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Decrease in oxidative stress and histological changes induced by physical exercise calibrated in rats with osteoarthritis induced by monosodium iodoacetate

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SUMMARY

Objective: The purpose of this study was to evaluate the effects of impact exercise on the joint cartilage of rats with osteoarthritis (OA) induced by monosodium iodoacetate (MIA).
Methods: Eighteen male rats were divided into three groups of six animals each: control, OA, and OA plus exercise (OAE). The OAE group trained on a treadmill for 8 weeks. Afterward, the right joints of the animals were washed with saline solution and joint lavage was used for biochemical analyses of myeloperoxidase (MPO) and enzyme superoxide dismutase (SOD) activities and total thiol content. The same limb provided samples of the articular capsule for analyses of MPO activity and total thiol content. The left joint was used for histological analysis.
Results: Our results indicate that MPO activity was increased in both OA groups in the lavage as well as the articular capsule, regardless of exercise status. SOD activity was increased in animals with OA, especially in the animals that had run on the treadmill. On the other hand, thiol content in the articular capsule and joint lavage decreased in the OA group, while the OAE group had values similar to those of the control group. The histological data indicate that animals that were submitted to running exercise showed a higher preservation rate of proteoglycan content in the superficial and intermediate areas of the joint cartilage.
Conclusion: Our results show that physical training contributes to the preservation of joint cartilage in animals with OA and to increase the defense mechanism against oxidative stress.

Introduction

Osteoarthritis (OA) is a degenerative disease characterized by the progressive degradation of joint cartilage and is accompanied by secondary inflammation of synovial membranes[1]. With synovial fluid, joint cartilage provides an almost frictionless articulation, enabling painless joint movement. The extracellular matrix of joint cartilage provides this tissue with great strength, resistance to deformation, and the ability to dissipate loads and handle the forces generated within the joint[2].

The pathophysiology of OA is thought to be multifactorial, with interplay between both systemic and local biomechanical factors. Among the systemic risk factors are ethnicity, age, gender and hormonal status, genetic factors, bone density, and nutritional factors. Local biomechanical factors include obesity, altered joint biomechanics (including ligamentous laxity, malalignment, impaired proprioception, and muscle weakness), prior joint injuries, occupational factors, and the effects of sports and physical activities[3]. Therefore, the combination of molecular damage and the inability to effectively manage physical forces leads to OA[2].

The symptoms of OA are often associated with significant functional impairment, as well as signs and symptoms of inflammation, including pain, stiffness and loss of mobility[4].

The articular cartilage is considered a hypoxic tissue, once it is an avascular tissue[2]. However, the implications of this hypoxic environment are poorly understood at the molecular level. A topic of great interest is the role of changes in oxygen (O_2) levels during cartilage degeneration process. Elevated production of reactive oxygen species (ROS) and/or depletion of antioxidants have been observed in a variety of pathological conditions, including inflammatory joint diseases[5].

It is possible though that those reactive species are involved in the physiopathological process of OA. Nevertheless, the systemic implications on OA of ROS production are still unknown and require investigation.

In agreement with the Brazilian consensus for the treatment of OA, treatment should be multidisciplinary and seek functional,

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mechanical and clinical improvement[6]. The treatment should be either pharmacological, using analgesic and anti-inflammatory drugs, or nonpharmacological, using physiotherapy and invigorating and stretching exercises in addition to aerobic exercises of low and medium intensity.

Historically, physical exercise has been believed to increase the risk of knee OA, because the disease was labeled as a "wear and tear" arthritis. However, regular physical exercise is now recognized as a safe, multifaceted therapeutic treatment to improve many of the factors that lead to disability in patients with knee OA[7] and may even be beneficial to the patient[8]. According to Sutton and colleagues[9], high levels of exercise increase the risk of OA, but moderate levels can be beneficial, reducing it.

Exercises of low and medium impact exert elastic and compressive forces on the joint cartilage, similar to those observed in studies including cartilage explants[10–15] and *in vitro* systems such as agarose[16–18], alginate[12] and other polymeric scaffolds[19]. At low magnitudes, tensile forces act as potent anti-inflammatory signals and inhibit interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and lipopolysaccharide-induced proinflammatory gene transcription[20–22].

Thus, the purpose of this study was to evaluate the effects of a physical-training program on histological parameters and oxidative stress markers in the cartilage of rats with OA induced by monosodium iodoacetate (MIA).

Materials and methods

Sample

Eighteen 4-month-old male Wistar rats (mean = 350 g) were used and cared for according to the European Communities Council Directive of November 24, 1986. Food (Nuvilab CR1, Nuvital Nutrientes S/A, Brazil) and water were available *ad libitum*. The room was kept at 70% humidity and 20 ± 2°C on a 12-h light/dark cycle with the lights on at 06.00 a.m. The rats were checked periodically to verify their pathogen-free condition. The animals were randomly divided into three groups ($n = 6$): control (C), OA and OA plus exercise (OAE).

OA exposure

The animals were anesthetized with ketamine (90 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.); OA was induced by direct infiltration of both knee joints with a 27-gauge needle and a 2 mL syringe containing 1.2 mg of iodoacetate diluted in 50 μL saline solution, according to Guzman[23].

