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Resisted exercise promotes repair in musculoskeletal structure in experimental model of rheumatoid arthritis

Exercício resistido promove reparo no musculoesquelético em modelo experimental de artrite reumatoide

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RESUMO
Distúrbios decorrentes da artrite reumatoide (AR) resultam em incapacitação funcional, com reflexos econômicos e sociais aos sistemas de saúde. Uma alternativa terapêutica que tem sido proposta é a atividade física na modalidade resistida. O presente estudo analisou os efeitos do exercício resistido de subida em escada (ERSE) na histomorfologia do músculo extensor longo dos dedos (EDL) de ratos *Wistar*, em modelo experimental de AR. Ratos machos (n=20) foram distribuídos aleatoriamente em quatro grupos. A lesão da AR foi induzida por administração intra-articular de Adjuvante Completo de Freund (CFA). Os resultados revelaram que a AR alterou a histomorfometria das fibras do EDL e que o ERSE promoveu reparo muscular, sugerindo sua eficiência na restauração da funcionalidade muscular. Ainda, o ERSE pode ser uma opção de tratamento voltado à melhoria na qualidade de vida dos portadores de AR.
Palavras-chave: Artrite reumatoide. Alteração histomorfométrica. Reparo muscular.

ABSTRACT
Disorders from rheumatoid arthritis (RA) result in functional disability, with economic and social impacts on health systems. A therapeutic alternative that has been proposed is physical activity in the resisted modality. Current study analyzed the effects of resistance exercise on stairs (ERSE) on the histomorphology of the extensor digitorum longus muscle (EDL) of Wistar rats in an experimental model of RA. Male rats (n=20) were randomly assigned to four groups. RA injury was induced by intra-articular administration of Freund's Complete Adjuvant (CFA). Results revealed that RA altered the histomorphometry of EDL fibers and that ERSE promoted muscle repair, suggesting its efficiency in restoring muscle functionality. Furthermore, ERSE may be a treatment option to improve the life quality of people with RA.
Keywords: Histomorphometric alteration. Muscle repair. Rheumatoid arthritis.

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INTRODUCTION

Rheumatoid arthritis (RA), a chronic inflammatory disease with systemic effects, is widely known for progressively destroying joint structural components due to its autoimmune nature[1-4] and the dispersion of inflammatory proteins in the synovial fluid.[2,4] The disease´s clinical scenario, featuring symmetrical polyarthritis, pain, deformities, bone erosion, rheumatoid nodules, edema and joint stiffness, causes serious limitations in patients´ mobility and an increase of other comorbidities which impair their life quality.[1,3]

Due to the disease´s systemic inflammatory features, some extra articular manifestations are also observed in RA patients.[1,4-6] According to the Brazilian Society of Rheumatology, structures such as skin, nails, kidneys, heart, lung, nervous system, eyes, blood and muscles[6-8] may also be affected by the disease and undergo changes.

Regarding the musculoskeletal system, studies have shown that inflammation compromises muscle physiology,[5,7-9] affects normal muscle renewal and responses to injury.[7.9] RA is related to a series of morphological and functional alterations that compromise tissue and musculoskeletal system,[5-7,9] triggering muscular atrophy processes via rheumatoid cachexia and sarcopenia, [7.10-12] and, consequently, reduced strength muscular.[5,7]

Compounding RA damage to the patient's functional capacity,[1,5-9] there is also a direct and indirect impact of the disease on the economy.[6] Due to early retirement, disability,[15] hospital, outpatient and medication treatments,[16] patients with the pathology are very burdensome to public coffers, more specifically to the Unified Health System and Social Security (SUS) in Brazil. In addition, it should be enhanced that pharmacological treatment may be complicated due to toxicity and drug contraindications.[16,17] Consequently, the search for alternative treatments that are less expensive and which control the disease without causing greater tissue damage to the patient is relevant.

