blood vessels with reduced vascular congestion and the decrease of inflammatory infiltrate in isolated muscle areas. Compared to RA, better cellular organization, and morphological appearance of the rectus femoris muscle of the exercise-treated group can be observed (**figure 3 D**). Organizational parameters like CON were found in the LPL of the EXRA (**figure 3 H**).

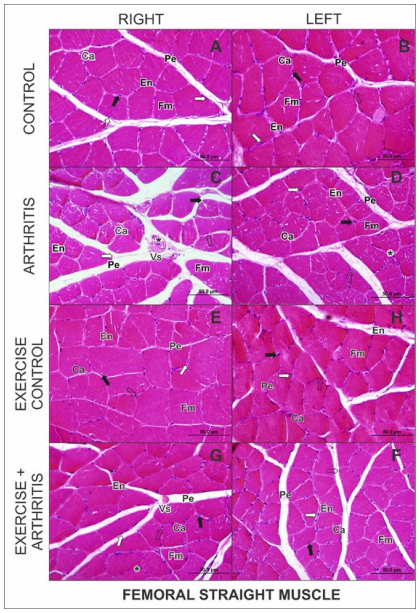

Figure 3. Photomicrographs of the right and left rectus femoris muscle of Wistar rats, cross section, hematoxylin and eosin stained.

For all groups of RPL **(A, B, C, E)** and LPL **(D, E, F, G)**, polygonal muscle fibers (Fm), blood capillaries (Ca), Perimysium (Pe), Endomysium (En), peripheral nuclei (hollow arrow), satellite cells (black arrow) and the presence of fibroblasts are visualized. Also, for RA **(B, F)** and EXRA **(D, H)**, there is presence of inflammatory cells (asterisk), congested Blood Vessels (Vs).

For the cross-sectional area of the rectus femoris muscle, there was a statistical difference between the right and left sides (WaldX2(1;28) = 15.860, $p < 0.001$) and interaction between groups and sides (WaldX2(3;28) = 5.381, $p < 0.05$).

The RPM of the RA group had a statistically significant reduction in the mean ($p < 0.001$) compared to the other groups. In the LPL, the EXRA showed decreased area ($p < 0.05$) compared to the COM and RA groups, but similar to the EX (**figure 4 A**). In the interaction between groups and right and left sides, only RA showed a statistical difference ($p < 0.001$), as can be seen in **figure 4 B**.

Regarding the smallest muscle fiber diameter, the statistics showed a significant difference between the right and left sides (WaldX2(1;32) = 14.645, $p < 0.001$) and in the interaction between groups and sides (WaldX2(3;32) = 5.474, $p < 0.001$).

The RA of the RPL obtained a reduction in the mean ($p < .05$) compared to the other groups on their respective sides. As for the LPL, the EXRA was similar to the EX; however, it was statistically different from the other groups ($p < .05$) as shown in **figure 7 A**. In the data corresponding to the interaction between groups and sides, the animals from the CON ($p < .05$) and RA ($p < .001$) showed statistical difference (**figure 4 B**).

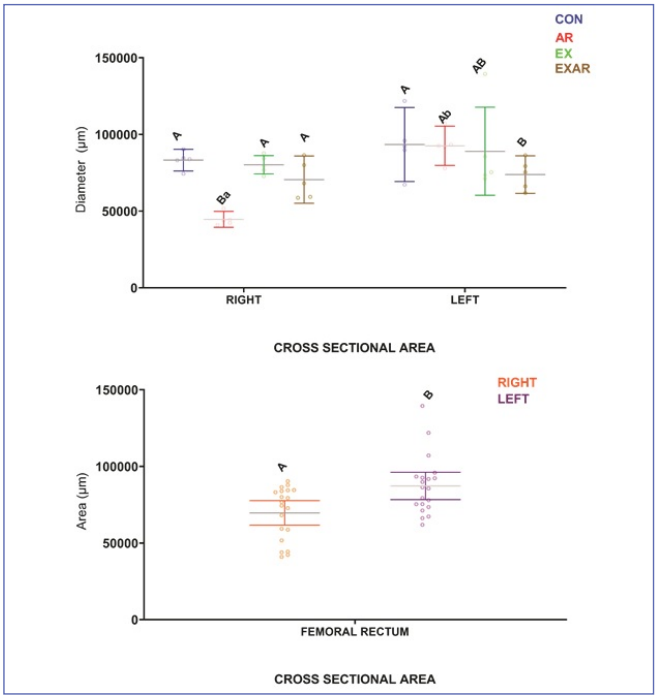

Figure 4. Analysis of the cross-sectional area of the rectus femoris muscle.