Training protocol

Twenty-four hours after OA induction, all groups were habituated on a motor-driven treadmill at a speed of 10 m min^{-1} for 10 min/day for 1 week to reduce their stress regarding the new environment. The rats did not receive any stimuli to run. After the adaptation period, a program of moderate physical training was undertaken. The trained groups were submitted to an 8-week training program on a treadmill (1% incline) for 3 days/week, 50 min/day, for a total of 60 days. The velocity of the treadmill was 13 m min^{-1} (Table I). According Leandro *et al.*[24], the velocity of training corresponded to approximately 60–70% VO_2max. The untrained animals were placed on the treadmill, which was not turned on, during the same 8 weeks as the exercise-trained groups.

Table I
Training protocol for rats

Week	Frequency	Speed (m min^{-1})	Inclination (%)	Duration (min)
Adaptation	1–3	10	1	10
1	1–3	16	1	30
2	1–3	16	1	30
3	1–3	16	1	30
4	1–3	16	1	30
5	1–3	16	1	50
6	1–3	16	1	50
7	1–3	16	1	50
8	1–3	16	1	50

Intra-articular lavage (IAL)

IAL of the right knee joints was performed with 2 × 50 μL of 0.9% NaCl under anesthesia (90 mg/kg ketamine and 20 mg/kg xylazine, i.p.). Approximately 70 μL IAL fluid (IALF) was recovered from each rat. The IALF was immediately diluted in 0.5 mL of distilled water and centrifuged at 300×*g* for 10 min. The IALF supernatants were stored at −70°C for later analysis. Immediately after recovering IAL, the animals were killed by decapitation, the right knee was shaved, and an incision below the patella was made to expose the joint. After washing the site with saline solution, approximately 40 mg of the articular capsule was removed. The sample was homogenized in phosphate buffer and frozen at −70°C for further analysis.

Histology

The soft parts and patella of the left joint of each animal were removed to facilitate fixation of the joint. The tissue was stored in 4% neutral-buffered formalin for 24 h. Afterward, they were kept in a running-water bath for 2 h to remove excess fixative. They were then placed in 10% Ethylenediaminetetraacetic acid (EDTA), pH 7.4, for decalcification. The EDTA was changed on a 48 h/48 h/72 h cycle for approximately 10 weeks until the bone was satisfactorily decalcified. Tissue blocks were placed in formalin, dehydrated in a graded series of ethanol and xylol, embedded in paraffin, cut into 6 mm-thick serial sections, and stained with hematoxylin–eosin (H&E) for analyses of the cartilage joint, subchondral bone and disk epiphyses. The femur and tibia were evaluated separately with respect to their histological appearances. For both tissues, the sections were divided into three areas of analysis, according to the illustration shown in Fig. 1. Proteoglycan (PG) preservation in the cartilage matrix was evaluated using a metachromasia scale, as

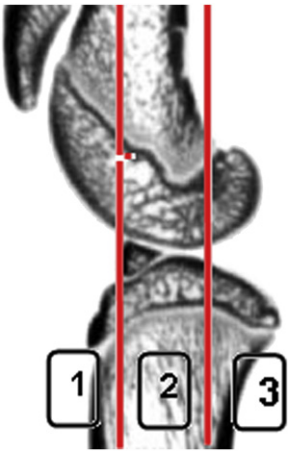

Fig. 1. Illustration showing the three areas of reference used for the histomorphological analysis. Anterior area (1) intermediate area (2) the posterior area (3) The patella is indicated by the arrow.

shown by toluidine blue staining. Thus, we consider (+) to be mild, (++) to be moderate, and (+++) to be intense metachromasia.

A semi-quantitative assessment of structural changes occurring in the knee joint was done following OARSI OA Cartilage Histopathology Assessment System[25]. The articular surface and subjacent tissues of medial femoral condyle were assessed for both grade and stage in Safranin O-stained sections and the surfaces of the knee cartilages were more easily assessed for the scores. The half of the knee cartilage surface of two serial sections for each animal was examined in at least three animals for comparison of the combined score (grade × stage)[25] among groups.

Biochemical assays

Myeloperoxidase (MPO) activity

IALF (10 μL/mL) was homogenized in 0.5% hexadecyltrimethylammonium bromide and centrifuged at 5000×*g* for 5 min. The suspension was then sonicated three times for 30 s each time. An aliquot of supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM H_2O_2. Activity was measured spectrophotometrically as the change in absorbance at 650 nm and 37°C[26].

Superoxide dismutase (SOD) activity

SOD activity of the articular capsule was determined according to the method described by McCord and Fridovich[27]. Enzymatic activity was assayed by adrenaline autoxidation inhibition, which was read at 480 nm on a spectrophotometer. Enzyme activity was expressed as U/mg protein. SOD of the joint lavage was not performed due to a lack of appropriate material.

Total thiol content

Total thiol content was determined using the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) method (Sigma). The reaction was started by the addition of 30 μL of 10 mM DTNB stock solution in phosphate buffered saline (PBS). Control samples did not include DTNB. After 30 min of incubation at room temperature, the absorbance at 412 nm was measured, and the amounts of TNB formed were calculated (equivalent to the amount of SH groups) using the Aksenov technique[28].

