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A STIMULUS-RESPONSE FRAMEWORK TO INVESTIGATE THE INFLUENCE OF CONTINUOUS VERSUS INTERVAL WALKING EXERCISE ON SELECT SERUM BIOMARKERS IN KNEE OSTEOARTHRITIS

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Abstract

Objective: Compare changes in the concentration of serum biomarkers in response to continuous versus interval walking exercise in participants with knee osteoarthritis (OA).

Design: A two-phase sequential design. Twenty-seven participants with unilateral knee OA completed two separate treadmill walking sessions: 1) continuous 45-minute walking exercise and 2) three 15-minute bouts of walking exercise separated by 1-hour rest periods for a total of 45 minutes in an interval format. Participants reported their knee pain using the numeric pain rating scale and serum levels of biomarkers associated with tissue turnover [cartilage oligomeric matrix protein (COMP)], inflammation [Tumor necrosis factor- α (TNF- α)] and pain [neuropeptide-Y (NPY)] were evaluated at baseline and every 15 minutes for both conditions.

Results: Continuous walking resulted in a cumulative increase in COMP concentration up to 45 minutes, whereas interval walking was associated with return of COMP concentrations back to baseline at 45 minutes. There were no significant changes in TNF- α and NPY concentration during walking. There was a significant increase in pain compared to baseline in the continuous walking regimen only.

Conclusion: Incorporating rest breaks in walking regimens may impact the potential deleterious effects of longer continuous bouts on the knee joint as well as limit pain during exercise.

INTRODUCTION

Knee osteoarthritis (OA) is considered one of the leading causes of disability in the United States with a lifetime risk of nearly 1 in 2 for developing symptomatic disease.¹ Articular cartilage is a mechano-sensitive structure, and maintenance of cartilage health requires loading, such as that which occurs during normal walking.² For example, moderate walking exercise has been shown to be associated with increases in articular cartilage thickness, stiffness and proteoglycan content.³ However studies have also shown that there may be a level of exercise beyond which there may be an increased risk of worsening cartilage damage. In a rat model for example, animals that were over-exercised were shown to have early degeneration of knee articular cartilage.⁴ In some individuals with knee OA, increased frequency and magnitude of joint loading has also been associated with increased pain and stiffness.⁵ This may be in part due to the fact that patients with knee OA have been shown to have increased net loads imparted on the knee joint during walking compared to healthy individuals.⁶ The limits of a safe walking program beyond which joint damage and worsening clinical symptoms are potentiated for individuals with knee OA has not been delineated. This is partly due to the difficulty in being able to follow real-time biological changes in response to walking exercise.

Disease progression does not occur at the same rate amongst patients with knee OA, with some experiencing significant progression over a few years, while others remain relatively stable over time. The application of serum derived biological markers (biomarkers) is a potential method to rapidly evaluate response to exercise to determine which patients will progress their joint disease and provide information that could enhance management strategies of the disease.^{7–11} Recent studies in participants with knee OA have used a stimulus-response framework which measures a biomarker response to a biomechanical stimulus to aid in outlining an individual's response to exercise. The most widely studied biological marker associated with OA that has been used for this indication is cartilage oligomeric matrix protein (COMP) that is a structural glycoprotein that binds and stabilizes type II collagen fibers. A change in the serum concentration of COMP has been shown to be sensitive to physical activity. For example, one hour after running exercise, changes in cartilage volume directly correlated with changes in serum COMP concentration.¹² Serum COMP is also sensitive to physiological loading of joints, with a significant increase in serum concentration following 30 minutes of walking exercise with changes in its serum concentration after 30 minutes of walking exercise have also shown to be closely associated with the 5-year rate of cartilage loss in participants with knee OA.¹³

There are minimal studies that have investigated changes in the serum concentration of inflammatory or pain related biomarkers using a stimulus-response framework such as during walking exercise. Tumor necrosis factor alpha (TNF- α) is considered a key inflammatory cytokine in OA and is also involved in multiple other mechanisms of potentiating OA such as blocking the synthesis of extracellular matrix components and stimulating cartilage degradative enzymes, such as matrix metalloproteinase-3 (MMP-3).^{14,15} Neuropeptide-Y (NPY) has been suggested to have role as a regulator of pain transmission and perception.^{16,17} Neither of these biomarkers associated with knee OA have been investigated as part of a stimulus-response framework in walking exercise, but may

represent important aspects of the response to activity in patients with knee OA beyond cartilage matrix changes.