Physical exercise has been investigated as a therapeutic modality of great relevance for the management of the disease.[1,3,4,7,9,18-20] Exercise in resistance mode is indicated for maintaining strength and gaining muscle mass.[4,9,18,19] It acts in the reversal to atrophy processes, besides being an attenuator of the inflammatory symptoms, especially pain, or rather, it promotes the functional improvement in the range of articular movement, muscular strength and resistance,[1,5,9] and gains in cardiovascular parameters.[1,8]

Owing to ethical issues and to the difficult control of research in humans, experimental models *in vivo* in animals have been developed, aiming at providing information and scientific knowledge necessary to foreground the issue. Freund's Complete Adjuvant (CFA) is a compound used in experimental models and accepted to simulate signs and symptoms of human RA.[21] CFA consists of paraffin oil with mannitol monooleate, as a suspended surfactant, and a dead mycobacterium.[22]

Although several studies have shown harmful effects of RA on different skeletal muscles, such as gastrocnemius, soleus,[19] and vastus lateralis muscles,[12, 23] the literature is scarce on the effects of the disease on the long extensor muscle of the fingers (EDL). Since glycolytic muscle fibers (type II) [24] are more prone to muscle atrophy, there is a lack of information about the histomorphometric effects of RA on the EDL muscle, and on ERSE as a promising alternative in tissue repair, with consequences on movement biomechanics. Current study analyzes the effects of ERSE on the morphology of the EDL muscle of Wistar rats in an experimental model of RA.

METHODOLOGY

ANIMALS

The sample group consisted of twenty 15-day-old male Wistar rats retrieved from the Central Animal Hospital of UNIOESTE and placed in polypropylene boxes, with access to water and feed *ad libitum*. Temperature was maintained at 22±2ºC, and the luminosity consisted of a light / dark photoperiod of 12 hours. The experimental procedures were previously approved by Committee for Ethics in the use of Animals (CEUA) of the Universidade Estadual do Oeste do Paraná (UNIOESTE), on the 27th October 2017.

CHARACTERIZATION OF THE EXPERIMENTAL GROUPS

The animals were randomly separated into four groups (n = 5):

- Control Group (CG): animals that were neither submitted to injury with CFA nor to treatment with physical exercise;
- Arthritis Group (GAR): animals induced to injury with CFA, without treatment;
- Exercise Control Group (GCE): animals that were not submitted to CFA injury protocol, but received treatment with resistance physical exercise;
- Arthritis Exercise Group (GARE): animals that underwent injury and treatment with exercise.

AIR INDUCTION PROTOCOL

RA was induced according to protocol described by Gomes *et al.*[22] with the use of Freund's Complete Adjuvant - CFA *Mycobacterium butyricum* (0.5 mg / mL, Difco®), administered by injection. Animals were manually restrained, or rather, the anterior knee area of the right pelvic limb was properly trichotomized and the injection site on the tibiofemoral joint region was submitted to asepsis with 1% iodized alcohol (Rialcool®). A 1 mL syringe and a 13 x 4.5 mm needle were used to apply the solutions.

CFA was used for groups GAR and GARE at two instances. Animals were first pre-sensitized by intradermal injection of 50μL of CFA, at the base of the tail; seven days after the first stimulus, an injection of 50μl (0.5 mg / mL) of CFA in the right tibiofemoral joint was given.

An isotonic sodium chloride solution (0.9%, Aster®) was injected, using the same methodology as the administration of the CFA, to simulate the same stress on the animals of the GC and GCE groups.

RESISTED EXERCISE PROTOCOL

Twenty-four hours after performing the RA induction protocol, the animals in the GCE and GARE groups were submitted to resistance exercise of climbing stairs. A vertical wooden ladder was used, with 67 iron steps, height 1.18 m, width 20.5 cm, with a 60° inclination. A box, height 20 cm and width 30 cm, was positioned at the top of the stairs for animal rest during the interval between series.[19]

The treatment protocol consisted of 3 weeks of resistance exercise by climbing stairs, with progressive repetitions performed series on alternate days, totaling 14 days of training. In the first week, the animals were submitted to 4 series of 5 repetitions. In the second week, they underwent 4 series of 7 repetitions and in the last week of treatment, 4 series of 10 repetitions. The rest interval between sets had duration of 60 seconds. To simulate the resistance effect of the exercise, a 100g overweight was adapted to the proximal region of the tail.[4]

EDL MUSCLE HISTOLOGICAL COLLECTION AND PREPARATION

At the end of the training period, namely, on the 28th day after the first dose of CFA, the animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (Ketalar - Brazil) (95 mg / Kg) and xylazine (Xilazin - Brazil) (12 mg / Kg). After euthanasia, the EDL muscle of the right pelvic limb of the animals was removed and fixed in Metacarn (70% Methanol + 20% chloroform + 10% glacial acetic acid) for 24 hours and later stored in 70% alcohol until routine histological processing for paraffin embedding.[22]

Transversal, semi-series, 5 μm thick slices of the EDL muscles were performed using microtome *Olympus CUT 4055*. Slides were stained with hematoxylin and eosin (HE) for morphological evaluations.