Protein content

Protein content from both IALF and articular capsule homogenates were assayed using bovine serum albumin as a standard, according to Lowry and collegues[29]. When the folin-phenol reagent (phosphomolybdic-phosphotungstic reagent) was added, the folin-phenol reagent bound to the protein. The bound reagent was slowly reduced and changed in color from yellow to blue. The absorbance was read at 750 nm.

Statistical analyses

Data are presented as mean ± 95% confidence intervals (CIs). Multiple comparisons among parametric data were performed using one-way ANOVA with Tukey's *post-hoc* tests. Kruskall–Wallis test followed by Dunns *post-hoc* test were employed to test for differences in the grade, stage and score of the OARSI OA Cartilage Histopathology Assessment System evaluation (median–range). A P value < 0.05 was considered to be significant. Statistical Package for the Social Sciences (SPSS), version 16.0 for Microsoft Windows was used for data analysis.

Reagents and spectrophotometer

EDTA, hexadecyltrimethylammonium bromide, adrenaline, 5,5′-dithiobis (2-nitrobenzoic acid), bovine serum albumin, and phosphomolybdic-phosphotungstic reagent were purchased from Sigma Chemical (St. Louis, MO, USA). The spectrophotometer used was an *Amersham* Ultrospec 1100 Pro UV/Vis, purchased from Amersham Biosciences of Brazil Ltda, São Paulo, Brazil.

Results

Histology

The histology of the joint cartilage in both the femur and shinbone of the control group is intact, with no anomalies and a homogeneous matrix [Fig. 2(A)]. However, in the OA group, the anterior surface of the joint cartilage of the shinbone shows groups of isogenic chondrocytes embedded in a homogeneous and slightly basophilic matrix. Also, in the back the cartilage matrix is more heavily stained and shows greater numbers of chondrocytes. In the anterior and posterior areas of the subchondral bone, there are small infiltration foci of loose connective tissue that are rich in fibroblasts and microvessels. This pattern is maintained in the intermediate area, where there is no cartilage, only bone tissue. The joint cartilage of the femur presents outbreaks of fibroblastoid infiltrate, mainly in the intermediate area [Fig. 2(B)], whereas the subchondral bone presents more discreet outbreaks. In the OAE group, the joint cartilage of the shinbone shows no alterations in its thickness and some infiltration foci of fibroblastoid cells [Fig. 2(C)] throughout its length. In some areas of connective tissue infiltration, we find osteoclasts. In the back, we can see the reactive proliferation of cartilage cells, evidenced by the increased numbers of focal chondrocytes. The subchondral bone did not show any changes. The surface of the joint cartilage at the back of the femur presents an irregular, thickened appearance, with a smaller number of chondrocytes, gaps and cracks, and small isogenic groups, thus taking on an aspect of fibrocartilage [Fig. 2(D)]. In the anterior area, the pattern is maintained but with reduced thickness and mixed with loose connective tissue. The subchondral bone does not show any changes. The metachromasia analysis was performed in the intermediate area of the joint cartilage of the femur, as shown in Table II. The control group shows intense metachromasia in the superficial and intermediate areas, whereas there was a mild metachromatic areas of deep and calcified cartilage. In the OA group, the superficial and intermediate areas show mild metachromasia, while there was intense metachromasia of deep areas with calcified cartilage. The OAE group presents moderate metachromasia in the superficial and intermediate areas, while the deeper, calcified area shows mild metachromasia.

OARSI system analysis of groups is shown in Table III. Control group has a mean score of zero, while OA group scored four and OAE group one and a half.

MPO activity and oxidative stress parameters

According Table IV, the MPO activity of the joint capsule was high in both groups with OA (control = 73.24 − 95% CI 63–95 mU/mg protein; OA = 210.59 − 95% CI 135–215 mU/mg protein; OAE = 133.21 − 95% CI 97–168 mU/mg protein, $P < 0.05$). Similarly, in joint lavage, MPO activity was increased in the groups with OA, irrespective of exercise (control = 3.61 − 95% CI 1.72–5.41 mU/mg protein; OA = 16.31 − 0.17–24 mU/mg protein; OAE = 11.95 − 3.15–20.8 mU/mg protein, $P < 0.05$). The SOD activity was increased in the joint capsules of animals with OA; exercise further exacerbated the antioxidant activity of SOD in this tissue (control = 3.02 − 95% CI