The goal of this study was to use a stimulus-response framework to compare the effects of a single continuous session of walking exercise to an interval session with multiple shorter bouts of walking on the serum biomarker profile and self-reported pain in individuals with knee OA. A previous study has evaluated the biomechanical changes that occur in response to these walking regimens.¹⁸ The present study investigates the biological responses to this paradigm. We hypothesized that the serum concentration of COMP, TNF- α and NPY, as well as self-reported pain will increase with walking time and that they will be significantly different during a continuous 45-minute bout of walking exercise as compared to completing the same volume of exercise in an interval format.

MATERIALS AND METHODS

Participants

All participants were recruited from local research registries and through community advertisements. Prior to testing, all participants signed an informed consent form approved by the institutional review board at our institution. We included participants who fit the American College of Rheumatology (ACR) radiographic and clinical criteria for knee OA that includes knee pain on most days, plus 3 of the following 6 criteria: age > 50 years, morning stiffness of <30mins, crepitus on active motion of the joint, tenderness of the bony margins of the joint, and bony enlargement of the joint.^{19,20} In addition participants had to have unilateral knee pain without pain in the contralateral limb.

Radiographic grading of knee OA was confirmed according to the Kellgren and Lawrence rating scale²¹ for the participant's painful knee with grading of 2 or higher warranting inclusion in the study. Participants were not excluded if there was evidence of radiographic knee OA was observed in their asymptomatic knee. This decision was based on previous evidence indicating that radiographic knee OA is an imprecise guide to the likelihood that knee pain or disability will be present.²² Participants were excluded if they had contralateral knee pain, had a past history of traumatic knee injury, total joint arthroplasty, hip or spine pain, cardiovascular disease or uncontrolled hypertension, were taking beta blockers, had a neurological disorder that affected their gait, required an assistive device for ambulation or had a history of two or more falls within the previous year. Subject characteristics are outlined in Table 1. There are demographic and clinical similarities of our subjects to our prior study, which assessed the biomechanical effects of these walking regimens in the same cohort.¹⁸

Study Design

As outlined in Figure 1, a two-phase sequential design was used for this study, with all participants performing two walking trials (continuous or interval walking) on two separate days more than 72 hours apart. All participants were screened prior to inclusion into the study to ensure that they were capable of maintaining a continuous 45-minute bout of walking exercise on a treadmill at a speed of 1.3 m/s. This walking speed was selected as the

average self-selected walking speed previously reported in patients with mild to moderate severity knee OA.²³ The American Geriatric Society advocates for of 20–30 minutes of aerobic exercise at low to moderate intensity (40–60% of heart rate reserve) two to five times per week. According to the recommendations from the American College of Sports Medicine, further participation in aerobic activities above the minimum recommended amount can provide additional health benefits.²⁴ These are minimum recommendations and hence 45 minutes was chosen as the duration of walking for the present study to investigate whether there is a deleterious effect beyond this recommendation. All participants also wore a heart rate monitor (Polar Electro Inc.®, Lake Success, NY, USA) during the walking exercise screening to ensure that they were walking at 40–60% of their heart rate reserve, which is the recommended intensity of aerobic exercise by the American Geriatric society for patients with knee OA.²⁵ This criterion was included to ensure recruitment of participants who had a similar level of fitness.

Power Analysis

A power analysis was performed for this study. Our sample size of 27 participants ensured adequate power to detect an estimated increase of at least 15% in the primary serum biomarker of COMP concentration after exercise. This is based on an estimated increase of 23% in serum COMP concentration previously reported after 30-minutes of walking exercise in healthy adults.²⁶ Therefore a total of 27 participants would be needed to detect a conservative 15% increase in serum COMP concentrations after 30 to 45 minutes of walking exercise with 80% power at an alpha level of 0.05.

