

Original Article

Individualized moderate aerobic exercise improves physical capacity and prevents weight loss in collagen-induced arthritis

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Abstract: Patients with rheumatoid arthritis (RA) suffer from joint pain, decreased physical capacity, muscle wasting, and fatigue. Physical exercise can improve these features, but the most appropriate training program is unclear. The objective of our study is to evaluate physical endurance and weight changes in collagen-induced arthritis (CIA) mice subjected to an individualized moderate aerobic exercise protocol on an incline treadmill. Male DBA1/J mice were randomly divided into 3 groups: nonarthritic control with exercise (CO-EXE, n=4), CIA with exercise (CIA-EXE, n=5), and CIA without exercise (CIA, n=4). Endurance exercise performance testing was performed in all groups prior to booster injections every 15 days after protocol initiation. CO-EXE and CIA-EXE were made to train on an incline treadmill ($\theta=5^\circ$), 60 minutes/day, 5 days/week for 6 weeks, at 60% of their own maximum velocity that induced exhaustion. The variables of interest were disease score, change in body weight (g), and maximum velocity (m/min) as a measure of exercise performance. Statistical significance was accepted at $P<0.05$. Clinical arthritis scores did not differ between CIA-EXE and CIA. Body weight was significantly different at week 6 when comparing CIA (0.9 ± 0.7 g) vs. CO-EXE (3.9 ± 0.4 g) and CIA-EXE (2.6 ± 1.6 g). Maximum velocity was significantly different at 4 weeks (CIA: 21 ± 3 m/min; CIA-EXE: 28 ± 4 m/min; CO-EXE: 35 ± 2 m/min) and 6 weeks (CIA: 21 ± 5 m/min; CIA-EXE: 28 ± 4 m/min; CO-EXE: 35 ± 2 m/min), demonstrating that CO-EXE and CIA had the highest and the lowest maximum velocity, respectively. Individualized moderate aerobic exercise on an incline treadmill appears to be an interesting intervention to treat decreased physical endurance without altered disease score in RA. This intervention had a positive impact on exercise endurance in CIA animals, although more limited than in non-arthritic controls.

Keywords: Arthritis, muscle, exercise, experimental, training, rheumatology

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by chronic, symmetric, and erosive synovitis [1, 2]. The clinical manifestations of RA include pain, stiffness, fatigue, acute joint swelling, joint deformity, weakness, muscle wasting, deconditioning, and a decrease in physical capacity [3]. Decreased physical capacity increases the risk of falls and fall-related injuries [4]. It is also associated with muscle wasting and even weight loss, which leads to a decrease in physical activities, creating a negative feedback effect on physical capacity and quality of life [5].

Weight loss also has important repercussions on musculoskeletal structure, leading to decreased physical capacity, and is a predictor of higher risk of death in RA [6]. These alterations are associated with reduced quality of life and are determinants of morbidity and mortality [7]. Exercise training is an effective tool against decrease of physical capacity and muscle wasting. In one study, a combination of strength and endurance training resulted in considerable improvements in RA patients' muscle strength and cardiorespiratory endurance, accompanied by positive changes in body composition and functional ability [8]. Other authors maintain that strength training has proved safe and effective in restoring lean mass and function in

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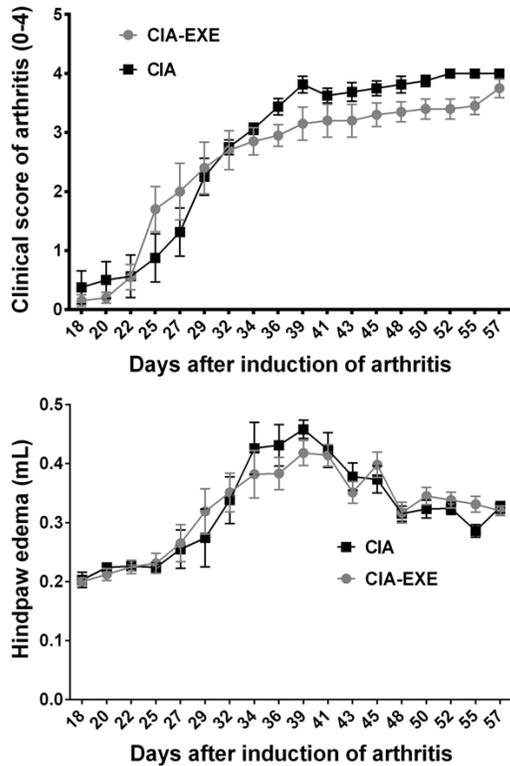