Data expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. **(A)** Capital letters denote statistical differences between groups and lower-case letters the interactions. **(B)** Capital letters denote statistical differences between sides.

In the morphological analysis of the knee joints, the tibial femur cartilage of the CON and EX groups presented normal aspects with a smooth and organized surface composed of four cellular layers. In the superficial zone, there was a greater density of flattened chondrocytes arranged in horizontal clusters. In the intermediate zone the cells assume a rounded morphology, isolated or in isogenic groups. In the deep zone, there is the presence of chondrocytes organized in lacunae, divided from the calcified zone by the tidemark and the presence of vascularization (**figure 5 A**, **E, C G**; **figure 6 A**, **E, C, G**).

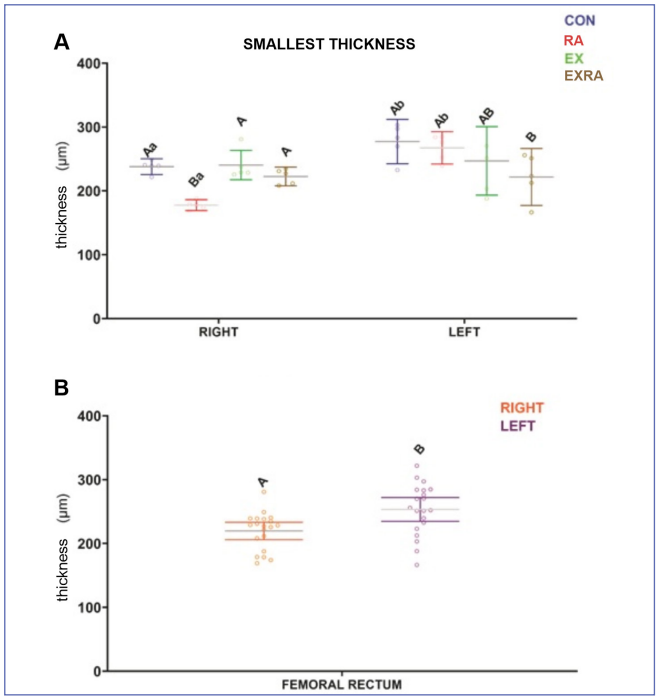

Figure 5. Analysis of the smallest diameter per fiber of the rectus femoris muscle. Data expressed as mean and standard deviation.

CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. **(A)** Capital letters denote statistical differences between groups and lower-case letters the interactions. **(B)** Capital letters denote statistical differences between sides.

In the RA group, both for the femur and the tibia, deleterious aspects were observed in the articular cartilage with some areas of less thickness and presence of flocculations on the surface, discontinuity of the tidemark, irregularly arranged chondrocytes, and subchondral bone invagination (**figure 5 B**, **F**; **figure 6 B**, **F**). Regarding the EXRA group, morphological changes were also observed in the femur and tibia of the injured and exercise-treated animals, showing areas of discontinuity of the tidemark and invaginations of the subchondral bone, however, with a better organization of the chondrocytes and an apparent recovery of the cartilage thickness similar to the CON group, demonstrating a recovery of the morphology of the right and left knees of the animals with rheumatoid arthritis (**figure 5 D**, **H**; **figure 6 D**, **H**).

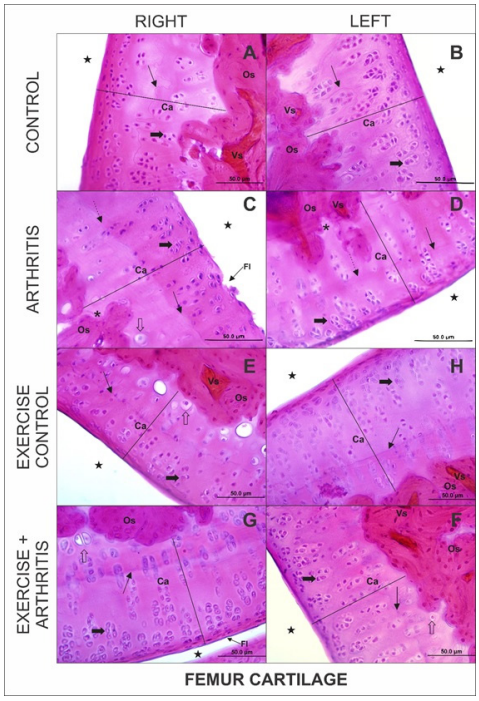

Figure 6. Morphological analysis of the femur bone in the periarticular region of the right and left pelvic limbs. Photomicrographs of the right and left femurs of Wistar rats in longitudinal section, hematoxylin and eosin stained.