Data Collection

For the continuous walking exercise session, participants completed a single 45-minute bout of walking exercise at a speed of 1.3m/s on a treadmill (Bertec Corp., Columbus, OH, USA). Walking speed was kept constant to ensure all participants covered the same distance, while minimizing the influence of variable gait speed on the knee joint. For the interval walking exercise session, at least 72 hours afterwards, participants performed three separate 15-minute bouts of walking exercise for a total of 45 minutes, with each bout separated by a 1-hour rest period.

On both days of testing, participants were seated in a chair for 30 minutes and their baseline heart rate and blood pressure were checked. An intravenous cannula was inserted into the antecubital vein (or the dorsal hand vein) by a registered nurse prior to the walking experiments. Participants were then attached to a ceiling mounted safety harness system before the initiation of the treadmill walking exercise to avoid accidental falls and injury.

Serum Biomarker Analysis

Five milliliter blood samples were drawn into BD vacutainer® tubes every 15 minutes of walking exercise. Blood samples were allowed to clot for 30 minutes on ice, centrifuged, serum decanted and then transferred for storage at –80°C until assayed. Serum levels of the biomarkers of interest (COMP, TNF- α or NPY) were determined in duplicate per sample using a commercially available solid-phase enzyme-linked immunosorbent assays (R and D Systems, Minneapolis, MN, USA) with a 50-fold dilution per the manufacturer's

instructions. Briefly, 50µL of the 7 standards and diluted sera from the participants were added to an antibody coated plate (mouse monoclonal antibodies to the specific biomarker of interest) and incubated with 200µL human analyte (COMP, TNF-α or NPY) conjugate peroxidase for 2 hours at room temperature. Following rinsing, the bound antibody was incubated with an enzyme substrate consisting of tetramethylbenzidine for 30 minutes at room temperature followed by incubation with a stop solution of sulfuric acid. The optical density of each well was determined within 30 minutes using a microplate reader set to a wavelength of 450nm. A standard curve was created by reducing the data using computer software that generated a four parameter logistic (4-PL) curve fit and allowed interpolation of serum concentrations of the samples in duplicate.

Numeric Pain Rating Scale

The participants' pain intensity during walking exercise for the affected knee was recorded on an 11-point (0–10) numerical pain rating scale (NPRS). The NPRS was anchored on the left with the phrase “No Pain” and on the right with the phrase “Worst Imaginable Pain.” NPRS was collected at baseline, and after 15 minutes, 30 minutes and 45 minutes of walking exercise during the continuous walking exercise session. During the interval walking exercise regimen, NPRS scores were collected at baseline and after each 15-minute bout of walking.

Statistical Analysis:

All data were analyzed using SPSS (SPSS, Inc., Chicago, IL). The data was firstly examined for normality of distribution and homogeneity of variance. To determine whether serum biomarker concentrations varied between continuous and interval walking conditions or with time, a 2×4 (condition x walking time) analysis of variance (ANOVA) with repeated measures was performed for the variables of interest. A paired T-test was performed to compare baseline serum biomarker concentrations between walking conditions. For all significant main effects, individual differences were compared using independent sample t-tests with multiple comparison adjustments using Bonferroni corrections. The NPRS scores for each testing condition were compared using the Friedman test secondary to not meeting the assumption of normal distribution.

