Short Communication

Walking Training Decreases the Plasma TBARS Concentration in Elderly Women with Knee Osteoarthritis

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Abstract

Knee osteoarthritis (kOA) is a common disease that primarily affects elderly populations worldwide, and thiobarbituric acid reactive substances (TBARS) appear to have a role in the cartilage degradation observed in kOA. The symptoms can be alleviated with physical exercise, which can also reduce lipid peroxidation. Therefore, if reactive oxygen species play a critical role in the cartilage degradation observed in OA, one might predict that walking training would decrease plasma TBARS concentrations and improve self-perceptions of pain, stiffness and physical function in OA patients. The aim of this study was to evaluate plasma TBARS concentrations in elderly kOA patients in response to walking exercise training. To accomplish this, 16 female individuals (mean age: 67.6 ± 4 years; mean body mass index: 27.8 ± 4.0 kg/m²) with clinical and radiographic diagnoses of kOA had their plasma TBARS concentrations measured before and after a physical exercise training program (12 weeks of aerobic training/ 50 min walk per day/3 times per week). In addition, the self-reported health status of each participant (using the Western Ontario McMaster Universities - WOMAC Osteoarthritis Index) was also evaluated. For the population studied, aerobic exercise training led to a 22% reduction in plasma TBARS concentrations (before 0.067 ± 0.007, after: 0.052±0.005 MDA nmol/protein mg; p = 0.03) and improved self-perceptions of pain (p = 0.0097), stiffness (p = 0.0179) and physical function (p = 0.0015). These data suggest that walking training programs might have antioxidant effects that could minimize or slow disease progression in elderly patients with kOA.

ABBREVIATIONS

OA: Osteoarthritis; Koa: Knee Osteoarthritis; ROS: Reactive Oxygen Species (ROS); TBARS: Thiobarbituric Acid Reactive Substances; TBA: Thiobarbituric Acid; WOMAC: Western Ontário McMaster Universities; MDA: Malondialdehyde; HR: Heart Rate, BMI: Body Mass Index; VO2max: Maximal Oxygen Consumption; KL Classification: Kellgren & Lawrence Classification; JSN: Joint Space Narrowing

INTRODUCTION

Osteoarthritis (OA) is a chronic, progressive degenerative osteoarticular disease that is characterized by arthralgia, stiffness and limitations of articular function, especially in the knee. Its etiology involves biomechanical, biochemical and genetic factors that contribute to an imbalance between the synthesis and destruction of articular cartilage [1]. Evidence suggests that oxidative stress is one of the primary causative factors in the pathogenesis of OA because plasma oxidative stress markers are increased and plasma antioxidant components are reduced in OA patients compared to healthy controls [2].

Reactive oxygen species can lead to oxidative damage in various components of the joint, including collagen, proteoglycans and hyaluronan [2]. Because reactive oxygen species (ROS) have extremely short half-lives, they are difficult to measure directly.
Instead, what can be measured are several products of the damage produced by oxidative stress, such as thiobarbituric acid reactive substances (TBARS) which are formed as a byproduct of lipid peroxidation [3].

Oxidative stress likely not only promotes cartilage destruction, but is also involved in inflammatory processes and in promoting the transition from clinically silent cartilage destruction to apparent OA [4]. Moreover, increased levels of ROS can damage DNA, including mitochondrial DNA, thereby affecting cell viability and contributing to the disruption of extracellular matrix homeostasis in chondrocytes of osteoarthritic cartilage [5]. Thus, further elucidation of the involvement of oxidative stress in the etiology of OA could provide novel insight into the mechanisms of disease progression and therefore reveal new approaches for symptom control.

To relieve the signs and symptoms of OA and, if possible, to delay its progression, several non-pharmacological treatments are available. These treatments are preferred as an initial option because they are not associated with deleterious side effects and are less aggressive for the patient [6,7]. In this context, regular physical exercise has been recognized as a safe treatment to improve functional activity and to reduce pain and stiffness in patients with kOA [5].