QUANTITATIVE ANALYSIS

Slides were photomicrographed under an Olympus ® DP71 microscope (USA) to measure

EDL´s morphometric parameters. Ten images were obtained for each animal, in a 40X objective, and measured by Image-Pro Plus 6.0. In addition, 10 fibers per image were standardized, preferably within the same issue, totaling 100 measurements per animal.

The histomorphological variables were: cross-sectional area, smaller diameter of the muscle fiber, count of total number of nuclei and total count of centralized nuclei. Centralized nuclei were considered to be those that were not close to the subsarcolemal membrane.

QUALITATIVE ANALYSIS

HE slides were observed under light microscopy (BX60 *Olympus*®, Tokyo, Japan), in 400X objective, for the descriptive analyses of EDL morphology. Parameters observed in the fibers comprised external contour and angulation, position of the myionuclei, general fascicular arrangement, fiber size and arrangement of blood capillaries.

STATISTICAL ANALYSIS

Data of quantitative variations were analyzed statistically, using parametric tests of analysis of variance - (ANOVA and Tukey's post-test), based on normality and homoscedasticity, at $p<0.05$. Results were expressed as mean ± standard deviation.

ETHICAL ASPECTS

All experimental procedures were performed according to the ethical precepts defined by the International Association for the Study of Pain (IASP, 1983), and approved by the Committee for Ethics in the Use of Animals (CEUA) of UNIOESTE, according to the memorandum of 26th September 2017.

RESULTS

MORPHOMETRIC ANALYSIS

Table 1 gives the morphometric results of RA induced by CFA and ERSE in EDL, indicating that the experimental model of RA by CFA and the treatment of ERSE promoted morphometric changes in the animals´ EDL muscle in the analyzed variables.

Regarding the cross-sectional area, results showed that RA induced by CFA promoted a significant reduction of the variable in animals of the GAR group (81.04 ± 3.77), when compared to the rates of the two control groups. In the CG group (94.00 ± 1.09), the reduction was 13.83%. albeit greater when contrasted to the GCE group (97.46 ± 2.02), with a 16.84% reduction. Further, although the ERSE treatment protocol did not show significant differences between the CG and GCE control groups, the data expressed in the GARE group (95.33 ± 5.19) revealed that physical exercise altered the lesion in approximately 15%, since area rates were statistically compared to the data of the two control groups GC and GCE, and differentiated from GAR ones.

In the case of the smallest diameter, data analysis revealed that RA promoted a significant reduction in the rates of the variable in GAR fibers (15.77 ± 1.59) when compared to CG (18.84 ± 0.21) and GCE (21.44 ± 0.96). ERSE alteration comprised an increase in the smallest diameter of the muscle fibers of the animals submitted to training. This factor was evident when mean rates between CG (18.84 ± 0.21) and GCE (21.44 ± 0.96) were contrasted, as well as in the comparison between GAR (15.77 ± 1.59) and GARE (19.08 ± 0.75). The most relevant factor in this assessment was that the change in diameter promoted by training with ERSE in the GARE group was able to statistically match its averages with those of the CG.

By contrasting GAR data (13.4 ± 3.78) with those of control, analysis of the central nucleus count (NC) showed CG (1.4 ± 0.55) and GCE (4.8 ± 2.95), or rather, in animals submitted to experimental RA, the number of NC was significantly more significant. Results of ERSAE treatment failed to indicate a significant difference between the two control groups GC (1.4 ± 0.55) and GCE (4.8 ± 2.95), nor between the groups GAR (13.4 ± 3.78) and GARE (8.6 ± 3.58). However, data analysis showed that the training of the animals of the GARE group was able to match the averages of the amount of NC of these animals with those of the GCE group, since there was no statistically significant difference between them.