RESULTS

All participants completed both walking sessions and there were no dropouts. Biomarker concentrations (COMP, TNF-α and NPY) did not differ at baseline between walking conditions. The ANOVA results for COMP revealed a significant walking condition x walking time interaction ($F_{3,78} = 2.76$, $P < 0.05$), but no significant walking condition effect ($F_{1,26} = 0.26$, $P = 0.61$) or a walking time effect ($F_{3,78} = 1.59$, $P = 0.20$). In general, during continuous walking, baseline COMP concentrations were elevated by 19%, 25%, and 26% after 15 (non-significant finding), 30 and 45 minutes of walking (which were significant), respectively (**Table 2**). In contrast, during interval walking, baseline COMP concentrations were elevated by 21% after 15 minutes (non-significant finding) but were reduced back to near baseline levels after 30 minutes and were lower by 2.3% after 45 minutes of walking. Although an increase in serum COMP was noted at 15 minutes for both regimens, this

finding was non-significant. When observing the response to walking exercise, there were no main effects for walking condition, walking time, or a walking condition x walking time interaction for the serum concentrations of TNF- α and NPY. Using the Friedman test, NPRS scores were significantly increased after 45 minutes of continuous walking ($X^2_{\text{F}} = 15.67$, $p = 0.001$) but not significant for the interval walking condition ($X^2_{\text{F}} = 1.57$, $p = 0.6621$).

DISCUSSION

In the present study, we used a stimulus response framework to assess the response of the knee joint in different domains of knee joint health; cartilage extracellular matrix integrity (COMP), inflammation (TNF- α) or pain (NPY). This study showed that TNF- α and NPY may not be optimal biomarkers in a stimulus response framework. In keeping with other studies, COMP appears to be a reasonable candidate biomarker for use in a stimulus-response model of knee OA.^{13,26} The findings of the current study demonstrated that the serum concentration of COMP was higher following a continuous 45-minute bout of walking exercise compared to completing the same volume of exercise in an interval format.

To this end, a stimulus-response model in which the mechanical loading of the knee joint through prolonged walking increases the serum concentration of COMP in healthy controls and individuals with knee OA has been previously proposed.^{13,26} Chondrocytes are known to be mechanosensitive and COMP is a known glycoprotein that transduces the mechanical signal from the extracellular matrix to the cell. Increased concentrations of COMP are thought to be indicative of cartilage turnover.^{13,26,27} This differences in loading profiles at the knee during the individual walking regimens maybe associated with the biomarker changes and pain experienced by participants that we observed in the present study. Therefore, the finding that completing the same volume of walking exercise in an interval compared to a continuous format can lead to lower levels of serum COMP concentration could have important clinical implications in terms of protection of the osteoarthritic knee joint when prescribing walking exercise for patients with knee OA. This assertion, however needs further systematic validation through long-term randomized control trials.

Changes in COMP are suggested to be representative of the total knee joint loading experienced.^{13,28} To this end, an increase in serum COMP was observed in our study for both continuous and interval-walking exercise formats after 15 minutes of walking. Such an increase in serum COMP after cyclical physiologic loading of the knee joint has been associated with enhanced diffusion of COMP fragments from the articular cartilage into systemic circulation.²⁶ However, whereas serum COMP concentrations continued to increase with continuous walking, a decrease to baseline levels was observed after 30 and 45 minutes of walking if a 1-hour rest break was taken after each 15-minute bout of walking. Delayed diffusion of COMP as an explanation for the differences seen with interval walking could not be ruled out. This observation is similar to patterns of decrease in serum COMP after cessation of joint loading activity reported previously.^{26,27} Interestingly, additional bouts of interval walking did not result in increased serum COMP levels. It is plausible that the low concentrations of free COMP fragments in the intra-articular space or possibly a change in the anabolic activity of the articular cartilage tissue after the initial bout of loading may be responsible for the absence of subsequent increases in serum COMP concentrations.

The findings of this study should be interpreted in light of number of limitations. We evaluated walking exercise on a treadmill as opposed to walking over-ground and also at a set speed as opposed to self-selected speed. The metabolic cost of treadmill walking has shown to be higher in older adults which may lead to premature fatigue compared to over-ground walking.²⁹ Self-selected speed may have been more relevant clinically and account for the individual's typical function but for this preliminary study this was standardized to ascertain a similar volume of exercise is completed by all participants. Validation of these results during an over-ground walking exercise or self-selected treadmill walking conditions is warranted. Our inclusion criteria necessitated participants walking on a treadmill for at least 45 minutes at a set speed, and therefore by design required participants that were likely to be more functionally capable compared to a typical cohort of participants with knee OA. In addition, biochemical analyses were performed on the serum and not the knee synovial fluid. Therefore, it is possible that other joints may be releasing and contributing to the serum COMP level. However, we attempted to reduce the effect of this limitation by excluding patients if they had symptoms in joints other than the affected knees. Finally, the participants were also not advised to limit activity on the day prior to exercise, and this potentially could have affected the baseline concentrations of the serum biomarkers tested.