The Control Group **(A, E)** and the Exercise Control **(C, G)** showed normal aspects of the articular cartilage **(Ca)**, presence of the continuous tidemark (continuous arrow), normal arrangement of the chondrocytes (filled arrow) with greater density in the superficial zone, subchondral bone (Os), blood vessels (Vs) and articular cavity (star) with normal aspects for both limbs. The Arthritis Group **(B, F)** presented morphological alterations of the articular cartilage (Ca) such as flocculations in the peripheral region (Fl) and cellular disorganization (filled arrow), regions of discontinuity of the tidemark (dotted arrow), and invagination of the subchondral bone (asterisk). The Arthritis + Exercise Group **(D, H)** demonstrated degenerative aspects such as subchondral bone invaginations (asterisk) and areas of tidemark discontinuity, but with less pronounced flocculations (Fl) present and with better organization of cell layers (black arrow).

In the analysis of the synovial membrane, the CON and EX groups denoted the expected organizational aspects, with synovial organization in two cell layers, synovial intima composed of synoviocytes and the subintima composed mostly of fat cells, without alterations in either the right or left limbs (**figure 7 A**, **C**, **E**, **G**). The RA group showed an intense inflammatory process of both the synovial intima, with consequent thickening, and the subintima with a reduction of fat cells and extensive synovitis (**figure 7 B**, **F**).

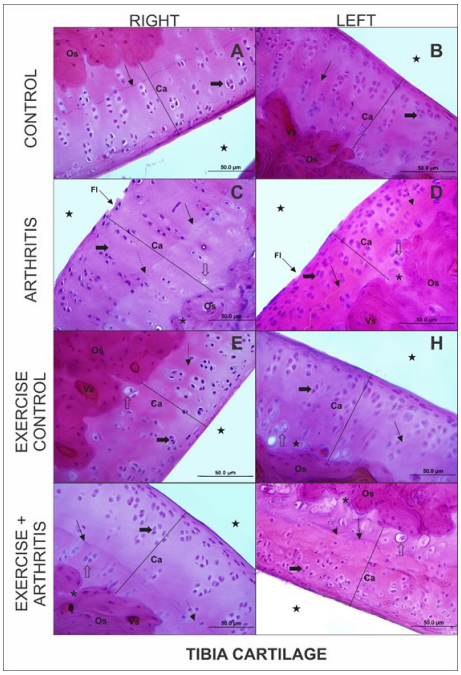

Figure 7. Morphological analysis of the tibia bone in the periarticular region of the right and left pelvic limbs. Photomicrographs of Wistar rats' right and left tibias in longitudinal section, hematoxylin and eosin staining.

The Control Group **(A, E)** and Exercise Control **(C, G)** show normal aspects of the articular cartilage (Ca), presence of continuous tidemark (continuous arrow), normality of the chondrocyte arrangement (filled arrow) with higher cell density in the superficial zone, extracellular matrix and subchondral bone (Os), blood vessels (Vs) and articular cavity (star) with normal aspects. The Arthritis Group **(B, F)** presented morphological alterations of the articular cartilage (Ca) such as flocculations in the peripheral region (Fl) and cellular disorganization (filled arrow) points of discontinuity of the tidemark (dotted arrow), invagination of the subchondral bone (asterisk). The Arthritis + Exercise Group **(D, H)** showed degenerative aspects such as presence of flocculation area (Fl) in the cartilage, subchondral bone invaginations (asterisk), however with greater cellular organization (black arrow) and better integrity of the tidemark (continuous arrow).

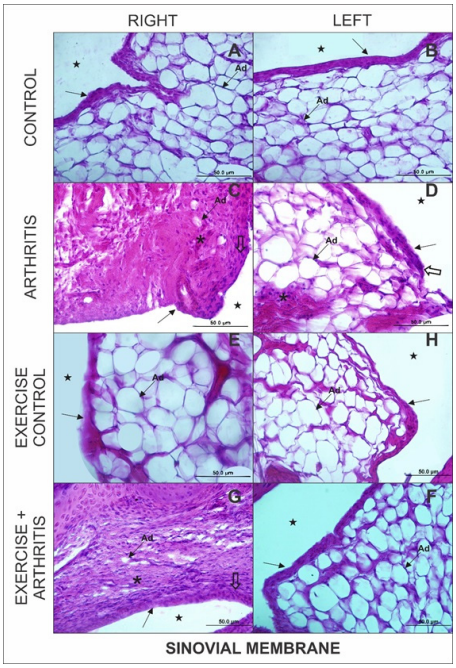

Figure 8. Morphological analysis of the synovial membrane of the right and left pelvic limbs. Photomicrographs of the synovial membrane of the right and left knee joint, longitudinal section, hematoxylin and eosin stained.