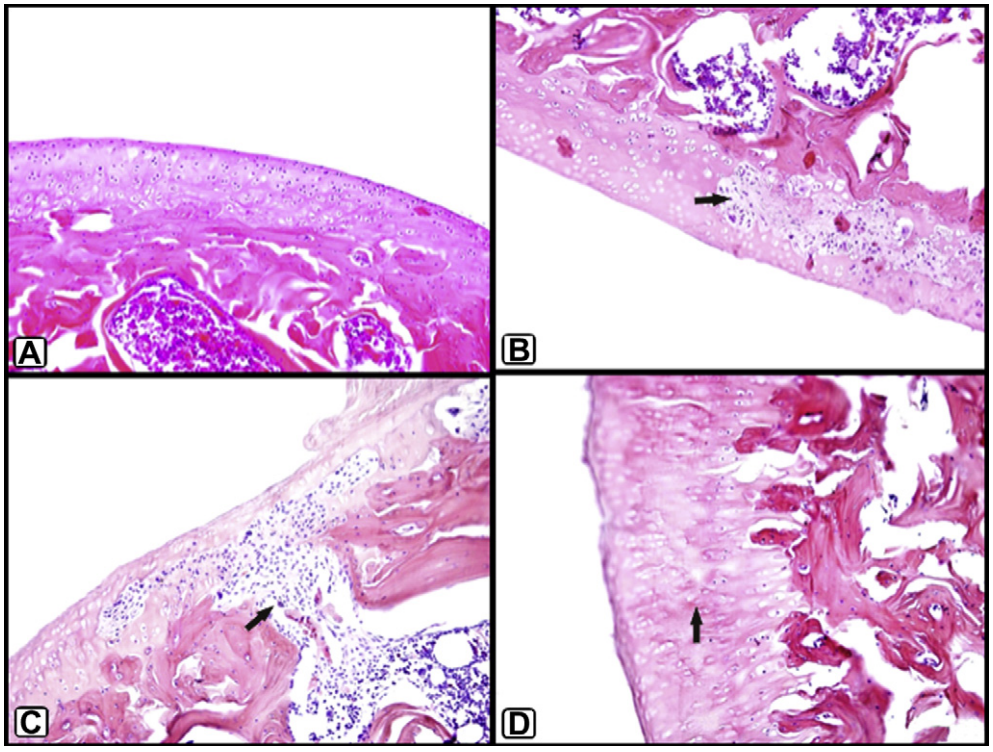

Fig. 2. Photomicrographs of histomorphological changes of joint cartilage stained by H&E. Normal appearance of joint cartilage (A). Invasion of fibroblastoid infiltrate in the cartilage (B) and the cartilage and subchondral bone (C). Chondrocytes forming strings with elongated collagen fibers in parallel bands giving it the appearance of fibrocartilage (D). Scale A–C = 100 mm, D = 50 mm.

1.88–4.15 nmol/mg protein; OA = 7.17 95% CI 4.41–10.58 nmol/mg protein; OAE = 13.90 – 95% CI 10.22–17.6 nmol/mg protein, $P < 0.05$). The total thiol content was significantly lower in the OA group compared to the control group. Interestingly, in the OAE group, total thiol content remained high in both the joint capsule (control = 44.21 – 95% CI 32.77–48.62 nmol DTNB/mg protein; OA = 13.89 – 95% CI 5.7–22 nmol DTNB/mg protein; OAE = 38.65 – 95% CI 34.37–42.92 nmol DTNB/mg protein, $P < 0.05$) and the joint lavage (control = 1.03 – 95% CI 0.7–1.35 nmol DTNB/mg protein; OA = 0.46 – 95% CI 0.26–0.62 nmol DTNB/mg protein; OAE = 0.97 – 95% CI 0.6–1.35 nmol DTNB/mg protein, $P < 0.05$).

Discussion

The present study is one of the few investigations that assess the effects of exercise on osteoarthritic joints in rats. Iodoacetate was used to induce OA, according with Guzman[23]. However, a possible limitation of this model is the fact that most animal models OA progresses rapidly, and it is likely that most or all models that have been studied have limited usefulness as tools for the study of human OA. Many caveats exist in using MIA models to correlate human OA with rat OA due to inherited variation, such as the biomechanical differences between two-legged and four-legged species. In addition, MIA models mimic the phenotype of OA, but their similarities to the underlying molecular components of human disease are typically unknown[30].

The histomorphological changes found in tissue slides show evidence of tissue damage that is characteristic of OA in groups where OA was induced by intra-articular administration of iodoacetate. The most significant changes were seen in the joint cartilage of the backs of both the femur and the shinbone, with increased basophilia, decreased chondrocytes, and fibroblastoid infiltrate, as

Table II
Metachromasia analyses of femur and tibia articular cartilages

Groups	Articular cartilage zones	Femur regions			Tibia regions		
		Anterior	Central	Posterior	Anterior	Central	Posterior
Control	Superficial	+++	+++	+++	+++	++	+++
	Middle	+++	+++	+++	++	++	+++
	Deep	+	+	++	++	+	++
	Calcified	+	+	+	+	+	++
OA 1, 2 mg	Superficial	+	+	+	++	+	++
	Middle	++	+	+	+	++	++
	Deep	+++	+++	+++	+++	+++	+++
	Calcified	+++	+++	+++	+++	+++	+++
OAE1, 2 mg	Superficial	++	++	+++	+++	+++	++
	Middle	++	++	++	+++	++	++
	Deep	++	+	++	++	+++	+++
	Calcified	++	+	+	++	+++	++

(+) Metachromasia mild; (++) metachromasia moderate; (+++) metachromasia intense. CTR = control.