Figure 1. Clinical score and hindpaw edema of mice from CIA-EXE group and CIA group during the experimental period.

patients with RA, and that endurance training appears to be the most efficacious training mode for maintaining and improving maximum aerobic power in the elderly and should be viewed as a complement to resistance training [9, 10]. However, the effects of aerobic exercise on an incline treadmill on weight loss, physical capacity, and, consequently, quality of life in arthritis are still unclear.

A study by our group with a mouse model of collagen-induced arthritis (CIA) showed weight loss and a decrease in spontaneous locomotion during the experiment period in CIA mice compared to control animals, demonstrating the relationship between weight loss and locomotion [11]. Furthermore, when exposed to aerobic exercise, these animals exhibited less cartilage erosion in the joints and an increase in muscle fiber area [12]. CIA models are widely used because of their similarities to human arthritis and the ease of control for confounding variables, and may be very valuable for studying moderate aerobic exercise on an

incline treadmill in RA [13]. Additionally, animal models of arthritis provide the advantage of easy collection of tissues. Within this context of incomplete understanding and given the clinical importance of better management of weight loss and impaired physical capacity in patients with RA, the aim of this study was to evaluate physical endurance and weight changes in CIA mice subjected to individualized moderate aerobic exercise on an incline treadmill.

Materials and methods

Animals

Male DBA/1J mice aged 8 to 12 weeks from the DBA mouse colony of the Hospital de Clínicas de Porto Alegre (HCPA) Animal Experimentation Unit were used. The mice were reared at 20°C, under a 12-h light-dark cycle, with free access to food and water. The animals were numbered and random group allocation was performed online, using the GraphPad QuickCalcs website (<http://www.graphpad.com/quickcalcs/>). Therefore, the animals were divided into three experimental groups: (i) non-arthritic control animals subjected to physical exercise (CO-EXE, n=4), (ii) animals with CIA subjected to physical exercise (CIA-EXE, n=5), and (iii) non-exercised animals with CIA (CIA, n=4). Arthritis was induced with bovine type II collagen (CII, Chondrex, Inc.; 2 mg/ml) dissolved in 0.1 M acetic acid at 4°C for 12 h and complete Freund's adjuvant (CFA; Sigma, St. Louis, MO, USA; 2 mg/ml) containing inactivated *Mycobacterium tuberculosis*. On day 0, 50 µL of emulsion (CII+CFA) was injected intradermally at the base of the tail to induce arthritis. On day 18, the animals received a booster injection of CII emulsified with incomplete Freund's adjuvant (IFA, without *Mycobacterium tuberculosis*) in another tail site [11]. During the procedures, mice were anesthetized with inhaled isoflurane (Abbott Lab., Abbott Park, IL, USA). Six weeks after the booster injection, with six weeks of physical exercise, the animals were anesthetized by isoflurane inhalation and killed by cervical dislocation. The gastrocnemius muscle was dissected and weighed. All experiments were performed according to the Guiding Principles for Research Involving Animals (NAS) and approved by the HCPA Research Ethics Committee.

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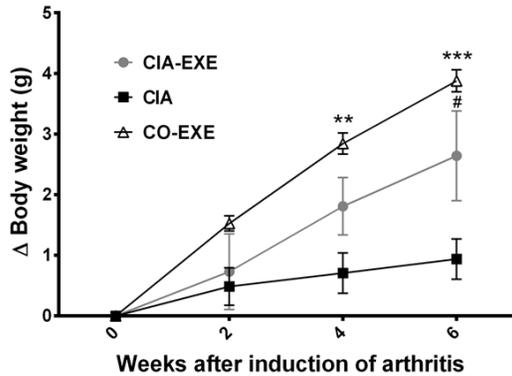


Figure 2. Body weights of mice from CIA-EXE group, CIA group and CO-EXE group during the exercise experimental period. Statistical significance for $P < 0.05$. #CIA-EXE vs CIA. *CO-EXE vs CIA.