The Control **(A, E)** and Exercise Control **(C, G)** groups showed normal morphological aspects of the synovial membrane, with the presence of a synovial intima with synoviocytes (arrow), subintima composed of adipocytes (Ad), and a joint cavity (star). For the Arthritis **(B, F)** and Arthritis + Exercise **(D)** groups, the presence of an intense inflammatory process in the subintima (asterisk) with thickening of the synovial intima (arrow) denoting synovitis. The EXRA showed a slight reorganization of adipocytes in the RPL and control of the inflammatory infiltrate in the left pelvic limb with recovery of the synovial subintima.

For the RPL of the EXRA group, it is possible to observe a large concentration of inflammatory cells in the synovial subintima; however, a subtle presence of fat cells can also be identified, denoting a subtle tissue repair (**figure 8 D**). In the contralateral limb analysis, the synovial membrane presented an aspect similar to the CON and EX groups, with a normal morphological aspect (**figure 8 H**).

In the analysis of cartilage thickness, there was a statistical difference between the groups (WaldX2(3;32) = 7.839, $p < 0.001$). The RA group showed reduced mean ($p < 0.001$) compared to the other groups, while the EXRA was statistically similar to the CON, differentiating from RA and EX as can be seen in **figure 9**. There was no statistical difference between the sides.

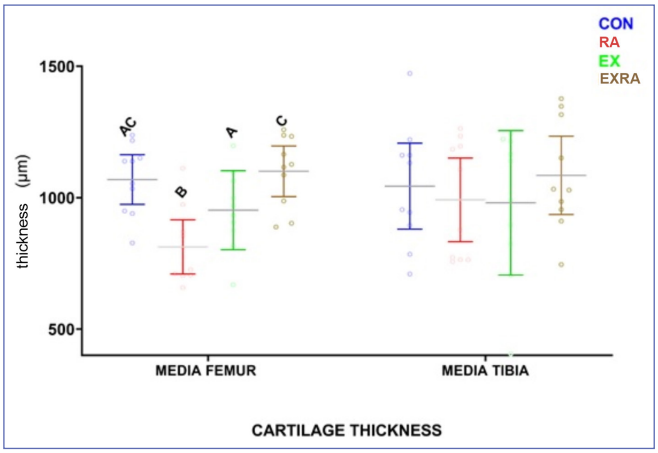

Figure 9. Articular cartilage thickness.

Data expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. Capital letters denote statistical differences between the groups.

For the chondrocyte count in the femur cartilage, statistical difference was observed between groups (WaldX2(3;28) = 7.297, $p < 0.001$) and between right and left sides (WaldX2(1;28) = 4.301, $p < 0.05$). The RA, EX, and EXRA animals reduced the number of chondrocytes compared to CON ($p < 0.05$). Between sides, the LPL had fewer chondrocytes compared to the RPL ($p < 0.05$). No statistical differences were observed for the tibia as can be seen in **figure 10**.

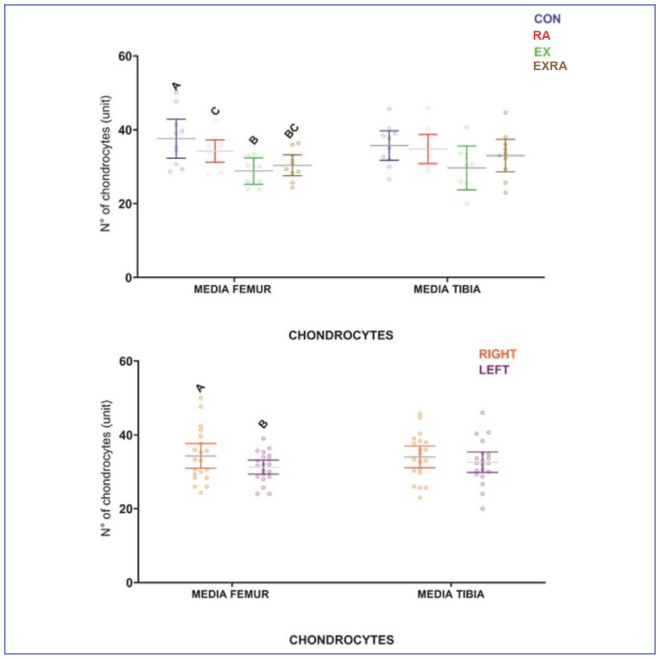

Figure 10. Number of chondrocytes in the femur and tibia cartilage.

Data expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. (A) Capital letters denote statistical differences between groups and lower-case letters the interactions. (B) Capital letters denote statistical differences between sides.