Table III
Assessment of structural changes in the knee joint of OARSI OA Cartilage Histopathology Assessment System

Group	Slide (animal)	Grade	Stage	Score
Control	554	0	1	0
	556	0	1	0
	558	0	1	0
	560	0	1	0
Mean (range)		0.0 (0–0)	1.0 (1–1)	0.0 (0–0)
OA	562	3	1	3
	564	4	1	4
	566	2	2	4
Mean (range)		3.0 (2–4)*	1.3 (1–2)	3.7 (3–4)***
OAE	578	1	1	1
	582	2	1	2
	584	1	1	1
	586	2	1	2
Mean (range)		1.5 (1–2)*	1.0 (1–1)	1.5 (1–2)***

ANOVA followed by Kruskall–Wallis, significantly different, $^{*}P = 0.0115$ and $^{**}P = 0.0088$.

well as an evident change of hyaline cartilage to fibrous cartilage (Fig. 2). These changes are consistent with results of other OA studies[31–34].

The OA group showed a decrease in metachromasia in the surface and intermediate areas of the joint cartilage, indicating greater destruction of PGs in this area, similar to the study by Galois *et al.*[35]. However, more intense metachromasia appeared in deep and calcified areas of this tissue in an attempt to restructure the matrix of the unaffected cartilaginous tissue (Table II).

In the OAE group, the surface and intermediate areas were better preserved compared to the OA group, suggesting that exercise may have contributed to preserving the PGs that act as a mechanical shock absorbing system, especially in joint cartilage. This preservation was also observed in the study by Roos and Dahlberg[36], in which moderate exercise improved GAG content in patients at risk for knee OA. Although none of them had high scores (above 12) in OARSI system, when considering only grade (G1–G6), OAE group showed an average grade of 1.5, which is considered an early or mild OA in the OARSI system. In comparison, OA group presented an average grade of 3 (Table III).

It is possible that the joint cartilage depends on a mechanotransduction mechanism to maintain homeostasis. According to Knobloch[37], biomechanical signals provide the bridge between gross morphologic signals and molecular gene expression. The combination of fluid-flow-generated signals coupled to matrix mechanotransduction generates a complex series of signaling cascades and ultimately leads to a biomechanical signal-dependent transcriptional response[38]. Gene expression in chondrocytes under compression is dependent on the magnitude[10,15,38], frequency[17], and duration[12,13,15,16,38–40] of applied compressive forces.

Our exercise protocol was prepared with the purpose of evaluating the effects of interspersed sessions of aerobic activity with impact on overload joints, such as osteoarthritic knees of rats. The intensity of effort reached moderate levels, in agreement with Leandro[24]. It seems that intensity, frequency and duration of aerobic exercise modulates chondrocyte response in some way, favoring the preservation of PG content in osteoarthritic joints at this stage of the disease[15], as seen in our study.

Dynamic compression upregulated the expression of genes such as anabolic *ACAN*, *COL2α1*, and *TIMP3*, while downregulated specific genes of the matrix metalloproteinase (*MMP*) family[41]. Furthermore, cyclic tensile strain could augment cartilage repair by facilitating the induction of *ACAN* mRNA and attenuating IL-1β-induced suppression of PG synthesis. More importantly, compressive forces at low magnitudes have been shown to be anti-inflammatory. These observations suggested that the anti-inflammatory cascade that was initiated by the application of dynamic tensile forces persisted despite the cessation of biomechanical stimuli[37].

One of the pathways that are regulated in chondrocytes following mechanical stimulation is the signaling cascade involved in inflammatory responses[37]. The nuclear factor (NF)-κB signaling pathway was considered as a possible link between tensile loading and chondrocytic responses to proinflammatory cytokines[38]. Biomechanical signals are transduced to cells by surface molecules such as β-integrins and focal adhesion kinases/protein tyrosine 2 kinases[41]. At lower magnitudes, biomechanical signals inhibit the nuclear translocation of NF-κB transcription factors and act as potent inhibitors of IL-1β and TNF-α dependent proinflammatory gene transcription[42–44]. As mentioned by Knobloch[38], compressive, tensile, and shear forces of appropriate low/physiologic magnitudes also promote the upregulation of PGs and collagen synthesis, which are drastically inhibited in inflamed joints. This could explain the greater preservation of PG content, as observed by more intense metachromasia on the superficial and intermediate areas of the joint cartilage in exercised rats in the present study.

Measuring tissue MPO activity is an established method of quantifying the presence of activated leukocytes (primarily neutrophils) and inflammatory responses in damaged tissue[45–47]. Significant elevations of muscle and tissue MPO activity have been reported following ischemia-reperfusion injury[48] as well as exercise[49]. According to Morozov[50], even moderate-intensity exercise stimulates blood neutrophil degranulation, although to a lesser degree. Thus, our results indicate that the OA protocol used in our study was adequate for inducing higher MPO activity in serum (data not shown) and specific tissues such as joints. The levels of MPO in the exercise group may be associated with the response to training. However, we believe that this is not a significant cause of tissue damage. Moreover, the link between neutrophil proteins and exercise stress relates to three specific functions of these proteins. First, they can improve blood bactericidal potency. Second, they can activate granulopoiesis in bone marrow and move neutrophils that are rich in lysosome cationic proteins into the circulation. Finally, neutrophil cationic proteins may have a regulatory significance[50].