Aerobic exercise programs, such as walking, are a popular form of physical activity and a convenient, low-cost option to prevent chronic diseases, and they have been studied in numerous trials in individuals with kOA. Recently, our group demonstrated that controlled walking training promoted changes in the concentration of soluble TNF-α receptors, which were correlated with functional improvements [8], increased plasma BDNF concentrations and improved clinical parameters [9]. However, there is little evidence in the literature regarding the effectiveness of this intervention modality on oxidative stress markers and self-perceptions of disease status in elderly women with kOA.

Regarding the beneficial effects of physical exercise on oxidative stress markers, the current literature reveals that moderate exercise treadmill training increases the release of superoxide in the extracellular space, which can cause a rapid reduction in muscle protein content and a rapid increase in the levels of antioxidant enzymes, thus leading to adjustments in the endogenous oxidant defense system [10].

Patients with kOA have higher plasma concentrations of reactive oxygen species and lower antioxidant reserves when compared to healthy controls, facts that can result in high levels of oxidative stress. Because reactive oxygen species may play critical roles in the degradation of cartilage that is observed in OA [2], it is believed that walking training would reduce plasma oxidative stress markers and improve self-perceptions of pain, stiffness and physical function in kOA patients. Therefore, the aim of this study was to investigate the effects of a walking program on TBARS levels (an oxidative stress marker) and the self-reported health status of elderly subjects with kOA.

METHODS

This is a quasi-experimental study that includes women from the general community of the city of Diamantina, Brazil, with clinically and radiographic diagnosed OA of the knee. All patients selected met the inclusion criteria: (1) aged 65 years and over, (2) diagnosed with OA in at least one knee based on clinical and radiographic criteria of the American College of Rheumatology [11] with radiographic classification [12], (3) has not undergone any surgical procedure in the lower limbs, (4) has no history of recent trauma on the knees, (5) does not make use of mobility aids (canes, crutches, and walkers), (6) did not undergo physical therapy or any other procedure for rehabilitation in the last 3 months, (7) presents with clinical conditions and cognitive requirements for the exercises, and (8) make use of any immunosuppressive medication. Patients unable to finish the test run, used to determine the physical capability required to complete this study, were removed from the study.

Radiographic evaluation

To ensure that the participants had knee OA and to reliably standardize the samples, radiological evaluation was performed on all volunteers. Anteroposterior, oblique, and lateral images of the affected knee were taken in standing [13]. Radiological classification was made in accordance with the Kellgren-Lawrence [12]: grade 0: no radiographic features of OA are present grade 1: doubtful joint space narrowing (JSN) and possible osteophyotic lipping grade 2: definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph grade 3: multiple osteophytes, definite JSN, sclerosis, possible bony deformity grade 4: large osteophytes, marked JSN, severe sclerosis and definitely bony deformity.

PROCEDURES

Of the 108 subjects who were initially contacted, 92 were excluded because they did not meet the inclusion requirements, and the remaining 16 subjects participated in the study and performed all experimental procedures. All parameters were evaluated before and after a physical exercise training program.

Demographic data were collected from all study volunteers who met the inclusion criteria. After providing informed consent, the patients were subjected to a maximal exercise test on a treadmill to determine their physical capacity. One week later, the patients were clinically and functionally assessed. In addition, blood samples were first collected prior to the start of the 12-week training program and again 24 hrs after the end of the training program.

Plasma thiobarbituric-acid-reactive substances (TBARS) concentration

For plasma processing, 6 mL of whole blood was collected from the anteceubital vein using aseptic techniques and EDTA as an anticoagulant. The blood was then centrifuged, and the plasma was removed and stored at -80°C. As an index of lipid peroxidation, the levels of TBARS were quantified during an
acids peroxidation [14]. The amount of malondialdehyde (MDA) produced was interpreted as reflecting the plasma TBARS concentration and was used to indicate the degree of lipid peroxidation.

**Self-reported health status and aerobic capacity**

Self-reported pain, stiffness, and physical function were assessed using the WOMAC Osteoarthritis Index. This index includes domains of pain, stiffness, and physical function obtained from patient self-reports, and the scores were standardized to a range of 0–100 [15,16]. To determine aerobic capacity, a progressive exercise test was performed on a treadmill until each volunteer reached a state of fatigue, as determined by the volunteer’s heart rate or by her Borg rating on the perceived exertion scale. The test was stopped and considered maximal if at least one of the following criteria was met: the volunteer reached a score of more than 18 on the Borg scale or reported volitional fatigue.