DISCUSSION

In the present study, we observed the repercussions of resisting exercise in an aquatic environment in Wistar rats with RA, considering grip strength, motor control, morphological and morphometric parameters induced in the knee joint by CFA. Both the groups RA and EXRA injured groups presented arthritic symptoms as described by Micheli *et al.* (29), which were analyzed by means of functional evaluation. Gomes *et al.* (23) compared two models of RA, with 2 injections of CFA, one containing *Mycobacterium tuberculosis* and the other *Mycobacterium butiricum*, report that the most suitable model for exercise analysis in a model of RA is with *Mycobacterium butiricum*.

In the functional analysis of strength, the EX and EXRA showed a deficit of the averages after CFA injury. The literature points out that contractile function can be impaired by what is called skeletal muscle disease in RA, characterized by reduced strength and endurance parameters, as well as the presence of sarcopenic obesity, defined by increased fat percentage and reduced contractile tissue in the intramuscular region (30, 31).

In the Uutela *et al.* (31), an investigation was carried out with 199 patients with RA, regarding the muscular performance of the upper and lower limbs and trunk, associated with several behavioral factors. The findings showed that 47% of the sample showed a high reduction in muscle strength, concluding that strength capacity is associated with the level of disease activity and that this risk factor may be modifiable through the practice of physical exercises. In the present study, we also observed a reduction in strength in the injured animals after the induction of arthritis, which was more pronounced immediately after the injury. However, the EXRA obtained a better recovery of strength, similar to the EX, demonstrating the effectiveness of the exercise protocol proposed for the preservation of muscle strength.

A lower limb aquatic exercise program, 3 times a week for 16 weeks, in 82 women with RA can promote beneficial effects in controlling disease activity and improving functional parameters (32). In the present study, the evaluation of the inclined plane in the horizontal position showed that the RA and EXRA groups had a reduction in angular tolerance after the injury; however, both groups recovered, exhibiting an average similar to the baseline parameters at the end of the experiment. Among the main comorbidities associated with RA, functional disability and significant loss of postural balance due to musculoskeletal changes and that in the long term also affect the vestibular and ocular systems, this being a relevant topic for investigation (33).

However, the model of RA induction used in the present experiment is predictive of the promotion of an accentuated inflammatory state during the first seven days after its application, justifying the result of motor control recovery of both injured groups during the experiment. Thus, it is suggested that new investigations be done contemplating specific evaluations for motor control.

Regarding the tissue analyses, it can be verified that the RA model promoted alterations in the rectus femoris muscle, with reduced cross-sectional area and smaller diameter of the injured limb in the RA group, besides the presence of inflammatory cells among the fibers and in the connective tissue region and higher concentration of red blood cells in medium caliber blood vessels. An adaptive response found in arthritic individuals is the presence of hypoxia resulting from synovial inflammation by hypoxia-induced

factors (HIFs) that alter the local oxygen supply (34) and the increase of proinflammatory cytokines such as TNFα that has a catabolic effect by altering protein synthesis and degradation (35).

Baker *et al.* (36) demonstrated by means of an evaluation of the calf muscle density using the computed tomography technique and force dynamometry in 50 individuals with RA and found an association of reduced strength in the presence of a smaller fiber area. Furthermore, the study concluded that lean mass deficits are associated with greater joint destruction.

In addition, local joint inflammation promotes endocrine repercussions that alter extra-articular tissues such as skeletal striated muscle (37). This hypermetabolic inflammatory state associated with physical inactivity, adiposity level, insulin resistance and endothelial dysfunction in RA, are determining factors for the development of long-term rheumatoid cachexia (30, 38).

The EXRA also showed a reduction in cross-sectional area and the smallest fiber diameter, but in the LPL, which can be explained by a possible biomechanical imbalance that may have generated a greater energy demand due to the intense inflammatory process in the RPL (39). Moreover, the presence of this contralateral atrophy may have occurred due to the increase in cortisol hormone, since pain promotes high levels of stress and has already been associated with the aquatic treatment protocol (40). However, for the injured limb, the proposed exercise protocol provided benefits in muscle morphometric parameters.

As for the repercussions on the knee joint, morphologic signs of degeneration of the articular cartilage and synovial membrane found on RA and EXRA were observed. The migration of synoviocytes, similar to fibroblasts, is the main pannus-forming event, which implies a severe inflammation of the synovial tissue, followed by the formation of numerous chemokines, cytokines, matrix metalloproteases that favor joint destruction (41, 42). These synoviocytes present in synovial inflammation produce enzymes involved in cartilage destruction and also the receptor activator ligand nuclear factor kappa B (RANKL) that regulates the differentiation of osteoclasts that carry out bone destruction and blood vessel neoformation (34).