According to Pinho and colleagues[51], exercise promoted important changes in antioxidant enzyme activities, reducing oxidative damage and increasing tissue resistance against free radicals. We also found increased SOD activity in the joint capsule of rats with OA that underwent a chronic aerobic training program with joint impact articulate. This important finding must be

Table IV
MPO and SOD activities and total thiol content in IALF and joint capsule

Groups	MPO (mU/mg protein)		SOD (U/mg protein)	Thiol content (nmol DTNB/mg protein)	
	Capsule	IALF	Capsule	Capsule	IALF
Control	73.24 (63–95)	3.61 (1.72–5.41)	3.12 (1.88–4.15)	44.21 (32.77–48.62)	1.03 (0.70–1.35)
OA	210.59* (135–285)	16.31* (0.17–24)	7.17* (4.41–10.58)	13.89* (5.70–22.10)	0.46* (0.26–0.62)
OAE	133.21*,# (98–169)	11.95* (3.15–21)	13.9*,# (10.22–17.58)	38.65# (34.37–42.92)	0.97# (0.59–1.35)

Values are expressed as mean with 95% CIs. The significant difference in relation to control group (*) and in relation to OA group (#); $P < 0.05$.

highlighted, because it is a common clinical practice to deprive individuals with hip and knee OA from programs of physical training, especially those involving impact exercise. Our study points to a potential therapeutic alternative for people with OA, as proposed by several studies[52,53].

Even moderate exercise may increase ROS production to levels exceeding the capacity of antioxidant defences[54]. Specific sources of ROS during exercise include leakage of electrons from the mitochondrial electron transport chain, xanthine oxidase reaction, hemoglobin oxidation and activated neutrophils[55,56].

Some studies have shown increases in SOD, Glutathione peroxidase (GPx) and Catalase (CAT) activities after aerobic exercise training in young rats[57,58].

ROS may modulate antioxidant enzyme activities by regulating their mRNA levels through activation of signaling pathways[59]. In fact, ROS plays a very important role in regulating several cellular functions, acting as second messengers and activating specific redox-sensitive transcription factors such as AP-1 and NF-κB[60–62].

Higher total thiol content in the OAE group, both in the joint capsule and the joint lavage, serves as evidence that biomechanical signals generated during joint movement are essential components of the cells' and tissues' abilities to repair and recover following physiologic insults, as well as to maintain homeostasis[37]. Davies[63] comments that in nonexhaustive daily training sessions (typical of endurance exercise training), the generation of free radicals could be the actual stimulus for exercise adaptation and mitochondrial biogenesis. NF-κB can bind to promoter areas for several hours to days after acute exercise and activate the production of more than one protective enzyme, thereby playing a very important role in against oxidative and radical damage[64].

Our group reached the conclusion that aerobic exercise performed on a treadmill (impact activity) can promote histological and biochemical changes that benefit the joints of rats with OA. These data indicate that exercise, or more precisely, biomechanical signals converted into biochemical responses in joint cartilage with OA, can induce anabolic changes in tissues. This is one of the few studies to evaluate the effects of low-impact physical training on OA in rats.

Author contributions

All authors participated of study and made substantial contributions in the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval.

Conflict of interest

No conflicts of interests are associated with this manuscript.