There was no statistically significant difference in total nucleus count (NT), between the groups: CG (231.80 ± 17.06), GCE (227.60 ± 27.22), GAR (241.40 ± 18, 48) and GARE (255 ± 26.3).

Table 1. Means and standard deviations of the cross-sectional area, smallest diameter (D. Minor), biggest diameter (D Major), number of nuclei (N Nuclei), number of central nuclei (NC) and total nuclei (NT) of EDL

GROUPS	Area (μm^2)	D Minor (μm)	NC	NT
GC	94.00 ± 1.09^{a}	18.84 ± 0.21^{b}	1.4 ± 0.55^{c}	231.80 ± 17.06^{a}
GCE	97.46 ± 2.02^{a}	21.44 ± 0.96^{a}	4.8 ± 2.95^{bc}	227.60 ± 27.22^{a}
GAR	81.04 ± 3.77^{b}	15.77 ± 1.59^{c}	13.4 ± 3.78^{a}	241.40 ± 18.48^{a}
GARE	95.33 ± 5.19^{a}	19.08 ± 0.75^{b}	8.6 ± 3.58^{ab}	255 ± 26.3^{a}

Different lower case letters represent significant differences ($p<0.05$) when compared to groups within the same column.

MORPHOLOGICAL ANALYSIS

In the control group (CG), morphological characteristics were reported within the normal range, muscular arrangements in fascicular pattern, homogeneous, fibers with polygonal external contour, rounded angles, myonuclei in subsarcolemal position and capillaries interspersed in the conjunctive (Figure 1A).

In the group that received resistance physical exercise (GCE), cellular hypertrophy was observed, providing a slightly heterogeneous aspect to the muscle fibers, with preservation of the polygonal shape and contours with rounded angles and capillaries interspersed with the conjunctive. The nuclei remained in a subsarcolemal position, with few centralized nuclei (Figure 1B).

On the other hand, GAR revealed microscopic signs of muscle damage, with a clear disorganization in the fascicular arrangement, especially in the peripheral regions, closer to the epimysium. The fibers showed irregular external contours and sharp angles. Another relevant issue was the heterogeneous aspect promoted by cells with varied diameters and visible disorganization of the fascicular arrangement (Figure 1C).

It has been verified at GARE that ERSE training stimulated the repair process, since it promoted the reorganization in the muscular fascicular arrangement, whose aspect of the bundles was more similar to the GCE group than to the GAR group. However, the irregular shape, the presence of centrally positioned nuclei and fibers in a degenerative process were still perceived in several fibers (Figure 1D)