**Training**

Training consisted of walking with a progressive increase in the exercise load. The training was performed three times per week on alternating days for 12 consecutive weeks. All volunteers were supervised by a responsible researcher to ensure the maintenance of exercise during the training period. In addition, each volunteer was wearing a heart monitor (F4, POLAR) with an audible alarm system that has been calibrated to ensure that heart rate training each subject (HR) remained within the desired range while she was performing the physical test. The exercise consisted of three distinct stages: a warm-up (5 min), aerobic exercise - walking (initially 30 min at 70% HRmax), and a cool down (5 min). The volume of aerobic exercise was individually prescribed and increased gradually in 5 min increments every 2 weeks (30 min for the first week and up to 55 min for the last week), with a similar progression for target heart rate training (70% HRmax for the first three weeks, 75% HRmax in weeks four-seven and 80% HRmax in weeks eight-twelve). The self-perception of pain, stiffness, and physical function, maximal oxygen consumption, and plasma TBARS levels of volunteers were evaluated before and after 12 weeks of training, each subject was his own control, by another researcher that didn’t participate of the training program.

**Statistical analysis**

After testing the normality of the data using the Shapiro-Wilk test, the Wilcoxon test was used to evaluate statistical significance for nonparametric variables and the Student t test was used to evaluate statistical significance for parametric variables. The data are presented as the median and interquartile range [for nonparametric variables - self-reported health status (WOMAC) and plasma TBARS concentrations] or as the median and standard deviation [for parametric variables - VO2max]. The significance level adopted was 5% (p < 0.05).

**RESULTS**

Samples were obtained and evaluated from 16 volunteers with a clinical and radiographic diagnosis of OA according to radiological KL classification (grade 1 = 31%, grade 2 = 25%, grade 3 = 25%, grade 4 = 19%). All subjects (mean age: 67 ± 4 years; mean body mass index: 27.8 ± 4.0 kg/m²) completed the 12-week training program, and the overall compliance rate was 93.6%. The training program did not significantly change the mean body mass index of the study participants (27.1 ± 2.0 kg/m²).

Physical performance was determined by obtaining maximum oxygen consumption (VO2max) values during the progressive tests to fatigue, which were performed on a treadmill, before and after training. Training led to an average increase of VO2max of 21% (mean before: 27.7 ± 5, mean after: 33.6 ± 5 mL/kg/min, p < 0.0001) (Table 1). In addition to the improvement in aerobic performance, plasma TBARS concentrations were also reduced approximately 22% (p = 0.03) (Figure 1) in response to training, and self- perceptions of pain, stiffness, and physical function increased approximately 47%, 57% and 53%, respectively (Table 2).

**DISCUSSION**

The present study was the first to demonstrate the effectiveness of an aerobic training program on lipid peroxidation (as measured by plasma TBARS concentrations after a training period) and on self-perceptions of pain, stiffness and physical function in elderly women with knee osteoarthritis. There is evidence demonstrating that some of the beneficial effects of regular exercise result from improved maintenance of redox balance. It is believed that this balance occurs by modulating the expression and activity of antioxidant enzymes and by decreasing the production of reactive oxygen species. These changes can

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**Table 1: Characteristics of volunteers (n=16). Data are presented as the median and standard deviation.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>67 ± 4</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>66.9 ± 11.80</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.54 ± 0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 ± 4.1</td>
</tr>
<tr>
<td>VO2max (mL O₂/kg/min)</td>
<td>27.7 ± 5.5</td>
</tr>
<tr>
<td>KL classification</td>
<td>grade 1 = 31%, grade 2 = 25%, grade 3 = 25%, grade 4 = 19%</td>
</tr>
</tbody>
</table>

**Abbreviations:** BMI: Body Mass Index; VO2max: Maximal Oxygen Consumption; KL classification: Kellgren & Lawrence classification.