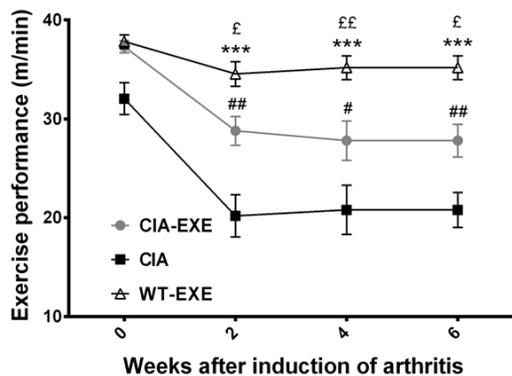


Figure 3. Running time of mice from CIA-EXE group, CIA group and CO-EXE group during the experimental period. Statistical significance for $P < 0.05$. *CIA-EXE vs CO-EXE. #CIA-EXE vs CIA.

Measures

Clinical severity and measurement of edema: Arthritis severity was determined clinically for each paw, three times a week, on a scale of 0 to 4 as follows: 0, no evidence of erythema or swelling; 1, mild erythema and swelling confined to the tarsals or metatarsals; 2, moderate erythema and swelling of the tarsal and metatarsal or tarsal and ankle joints; 3, severe erythema and swelling extending from the ankle to the metatarsal joints; and 4, severe erythema and swelling encompassing the ankle, foot, and digits, or ankylosis of the limb [14]. Hindpaw edema was measured prior to the booster injection and three times a week thereafter using a plethysmometer (Insight Ltda).

Animal weight: Body weights were measured weekly starting from the time of booster injection.

Weight values were subtracted from the body weight of each animal prior to inductive analysis to assess the change in weight (delta).

Exercise performance testing: All exercise performance tests and aerobic training were performed at the start of the dark cycle, when animals are most active. The exercise performance test consists of leading mice into exhaustion. The mice were set to run on an incline treadmill (slope, 5°) developed by HCPA. One week before the booster injection, mice were placed on the treadmill for 10 min/day, for 5 days, at a speed of 10 m/min for adaptation. One day before the booster, the animals were subjected to the first test and tested every two weeks thereafter until the experiment had finished. Prior to each exercise performance test, mice were made to stay still for 5 minutes on the treadmill, for adaptation. The test was then started at a speed of 8.5 m/min for 9 minutes. Speed was then increased by 2.5 m/min every 3 minutes. When exhaustion was reached and mice were no longer able to run (defined as staying near the rear of the treadmill for more than 10 seconds), the animals were returned to their cages. The velocity, total time, and distance traveled at the time of exhaustion were considered the maximum velocity, running time, and distance traveled.

Aerobic training: In this protocol, mice were first made to stay still for 5 minutes on the treadmill for adaptation before aerobic exercise training. The aerobic training protocol consisted of 60 minutes/day of exercise: a 10-minute warm-up at 10 m/min, running at 60% of the exhaustion velocity for 45 minutes, and a 5-minute cooldown at 10 m/min. This was applied 5 days/week for six weeks, starting on the day of booster injection.

Euthanasia

Six weeks after the booster injection, i.e., after six weeks of physical exercise, the animals were anesthetized by isoflurane inhalation (Abbott) and killed by cervical dislocation. The gastrocnemius muscle was dissected for weighing.

Statistical analysis

Sample size was based on previous CIA and exercise research by our group (12). Quantitative data produced were described as mean \pm stan-

dard error of the mean (SEM). Comparison of single variables between groups was performed with one-way analysis of variance (ANOVA) followed by Tukey's test, whereas comparisons for two variables were performed by two-way ANOVA followed by Bonferroni's test. All statistical tests were performed in the GraphPad Prism v. 6 software environment. Statistical significance was accepted at $P \leq 0.05$.