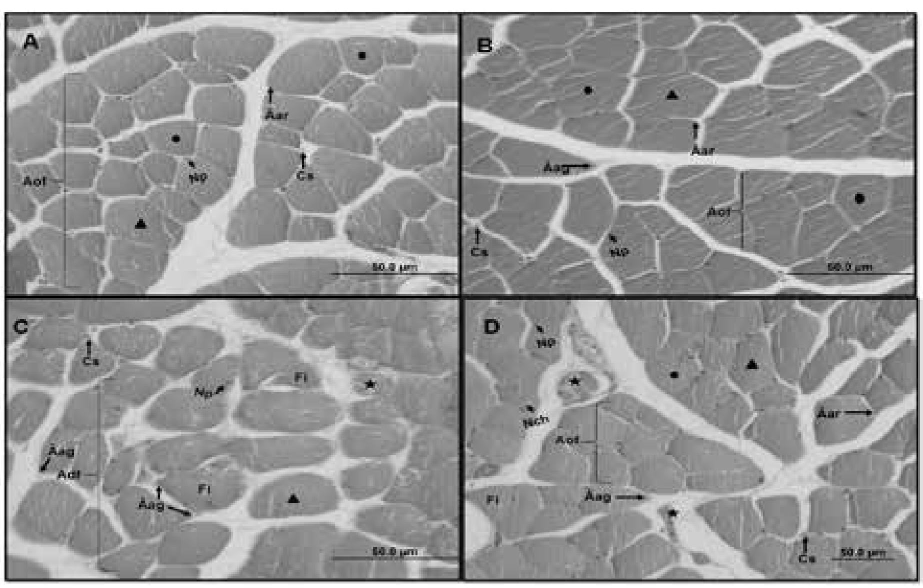

FIGURE 1. Photomicrographs of cross sections of EDL muscle of Wistar rats, 400x, with hematoxylin and eosin staining. Control group (**A**), polygonal fibers (black dot) with rounded angles (Âar), peripheral myonuclei (Np), blood capillaries in the conjunctiva (Cs) and organized fascicular arrangement (Aof). Exercise control group (**B**), hypertrophic muscle fibers and other characteristics preserved. Arthritis group (**C**), disorganized fascicular arrangement (Adf), fibers with irregular contours (Fi), acute angles (Âag) and degenerating cells (star). Arthritis exercise group (**D**), polygonal (dot), hypertrophic (triangle) and irregularly shaped (Fi) fibers, presence of central nuclei with halo (Nch) and cells in the process of degeneration (star), blood capillaries (Cs) and reorganization in the muscular fascicular arrangement (Aof).

DISCUSSION

In addition to the inflammatory process of RA damaging the joints and strongly contributing to the functional limitations of patients, several deleterious effects resulting from chronic systemic inflammation have been acknowledged in skeletal muscle tissue.[7,19] However, the literature provides scanty information on possible histomorphological changes in the EDL of RA specimens. In current study, the authors analyzed the morphological and morphometric effects of RA on EDL and ERSE activity as a treatment for the repair of damaged fibers.

RA altered morphological and morphometric parameters of EDL muscle fibers, revealing that the model, through two applications of CFA containing *Mycobacterium butyricum* in Wistar rats, was efficient, as indicated in another experimental study of the disease.[22] Furthermore, the use of an animal model is justified by the fact that CFA triggers an autoimmune response mediated by T cells, similar to that in humans.[24]

Morphological changes in the fibers of ELD muscle were verified by significant reductions in the cross-sectional area (13.83%) and in the smallest diameter (16.29%). These results were similar to those obtained in a study on muscle architecture, in which the authors compared the cross-sectional area of the vastus lateralis muscle of 23 healthy individuals and of 23 RA patients. RA patients showed a 13.9% decrease in the variable.[21] Such reduction in the cross-sectional area of the fibers is characteristic of a process of muscle atrophy, which occurs in an orderly and regulated manner and which affects RA patients with loss of strength, resistance[6,7,11] and muscle mass,[6, 8,9] greatly impairing the mobility of these individuals.[6,9,7,11]

Further, results showed that in animals with RA, the number of centralized nuclei was higher. Their presence reveals the repair action of the muscle fiber and indicates that RA caused tissue damage. Studies have elucidated that the plastic capacity of muscle tissue, in response to inflammation and degeneration, resulting from a harmful process, depends, among others, on the functional role of satellite cells.[25,26] These myogenic cells remain in a quiescent state in the healthy muscle until a stimulus induces their chemotaxis, proliferation and differentiation in myoblasts.[20,25] Furthermore, there is evidence that by merging with the muscle fiber, the satellite cells become capable of promoting the repair of the injured segment or, still, they may form myotubules, differentiating and originating a distinct muscle fiber.[20,25]

Another factor that negatively affects the musculature of RA patients is the reduction of physical activities which had to be decreased due to the joint pain caused by the disease´s inflammatory process.[7,24] Sedentary lifestyle due to RA compromises muscle health, reduces flexibility, strength and muscle weight, and results in functional disability, triggering the onset of chronic and degenerative diseases.[7,9]

Physical activity in the resisted modality, with the use of adequate loads, improves the functional capacities of individuals, especially in people with a reduced muscle mass.[9] Another study has shown that the improvement of the functional scenario of individuals is linked to the process of muscle hypertrophy, since the contraction of skeletal muscle stimulates the release of neurotrophic factors which are crucial for tissue repair.[26] Developing an animal model of resistance training, similar to training in humans, is of utmost importance.[18] In order to enhance the muscle repair process, resulting from the changes promoted by RA, ERSE has been employed in current study as a treatment model for the disease, based on the similarity of its applicability in humans,[18,26,27] and the use of an overload for the development of muscle hypertrophy.[4,7,9,18,19,26]