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**Figure 1** Plasma TBARS levels in elderly women with kOA (n = 16) before and after a 12-week training period. Statistical analysis was performed using the Wilcoxon test, and the p values are shown above.
become evident through reductions in the levels of oxidative stress markers, such as plasma TBARS concentrations [13,14].

A recent study has demonstrated that plasma TBARS concentrations are higher in the early stages of kOA (grade I and II). The findings of that study are in accordance with the results of the present study, in which higher concentrations of plasma TBARS were observed in the earlier stages of knee OA (rs = 0.85, p = 0.0014). Based on these observations, it is believed that this oxidative stress marker could be used as a marker of disease progression in addition to being used to evaluate the effects of therapeutic interventions [17-19]. Despite these results and the growing interest in studying oxidative stress in specific populations subjected to chronic exercise, we are unaware of any studies of human knee OA that have investigated the effects of exercise training on oxidative stress parameters, especially plasma TBARS concentrations, which are indicative of lipid oxidation byproducts. However, increased plasma TBARS concentrations have been reported following a single session of isokinetic exercise in a study examining the inflammatory and oxidative stress parameters of obese women with kOA compared to a control group. The authors hypothesized that this result could be attributed to the action of nitric oxide, which is secreted by chondrocytes [16,17]. Furthermore, Cifuentes and colleagues performed a study that aimed to evaluate the effects of physical training on articular cartilage in rats with knee osteoarthritis. Histological analyses revealed that rats subjected to physical training of moderate intensity (an intensity of approximately 60-70% VO2 max, 8 weeks of training on a treadmill; 3 days/week during 50 minutes/day) had a higher rate of proteoglycan content preservation in the superficial and intermediate areas of articular cartilage, indicating a role of physical training in the preservation of articular cartilage, at least in an animal model [20-22].

Given the fact that a small number of studies have found associations between plasma TBARS levels and inflammatory diseases, the possibility remains that TBARS plasma levels are not related to OA but are instead associated with chronic inflammatory conditions resulting from aging. However, even in the light of this possibility, the present pilot study brings novel data to this discussion. The current study has several limitations that should be considered when interpreting the results. Although this manuscript describes a pilot study with limited sample size, significant differences were found with training exercise. With a larger sample size, it may have been possible to establish a significant correlation between self-perceptions of health status and plasma TBARS levels.

The lack of a control group is another limitation of the present study, and a study with a control group should be developed such that the relation of cause and effect is confirmed. However, it is important to emphasize that although the strength of the “before and after” trial decreases as it is adjusted for internal conditions, it is plausible that the events were not random. Authors claim that by respecting the limitations of cause and effect and taking necessary precautions, the type of design used in our study (quasi-experimental study) is a great alternative to knowledge of phenomena involving the study object [23]; in this case, it was evaluated plasma TBARS concentration and clinical parameters in elderly with osteoarthritis of the knee during exercise.

Additional limitation includes the lack of reliable parameters to establish the basal concentrations of plasma TBARS levels. It remains to be determined whether basal plasma TBARS levels differ among patients with arthritic diseases.

**CONCLUSION**

Our results demonstrate that aerobic training programs can effectively decrease plasma TBARS concentrations and improve self-perceptions of health status in elderly women with knee osteoarthritis.

**ACKNOWLEDGMENTS**

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**REFERENCES**

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**Table 2:** Self-reported health status (WOMAC) in elderly women with kOA (n = 16) before and after the 12-week training period.

<table>
<thead>
<tr>
<th>WOMAC (score)</th>
<th>Before training</th>
<th>After training</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-reported pain</td>
<td>138 (100-268)</td>
<td>38 (0-136)</td>
<td>0.0097</td>
</tr>
<tr>
<td>Self-reported stiffness</td>
<td>50 (0-118)</td>
<td>0 (0-44)</td>
<td>0.0179</td>
</tr>
<tr>
<td>Self-reported physical function</td>
<td>663 (213-838)</td>
<td>125 (50-500)</td>
<td>0.0015</td>
</tr>
<tr>
<td>Total</td>
<td>988 (306-1344)</td>
<td>188 (56-631)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

Data are presented as the median and interquartile range.