Results

Clinical severity and measurement of edema

Clinical arthritis scores and hindpaw edema were not statistically different between the CIA-EXE and CIA groups during the experimental period (**Figure 1**). Clinical parameters indicate that the exercise protocol did not worsen arthritis.

Body weight

Animal body weight was higher in CO-EXE compared with CIA animals after 4 and 6 weeks of exercise ($P < 0.05$). At week 6 of exercise, CIA-EXE animals had higher body weight than CIA mice ($P < 0.05$) (**Figure 2**). This weight gain observed in mice from the CIA-EXE group demonstrates the beneficial effects of exercise on this parameter despite development of arthritis.

Exercise performance testing

Exercise performance at 4 and 6 weeks was significantly different across all experimental groups and all variables; CO-EXE and CIA animals had, respectively, the highest and the lowest running time ($P < 0.05$) (**Figure 3**), maximum velocity, and distance traveled (**Table 1**).

Muscle weight

At the end of the experimental period, gastrocnemius muscle weight was significantly higher in the CO-EXE group than in the CIA-EXE and CIA groups (**Figure 4**). CIA-EXE muscles were heavier than CIA muscles, but this difference did not reach statistical significance. Nevertheless, CIA-EXE mice exhibited slightly greater muscle weight than CIA mice.

Discussion

To our knowledge, this was the first study to examine the effects of individualized, controlled, moderate aerobic exercise on an incline tread-

mill in collagen-induced arthritis. Our results demonstrated that the tested exercise program was able to improve physical capacity and reduce weight loss in mice. Thus, an aerobic training protocol at 60% intensity appears to be an interesting intervention to manage decreased physical capacity in RA.

Previous studies have shown that patients with RA can tolerate physical training as an intervention that has no detrimental effects on joint as long as inflammation is medically controlled [15]. However, long-term, high-intensity weight-bearing exercises may induce additional large-joint damage in patients with preexisting extensive damage [16]. CIA models enable the investigation of the effects of exercise on the natural history of the disease, as human patients usually receive antirheumatic medication [17]. We showed that exercise training did not change clinical parameters of experimental arthritis, such as clinical score and hindpaw edema, even though the animals had not been under drug treatment. These data confirm the safety of moderate treadmill exercise training, and suggest that moderate exercise does not increase cartilage degradation and can in fact be used as adjuvant therapy for RA.

Another important feature that could be improved by exercise is body weight. A previous study from our group showed that healthy mice gain weight during the experimental period, while mice with CIA do not exhibit this weight gain, and, in some points of the experiment, actually weigh less than at baseline [11]. However, physical exercise is believed to be an effective countermeasure against muscle wasting [12]. In our study, despite exercise, animals from the CIA-EXE group weighed less than animals from the CO-EXE group throughout the experimental period, perhaps due to chronic inflammation. Nevertheless, comparison of CIA-EXE vs. CIA animals showed the lowest weights throughout the experimental period in the CIA group, demonstrating that individualized moderate aerobic exercise controlled by maximum velocity may be useful against weight loss in mice with CIA. In addition, in a pilot experiment by our group, daily food intake did not differ between healthy controls and CIA animals [11].

Analyses of lean weight in RA have reported increased muscle protein catabolism leading to

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Table 1. Fatigue velocity and distance traveled of mice from CIA-EXE group, CIA group and CO-EXE group during the experimental period

	At booster of arthritis			2 weeks after booster of arthritis			4 weeks after booster of arthritis			6 weeks after booster of arthritis		
	CO-EXE (N=4)	CIA-EXE (N=5)	CIA (N=4)	CO-EXE (N=4)	CIA-EXE (N=5)	CIA (N=4)	CO-EXE (N=4)	CIA-EXE (N=5)	CIA (N=4)	CO-EXE (N=4)	CIA-EXE (N=5)	CIA (N=4)
Maximum velocity (m/min)	35.6±1.6	35.3±1.9	30.2±3.6	33.5±1.6	27.7±4.0	18.5±4.8	34.0±1.6	25.7±4.2	19.7±5.2	33.3±2.1	26.3±3.8	19.2±4.0
Distance traveled (m)	837.5±68.2	825.1±83.3	621.4±138.8	747.4±67.0	532.3±141.8	265.6±120.5	769.4±69.5	463.6±130.9	297.1±146.4	739.4±85.5	484.3±126.6	277.6±105.7