Consequently, animals submitted to the fourteen days of treatment with ERSE had an overload of a weight of 100 g attached to the tail. The treatment resulted in an increase in the cross-sectional area and in the smallest diameter of EDL fibers, suggesting a process of muscle hypertrophy.[18,19] This factor is related to a greater activity of muscle protein synthesis and an increase in the amount of myofibrils and contractile filaments, essential for the production of strength during a maximum contraction, required in the practice of physical activity.[19]

Employing the same protocol for climbing exercises in muscle assessment, researchers indicated that the practice of physical activity is essential, both in the acute phase and in the chronic phase of inflammation, emphasizing that the immediate onset helps to prevent the disease´s progress.[19] In another study,[4] ERSE had positive effects in increasing the nociception threshold in the acute phase and also in the chronic inflammatory period of the disease.

The results of this study indicated that the effects of ERSE treatment on muscle physiology were fundamental for the recovery of the morphological aspects of the EDL muscle of animals submitted to RA and later exercised. The regenerative processes of the muscle fibers of animals trained with ERSE have also been shown in other studies[18,19,27] by comparison with the arthritis group, without exercises.

For health promotion, the relevant regenerative action that ERSE performed on the morphology of the EDL muscle should be underscored, corroborating other studies[1,4,5,7,9,19,28] that indicated the exercise program, properly applied, as an effective therapeutic alternative to gain strength and restore its functional capacity. In summary, ERSE may become a strong ally to health promotion because health and quality of life are closely related and are directly proportional themes. Health promotion enables life quality. Current study provides relevant information on RA effects on the morphology and morphometry of fast glycolytic muscles, such as EDL, and reinforces other data that point out ERSE as a determinant in maintaining the individual's physical function.[28] An other topic in current research is the proposal to use a non-drug treatment protocol, easily accessible and applicable, in addition to being inexpensive. These factors make ERSE a promising therapeutic intervention tool, being of great relevance to the public health system, since its viability as a treatment may have direct effects on health, reduce pain and increase muscle function and life quality to people with RA. It also implies in significant cost reductions in the treatment of RA patients.

Current study features some limitations related to the time of the experiment. Or rather, a longer period would reveal more consistent data on structural recovery of the EDL muscle in ERSE treatment. Experimental designs with the use of markers for satellite cells would allow a direct analysis of the cellular function in muscle regeneration. Similarly, in the use of inflammatory markers, for example, pro-inflammatory cytokines, associated with physical activity.

Current paper intends to encourage future in-depth studies on line of research or bring new perspectives to the theme. It is understood that new investigations covering human studies and analyzing other variables, such as metabolic, physiological and psychological variables, as well as the use of evaluative instruments to measure the effectiveness of this protocol in restoring range of motion and the effect of the intervention on the life quality of animals submitted to RA and treated with ERSE, may be highly promising for the health system and for the academic and scientific community.

CONCLUSION

CFA-induced RA altered morphological and morphometric parameters of the EDL muscle, producing a picture of muscle atrophy. The deleterious effects of RA on muscle tissues are not yet fully elucidated, and the search for treatments that may modulate the inflammatory profile and promote tissue remodeling is pertinent. The proposed treatment protocol, using ERSE, restored the histomorphological structural patterns of the fibers affected by the disease, a fact reported when the parameters of the arthritic groups, submitted to exercise, were compared to those of the control groups.

PRACTICAL APPLICATIONS

Functional disability due to RA not only promotes damage to the physical and mental health of patients, but it also has a profound impact on their life quality, since it interferes in their daily activities by limiting autonomy. Simple actions such as eating, bathing and dressing, once performed without the dependence of others, tend to be outsourced with the progression of immobility resulting from the pathology, generating discomfort and frustration to the patient. The referred "losses" may be mitigated when the practice of physical activity is introduced in the routine of the RA-affected person.

One may observe that an adequate physical exercise protocol is an important ally in maintaining the functionality and mobility of patients, allowing patients´ autonomy in certain activities, whereas ERSE promotes the improvement of the structural and functional properties of the musculoskeletal system. Moreover, regular physical activity prevents RA patients from developing other comorbidities related to immobility, thus promoting an improvement in health.[5,9,27]

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