The RA group also showed reduced cartilage thickness and amount of chondrocytes in the femur. Bone erosion associated with cartilage destruction are common radiological findings in patients with chronic RA, although cartilage damage seems to be more associated with physical disability (43). The aquatic resistance exercise protocol preserved the integrity of the tibial and femoral cartilage and promoted the permanence of fat cells in the synovial subintima, evidencing the need for exercise for joint maintenance.

In the study of Vieira *et al.* (44), the effect of resistance exercise in stair climbing (five days a week, three weeks, and overload of 100 g) on morphological parameters of the ankle joint of Wistar rats submitted to a model of sciatic pain was observed. The groups that suffered nerve damage showed an increase in the number of chondrocytes, which was justified by a possible hypertrophy due to exercise or due to joint kinematics, since the weight load on the pelvic limb was altered. These data differ from those presented in the present study, being similar in the use of resistance training for treatment, but performed in an aquatic environment, 3 times a week with 50% of the animals' weight as overload. Both RA and EXRA obtained a reduction in the number of chondrocytes in the femur, although the exercised groups presented similarities and lower statistical means.

It is hypothesized that the physical properties of the water may have interfered with the proliferative demand of chondrocytes, as well as the reduced weight load characteristic of aquatic exercise. However, this reduced mean number of chondrocytes was not related to the findings regarding the femur cartilage thickness of the animals in the EX group. These were similar to the CON and EXRA groups, with no influence on the cartilage thickness, reinforcing the evidence that exercise had a chondroprotective effect.

Finally, the present study showed increased chondrocyte numbers in RPL compared to LPL. Chondrocyte proliferation correlates with proinflammatory regulation. In a stress situation, the production of cytokines is of greater relevance to the immune response than that of type II collagen and glycosaminoglycans (GAGs). This stress may be associated with systemic inflammatory conditions, physical activity, or even the presence of physical properties such as hydrostatic pressure that act in modulating the number of cartilage chondrocytes (41, 45).

In the present research, it was possible to observe repercussions of the RA induced by CFA, in relation to the parameters of strength, motor control, and symmetrical joint manifestations, and it was demonstrated that resisting physical exercise performed in an aquatic environment improved muscle strength and the morphological characteristics of both the rectus femoris muscle and the knee joint of Wistar rats with rheumatoid arthritis. Based on the results obtained, future investigation of inflammatory markers, such as tissue cytokines, is suggested, in addition to this, which is also a limitation of the study, an exercise protocol with only one water temperature was performed, since this characteristic can generate different therapeutic effects, it is suggested that exercises with different protocols at different water temperatures should also be performed. Obviously, the extrapolation of these results to humans is a limitation, but they allow us to suppose that resistance exercise in aquatic environment can also promote, besides muscle strength

gains, a greater joint preservation in individuals with RA, which should be explored in future clinical research.

CONCLUSIONS

Resistance jumping exercise in an aquatic environment promoted benefits in muscle strength and preserved morphometric aspects of the rectus femoris muscle and knee joint components of Wistar rats with RA experimentally induced model.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUITIONS

ALFT: funding, conception, data collection, data analysis, manuscript writing. MN, TCS, LAP: conception, data collection, manuscript reviewing. TSSL, LFCR, GRFB: funding, conception, data analysis, manuscript reviewing.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Pinto ACPN, Natour J, Castro CH de M, Eloi M, Junior IL. Acute effect of a resistance exercise session on markers of cartilage breakdown and inflammation in women with rheumatoid arthritis. Int J Rheum Dis 2017;20(11):1704–13.
2. Okada Y, Eyre S, Suzuki A, Kochi Y, Yamamoto K. Genetics of rheumatoid arthritis: 2018 status. Ann Rheum Dis 2019;78(4):446–53.
3. McKenna S, Kelly G, Kennedy N. A survey of physiotherapists' current management and the promotion of physical activity, in people with rheumatoid arthritis. Disabil Rehabil 2019;41(18):2183–91.
4. Li WC, Bai DL, Xu Y, *et al.* Identification of differentially expressed genes in synovial tissue of rheumatoid arthritis and osteoarthritis in patients. J Cell Biochem 2019;120(3):4533–44.
5. Olson AL, Swigris JJ, Sprunger DB, *et al.* Rheumatoid arthritis-interstitial lung disease-associated mortality. Am J Respir Crit Care Med 2011;183(3):372–8.
6. Kiadaliri AA, Englund M. Mortality with musculoskeletal disorders as underlying cause in Sweden 1997-2013: A time trend aggregate level study. BMC Musculoskelet Disord 2016;17(1):163.
7. de Santana FS, Nascimento Dda C, de Freitas JP, *et al.* Avaliação da capacidade funcional em pacientes com artrite reumatoide: implicações para a recomendação de exercícios físicos [Assessment of functional capacity in patients with rheumatoid arthritis: implications for recommending exercise]. Rev Bras Reumatol 2014;54(5):378–85. Portuguese.
8. Blum D, Rodrigues R, Geremia JM, Brenol CV, Vaz MA, Xavier RM. Quadriceps muscle properties in rheumatoid arthritis: Insights about muscle morphology, activation and functional capacity. Adv Rheumatol 2020;60(1):28.
9. Buckner SL, Jessee MB, Dankel SJ, Mattocks KT, Abe T, Loenneke JP. Resistance exercise and sports performance: The minority report. Med Hypotheses 2018;113(2017):1–5.
10. Hooper DR, Tunde KS, DiStefano LJ, *et al.* Effects of resistance training fatigue on joint biomechanics. J strength Cond Res 2013;27(1):146–53.
11. Mackey AL, Kjaer M. Connective tissue regeneration in skeletal muscle after eccentric contraction-induced injury. J Appl Physiol 2017;122(3):533–40.
12. Louder T, Dolny D, Bressel E. Biomechanical comparison of countermovement jumps performed on land and in water: Age effects. J Sport Rehabil 2018;27(3):249–56.
13. Beckwée D, Vaes P, Cnudde M, Swinnen E, Bautmans I. Osteoarthritis of the knee: Why does exercise work? A qualitative study of the literature. Ageing Res Rev 2013;12(1):226–36.
14. Mattos F, Leite N, Pitta A, Bento PC. Effects of aquatic exercise on muscle strength and functional performance of individuals with osteoarthritis: a systematic review. Rev Bras Reumatol Engl Ed 2016;56(6):530–42. English, Portuguese.
15. Pan CY. Effects of water exercise swimming program on aquatic skills and social behaviors in children with autism spectrum disorders. Autism 2010;14(1):9–28.
16. Methajarunon P, Eitivipart C, Diver CJ, Foongchomcheay A. Systematic review of published studies on aquatic exercise for balance in patients with multiple sclerosis, Parkinson's disease, and hemiplegia. Hong Kong Physiother J 2016;35:12–20.
17. Vasile L, Stănescu M. The aquatic therapy in balance coordination disorders. Procedia - Soc Behav Sci 2013;92:997–1002.
18. Delevatti R, Marson E, Kruel LF. Effect of aquatic exercise training on lipids profile and glycaemia: A systematic review. Rev Andaluza Med del Deport 2015;8(4):163–70.
19. Meredith-Jones K, Waters D, Legge M, Jones L. Upright water-based exercise to improve cardiovascular and metabolic health: A qualitative review. Complement Ther Med 2011;19(2):93–103.
20. Cugusi L, Cadeddu C, Nocco S, *et al.* Effects of an aquatic-based exercise program to improve cardiometabolic profile, quality of life, and physical activity levels in men with type 2 diabetes mellitus. PM R 2015;7(2):141–8.
21. Viña J, Rodriguez-Mañas L, Salvador-Pascual A, Tarazona-Santabalbina FJ, Gomez-Cabrera MC. Exercise: The lifelong supplement for healthy ageing and slowing down the onset of frailty. J Physiol 2016;594(8):1989–99.