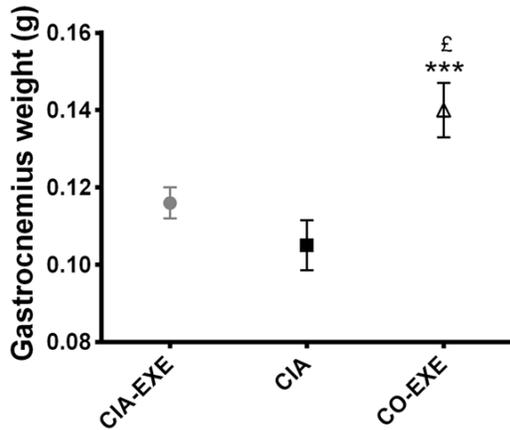


Figure 4. Gastrocnemius muscle weight of mice from CIA-EXE group, CIA group and CO-EXE group after death. Statistical significance for $P < 0.05$. *CIA-EXE vs CO-EXE.

loss of skeletal muscle mass, and, consequently, loss of muscle strength and power [18, 19]. Thus, we also evaluated the ability of our exercise program to improve muscle mass. Our results showed increased gastrocnemius muscle mass in CO-EXE mice only. Therefore, it is feasible that exercise training has not been able to slow loss of muscle mass induced by chronic inflammation in mice with CIA, whereas mice from the CIA-EXE group did not lose as much weight, and, consequently did not lose as much lean mass. This suggests that adding a treatment capable of controlling inflammation may facilitate muscle gain and bring further body weight benefits.

Regarding physical capacity in RA, patients report a vicious circle characterized by fatigue, reduced physical activity, loss of aerobic capacity, and increasing physical deterioration [20-22]. It is widely accepted in the literature that exercise training can prevent this physical detriment to RA patients [20-22]. In animal experimentation, as has been shown in healthy mice, the chronic effect of treadmill training results in an increased maximal oxygen uptake (VO_{2max}), which allows mice to run at higher speeds [23]. In our study, we found improvements in exercise performance after 4 and 6 weeks of training on comparison between the CIA-EXE and CIA groups, which demonstrates that exercise helps enhance running endurance in CIA mice despite chronic disease. All parameters of exercise performance testing (time, distance, and velocity) remained practically stable during the

experimental period in mice from CO-EXE group. On the other hand, the same parameters decreased in the second week and increased slightly in the third and fourth weeks in mice from the CIA-EXE and CIA groups. This finding may be related to inflammation, which is more intense around the second week of disease and can impair mobility. From the third week of disease, inflammation becomes chronic, reducing its influence on animal locomotion.

Our findings related to physical capacity may be explained by the novelty of the training protocol, in which running endurance was evaluated every two weeks to adjust the intensity of the exercise for each individual. Thus, training was truly individualized, taking into account the changes in physical capacity arising from the practice of exercise. Additionally, disease activity at the time of exercise performance testing also helped ensure that training was not excessive and, hence, damaging to the subject. Furthermore, our aerobic training protocol was applied 5 days/week on an incline treadmill. Although previous authors reported that wheel running induces improved exercise capacity more effectively, this was always assessed in healthy animals [24]. As inactivity is well documented in patients with RA and in animal models of arthritis, forced treadmill running was the best option for our study [11, 20].

Finally, physical capacity appears to be a reliable outcome measure in the clinical management of RA, especially considering exercise prescription. Thus, animal models of arthritis are important tools for the establishment of the best exercise protocol to achieve physical improvements. Accordingly, inclined controlled aerobic exercise appears to be an interesting intervention to prevent decreased physical capacity and weight loss in RA. Further studies, particularly molecular analyses, are needed to better understand the role of exercise in arthritis development. Future research could also add drug treatments to the protocol so as to better resemble clinical practice in humans and, perhaps, provide added benefit regarding muscle involvement.

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Disclosure of conflict of interest

None.

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