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References

1. Shi Q, Vaillancourt F, Cote V, Fahmi H, Lavigne P, Afif H, *et al*. Alterations of metabolic activity in human osteoarthritic osteoblasts by lipid peroxidation end product 4-hydroxynonenal. Arthritis Res Ther 2006;8:1–14.
2. Poole AR. Can serum biomarker assays measure the progression of cartilage degeneration in osteoarthritis? Arthritis Rheum 2002;46:2549–52.
3. Garstang SV, Stitik TP. Osteoarthritis: epidemiology, risk factors, and pathophysiology. Am J Phys Med Rehabil 2006;85: S2–11.
4. Goldring SR, Goldring MB. Clinical aspects, pathology and pathophysiology of osteoarthritis. J Musculoskelet Neuronal Interact 2006;6:376–8.
5. Henrotin Y, Kurz B, Aigner T. Oxygen and reactive oxygen species in cartilage degradation: friends or foes? Osteoarthritis Cartilage 2005;13:643–54.
6. Coimbra IB, Pastor EH, Greve JMD, Puccinelli MLC, Fuller R, Cavalcanti R, *et al*. Consenso brasileiro para o tratamento da osteoartrite. Rev Bras Reumatol 2002;429:371–4.
7. Farr JN, Going SB, Lohman TG, Rankin L, Kasle S, Cornett M, *et al*. Physical activity levels in early knee osteoarthritis patients measured by accelerometry. Arthritis Rheum 2008;15:1229–36.
8. Roddy E, Zhang W, Doherty M. Aerobic walking or strengthening exercise for osteoarthritis of the knee? A systematic review. Ann Rheum Dis 2005;64:544–8.
9. Sutton AJ, Muir KR, Mockett S, Fentem P. A case–control study to investigate the relation between low and moderate levels of physical activity and osteoarthritis of the knee using data collected as part of the Allied Dunbar National Fitness Survey. Ann Rheum Dis 2001;60:756–64.
10. Fehrenbacher A, Steck E, Rickert M, Roth W, Richter W. Rapid regulation of collagen but not metalloproteinase 1, 3, 13, 14 and tissue inhibitor of metalloproteinase 1, 2, 3 expression in response to mechanical loading of cartilage explants *in vitro*. Arch Biochem Biophys 2003;410:39–47.
11. Fitzgerald JB, Jin M, Grodzinsky AJ. Shear and compression differentially regulate clusters of functionally related temporal transcription patterns in cartilage tissue. J Biol Chem 2006;281:24095–103.
12. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage oligomeric matrix protein (COMP). Biorheology 2003;40:101–9.
13. Murata M, Bonassar LJ, Wright M, Mankin HJ, Towle CA. A role for the interleukin-1 receptor in the pathway linking static mechanical compression to decreased proteoglycan synthesis in surface articular cartilage. Arch Biochem Biophys 2003;413:229–33.
14. Valhmu WB, Raia FJ. Myo-inositol 1,4,5-trisphosphate and Ca2+/calmodulin-dependent factors mediate transduction of compression-induced signals in bovine articular chondrocytes. Biochem J 2002;361:689–96.
15. Valhmu WB, Stazzone EJ, Bachrach NM, Saed-Nejad F, Fischer SG, Mow VC, *et al*. A load controlled compression of articular cartilage induces a transient stimulation of aggrecan gene expression. Arch Biochem Biophys 1998;353:29–36.
16. Mio K, Saito S, Tomatsu T, Toyama Y. Intermittent compressive strain may reduce aggrecanase expression in cartilage: a study of chondrocytes in agarose gel. Clin Orthop Relat Res 2005;433:225–32.
17. Mauck RL, Byers BA, Yuan X, Tuan RS. Regulation of cartilaginous ECM gene transcription by chondrocytes and MSCs in 3D culture in response to dynamic loading. Biomech Model Mechanobiol 2007;6:113–25.
18. Kisiday J, Jin M, Kurz B, Hung H, Semino C, Zhang S, *et al*. Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. Proc Natl Acad Sci U S A 2002;99:9996–10001.
19. Grodzinsky AJ, Levenston ME, Jin M, Frank EH. Cartilage tissue remodeling in response to mechanical forces. Annu Rev Biomed Eng 2000;2:691–713.