22. Gomes RP, Bressan E, Silva TM, Gevaerd Mda S, Tonussi CR, Domenech SC. Effects of one minute and ten minutes of walking activity in rats with arthritis induced by complete Freund's adjuvant on pain and edema symptoms. Rev Bras Reumatol 2014;54(2):83–9.
23. Gomes RP, Bressan E, Silva TM, Gevaerd Mda S, Tonussi CR, Domenech SC. Standardization of an experimental model suitable for studies on the effect of exercise on arthritis Padronização de modelo experimental adequado a estudos do efeito do exercício na artrite. Einstein 2013;11(55 48):76–82.
24. Kunz RIR, Coradini JGJ, Silva LIL, Bertolini GGRF, Brancalhão RRMC, Ribeiro LLFC. Effects of immobilization and remobilization on the ankle joint in Wistar rats. Brazilian J Med Biol Res 2014;47(10):842–9.
25. Coradinia JG, Kakihata CM, Kunz RI, Errero TK, Bonfleur ML, Bertolini GR. Avaliação da força de preensão em ratos Wistar, normais e obesos, submetidos à natação com sobrecarga após compressão do nervo mediano [Evaluation of grip strength in normal and obese Wistar rats submitted to swimming with overload after median nerve compression]. Rev Bras Reumatol 2015;55(1):43–7. Portuguese..
26. Dunk NM, Nicholson KJ, Winkelstein BA. Impaired performance on the angle board test is induced in a model of painful whiplash injury but is only transient in a model of cervical radiculopathy. J Orthop Res 2011;29(4):562–6.
27. Brito MKM de, Camargo Filho JCS, Vanderlei LCM, Tarumoto MH, Dal Pai V, Giacometti JA. Dimensões geométricas das fibras do músculo sóleo de ratos exercitados em esteira rolante: a importância da análise por meio de imagens digitalizadas. Rev Bras Med do Esporte 2006;12(2):103–7.
28. Kunz RI, Silva LI, Roncini J, *et al.* Histomorphometric changes in the knee joint of Wistar rats after remobilization in a water enviroment. Fisioter e Pesqui 2015;22(3):317–24.
29. Micheli L, Bozdag M, Akgul O, *et al.* Pain relieving effect of-NSAIDS-CAIs hybrid molecules: Systemic and intra-articular treatments against rheumatoid arthritis. Int J Mol Sci 2019;20(8):1923.
30. Andonian BJ, Huffman KM. Skeletal muscle disease in rheumatoid arthritis: The center of cardiometabolic comorbidities? Curr Opin Rheumatol 2020;32(3):297–306.
31. Uutela TI, Kautiainen HJ, Häkkinen AH. Decreasing muscle performance associated with increasing disease activity in patients with rheumatoid arthritis. PLoS One 2018;13(4):e0194917.
32. Siqueira US, Orsini Valente LG, De Mello MT, Szejnfeld VL, Pinheiro MM. Effectiveness of aquatic exercises in women with rheumatoid arthritis: a randomized, controlled, 16-week intervention-The HydRA Trial. Am J Phys Med Rehabil 2017;96(3):167–75.
33. Silva MCR da, Koch DB, Mochizuki L, Gevaerd MDS, Zanella ÂK, Domenech SC. Avaliação do equilíbrio postural em pessoas com artrite reumatoide. Rev Ter Ocup da Univ São Paulo 2019;29(3):280–7.
34. Ryu JH, Chae CS, Kwak JS, *et al.* Hypoxia-inducible Factor-2α is an essential catabolic regulator of inflammatory rheumatoid arthritis. PLoS Biol 2014;12(6):e1001881.
35. Lim H, Lee SH, Lee HT, *et al*. Structural biology of the TNFα antagonists used in the treatment of rheumatoid arthritis. Int J Mol Sci 2018;19(3):768.
36. Baker JF, Von Feldt J, Mostoufi-Moab S, *et al*. Deficits in muscle mass, muscle density, and modified associations with fat in rheumatoid arthritis. Arthritis Care Res 2014;66(11):1612–8.
37. Stavropoulos-Kalinoglou A. Muscle wasting in rheumatoid arthritis: The role of oxidative stress. World J Rheumatol 2014;4(3):44–53.
38. van der Kolk BW, Goossens GH, Jocken JW, Blaak EE. Altered skeletal muscle fatty acid handling is associated with the degree of insulin resistance in overweight and obese humans. Diabetologia 2016;59(12):2686–96.
39. Iop R da R, Shiratori AP, Ferreira L, Borges Júnior NG, Domenech SC, Gevaerd M da S. Production capacity of maximal isometric grip strength in women with rheumatoid arthritis: a pilot study. Fisioter e Pesqui 2015;22(1):11–6.
40. Orsini M, Hasue RH, Leite MAA, Menezes SLS de, Silva JG, Oliveira AB. Neuromuscular diseases: revisiting the "overtraining." Fisioter e Pesqui 2014;21(2):101–2.
41. Huh YH, Lee G, Lee KB, Koh JT, Chun JS, Ryu JH. HIF-2α-induced chemokines stimulate motility of fibroblast-like synoviocytes and chondrocytes into the cartilage-pannus interface in experimental rheumatoid arthritis mouse models. Arthritis Res Ther 2015;17(1):302.
42. Neves M, Tavares AL de F, Retameiro ACB, Leal TS da S, Ribeiro L de FC, Bertolini GRF. Low-level laser therapy in periarticular morphological aspects of the knee of Wistar rats in rheumatoid arthritis model. Brazilian J Pain 2020;3(1):8–13.
43. Fujimori M, Nakamura S, Hasegawa K, *et al*. Cartilage quantification using contrast-enhanced MRI in the wrist of rheumatoid arthritis: Cartilage loss is associated with bone marrow edema. Br J Radiol 2017;90(1077):20170167.
44. Vieira L, Lovison K, Kunz RI, *et al*. Resistance exercise recovers the structure of cartilage and synovial membrane of the ankle joint of rats after sciatic compression. Mot Rev Educ Física 2017;23(3):e101707.
45. Blazek AD, Nam J, Gupta R, *et al*. Exercise-driven metabolic pathways in healthy cartilage. Osteoarthr Cartil 2016;24(7):1210–22.