20. Park S, Hung CT, Ateshian GA. Mechanical response of bovine articular cartilage under dynamic unconfined compression loading at physiological stress levels. Osteoarthritis Cartilage 2004;12:65–73.
21. Long P, Gassner R, Agarwal S. Tumor necrosis factor α-dependent proinflammatory gene induction is inhibited by cyclic tensile strain in articular chondrocytes *in vitro*. Arthritis Rheum 2001;44:2311–9.
22. Madhavan S, Anghelina M, Rath-Deschner B, Wypasek E, John A, Deschner J, *et al*. Biomechanical signals exert sustained attenuation of proinflammatory gene induction in articular chondrocytes. Osteoarthritis Cartilage 2006;14:1023–32.
23. Guzman RE, Evans M, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol 2003;31:619–24.
24. Leandro CG, Levada AD, Hirabara SM, Manhaes-De-Castro R, De-Castro CB, Curi R, *et al*. A program of moderate physical training for Wistar rats based on maximal oxygen consumption. J Strength Conditioning Res 2007;21:751–6.
25. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, *et al*. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
26. Young lM De, Kheifets JB, Ballaron SJ, Young JM. Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacologic agents. Agents Actions 1989;26:335–41.
27. Mccord JM, Fridovich I. Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049–55.
28. Aksenov MY, Markesbery WR. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett 2001;302:141–5.
29. Lowry OH, Rosebough NG, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
30. Barve RA, Minnerly JC, Weiss DJ, Meyer DM, Aguiar DJ, Sullivan PM, *et al*. Transcriptional profiling and pathway analysis of monosodium iodoacetate-induced experimental osteoarthritis in rats: relevance to human disease. Osteoarthritis Cartilage 2007;15:1190–8.
31. Shrier I. Muscle dysfunction versus wear and tear as a cause of exercise related osteoarthritis: an epidemiological update. Br J Sports Med 2004;38:526–35.
32. Tokuda M. Histological study of spontaneous osteoarthritis in the knee joint of guinea pigs. J Orthop Sci 1997;2:248–58.
33. Yamamoto K, Shishido T, Masaoka T, Imakiire A. Morphological studies on the ageing and osteoarthritis of the articular cartilage in C57 black mice. J Orthop Surgery 2005;13:8–18.
34. Kuettner KE, Thonar EJMA. Cartilage integrity and homeostasis. In: Klippel JH, Dieppe PA, Eds. Rheumatology. 2nd edn. London: Mosby; 1998.
35. Galois L, Etienne S, Grossin L, Watrin-Pinzano A, Cournil-Henrionnet C, Loeuille D, *et al*. Dose–response relationship for exercise on severity of experimental osteoarthritis in rats: a pilot study. Osteoarthritis Cartilage 2004;12:779–86.
36. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage. Arthritis Rheum 2005;52:3507–14.
37. Knobloch TJ, Madhavan S, Nam J, Agarwal Jr S, Agarwal S. Regulation of chondrocytic gene expression by biomechanical signals. Crit Rev Eukaryot Gene Expr 2008;18:139–50.
38. Hunter CJ, Imler SM, Malaviya P, Nerem RM, Levenston ME. Mechanical compression alters gene expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels. Biomaterials 2002;23:1249–59.
39. Stoddart MJ, Ettinger L, Hauselmann HJ. Enhanced matrix synthesis in de novo, scaffold free cartilage-like tissue subjected to compression and shear. Biotechnol Bioeng 2006;95:1043–51.
40. Fitzgerald JB, Jin M, Dean D, Wood DJ, Zheng MH, Grodzinsky AJ. Mechanical compression of cartilage explants induces multiple time-dependent gene expression patterns and involves intracellular calcium and cyclic AMP. J Biol Chem 2004;279:19502–11.
41. Angele P, Yoo JU, Smith C, Mansour J, Jepsen KJ, Nerlich M, *et al*. Cyclic hydrostatic pressure enhances the chondrogenic phenotype of human mesenchymal progenitor cells differentiated *in vitro*. J Orthop Res 2003;21:451–7.
42. Ferretti M, Srinivasan A, Deschner J, Gassner R, Baliko F, Piesco N, *et al*. Anti-inflammatory effects of continuous passive motion on meniscal fibrocartilage. J Orthop Res 2005;23:1165–71.
43. Angele P, Schumann D, Angele M, Kinner B, Englert C, Hente R, *et al*. Cyclic, mechanical compression enhances chondrogenesis of mesenchymal progenitor cells in tissue engineering scaffolds. Biorheology 2004;41:335–46.
44. Trindade MC, Shida J, Ikenoue T, Lee MS, Lin EY, Yaszay B, *et al*. Intermittent hydrostatic pressure inhibits matrix metalloproteinase and pro-inflammatory mediator release from human osteoarthritic chondrocytes *in vitro*. Osteoarthritis Cartilage 2004;12:729–35.
45. Grisham MB, Hernandez LA, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am J Physiol 1986;251:G567–74.
46. Biasucci LM, D'Onofrio G, Liuzzo G, Zini G, Monaco C, Caligiuri G, *et al*. Intracellular neutrophil myeloperoxidase is reduced in unstable angina and acute myocardial infarction, but its reduction is not related to ischemia. JACC Cardiovasc Imaging 1996;27:611–6.
47. Xiao F, Eppihimer M, Willis B, Carden DL. Complement-mediated lung injury and neutrophil retention after intestinal ischemia-reperfusion. J Appl Physiol 1997;82:1459–65.
48. Smith JK, Grisham MB, Granger ND, Korthuis RJ. Free radical defense mechanisms and neutrophil infiltration in post-ischemic skeletal muscle. Am J Physiol Heart Circ Physiol 1989;256:H789–93.
49. Belcastro AN, Arthur G, Albisser T, Raj DA. Heart, liver and skeletal muscle myeloperoxidase activity during exercise. J Appl Physiol 1996;80:1331–5.
50. Morozov VI, Pryatkin SA, Kalinski MI, Rogozkin VA. Effect of exercise to exhaustion on myeloperoxidase and lysozyme release from blood neutrophils. Eur J Appl Physiol 2003;89:257–62.
51. Pinho RA, Andrades ME, Oliveira MR, Pirola AC, Zago MS, Silveira PCL, *et al*. Imbalance in SOD/CAT activities in rat skeletal muscles submitted to treadmill training exercise. Cell Biol Int 2006;30:848–53.
52. Petrella Robert J. Is exercise effective treatment for osteoarthritis of the knee? Br J Sports Med 2000;34:326–31.
53. Roos EM, Dahlberg L. Physical activity as medication against arthrosis-training has a positive effect on the cartilage. Lakartidningen 2004;101:2178–81.
54. Alessio HM. Exercise-induced oxidative stress. Med Sci Sports Exerc 1993;25:218–24.
55. Ji L. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 1999;222:283–92.
56. Sjodin B, Hellsten WY, Apple FS. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med 1990;10:236–54.

57. Meydani M, Evans W, Handelman G, Fielding RA, Meydani SN, Fiatarone MA, *et al*. Antioxidant response to exercise-induced oxidative stress and protection by vitamin E. Ann N Y Acad Sci 1992;669:363–74.
58. Powers SK, Criswell D, Lawler J, Ji LL, Martin D, Herb RA, *et al*. Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am J Physiol 1994;266: R375–80.
59. Fulle S, Protasi F, Di Tano G, Pietrangelo T, Beltramin A, Boncompagni S, *et al*. The contribution of reactive oxygen species to sarcopenia and muscle ageing. Exp Gerontol 2004;39:17–24.
60. Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 1999;39:67–101.
61. Zhou LZ, Johnson AP, Rando TA. NF kappa B and AP-1 mediate transcriptional responses to oxidative stress in skeletal muscle cells. Free Radic Biol Med 2001;31:1405–16.
62. Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, *et al*. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. J Physiol 2003;549:645–52.
63. Davies KJA, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982;107:1198–205.
64. Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Viña J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 2005;567:113–20.