In conclusion, the continuous walking regimen evaluated in our study appeared to potentiate an increase in serum COMP concentration while an intermittent walking regimen of similar walking volume that involved rest breaks did not. The incorporation of rest breaks in walking regimens may impact the potential deleterious effects of longer continuous bouts on the knee joint as well as limit pain during exercise.

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Figure 1:

Study outline. On day one, participants walked 45 minutes on the treadmill (CONTINUOUS REGIMEN) at a set speed of 1.3m/s with blood/serum drawn and reporting of their pain score every 15 minutes. On day two, more than 72 hours later participants returned and walked a total 45 minutes with two 1-hour rest breaks every 15 minutes (INTERVAL REGIMEN). Blood/serum was again drawn every 15minutes at the completion of a walking bout and pain score reported at that timepoint.

Table 1 –**Subject Characteristics**

Subject Demographics	Mean (SD)
Age (years)	63.5 (7.7)
Female, n (%)	20 (66.7%)
Height (cm)	169.0 (9.8)
Weight (kg)	80.1 (18.5)
Body Mass Index (kg/m ²)	27.7 (4.0)
Tibiofemoral Compartment Radiographic Severity	n (%)
Grade 2	12 (40%)
Grade 3	8 (27%)
Grade 4	10 (33%)

Table 2 –

Mean biomarker Concentration and Numeric Pain Rating Scale (NPRS) at Measured Timepoints

Walking Regimen	Baseline	15 minutes	30 minutes	45 minutes
TNF-α concentration (ng/ml, mean $\pm$ standard deviation)				
Continuous	11.06 $\pm$ 7.10	9.66 $\pm$ 4.79 (p=0.196)	10.52 $\pm$ 5.55 (p=0.372)	10.93 $\pm$ 6.38 (p=0.472)
Interval	9.72 $\pm$ 6.38	9.31 $\pm$ 4.77 (p=0.387)	10.34 $\pm$ 5.21 (p=0.339)	10.24 $\pm$ 6.52 (p=0.385)
NPY concentration (ng/ml, mean $\pm$ standard deviation)				
Continuous	28.65 $\pm$ 10.78	25.63 $\pm$ 6.54 (p=0.261)	28.19 $\pm$ 10.54 (p=0.516)	37.07 $\pm$ 10.58 (p=0.181)
Interval	28.19 $\pm$ 7.65	26.06 $\pm$ 7.27 (p=0.365)	16.82 $\pm$ 8.56 (p=0.096)	28.65 $\pm$ 4.50 (p=0.456)
COMP concentration (ng/ml, mean $\pm$ standard deviation)				
Continuous	935.02 $\pm$ 422.44	1109.20 $\pm$ 586.56 (p=0.096)	1164.55 $\pm$ 665.41* (p=0.048)	1178.03 $\pm$ 523.43* (p=0.026)
Interval	1026.01 $\pm$ 404.17	1207.34 $\pm$ 641.84 (p=0.098)	1010.40 $\pm$ 539.61 (p=0.450)	1002.31 $\pm$ 447.22 (p=0.415)
Numeric pain rating scale (mean $\pm$ standard deviation)				
Continuous	1.13 $\pm$ 1.57	1.51 $\pm$ 1.79 (p=0.100)	1.97 $\pm$ 2.19* (p=0.028)	2.18 $\pm$ 2.19* (p=0.0062)
Interval	1.20 $\pm$ 1.37	1.50 $\pm$ 1.64 (p=0.201)	1.55 $\pm$ 1.63 (p=0.361)	1.42 $\pm$ 1.69 (p=0.715)

*
p<0.05 – significant increase in specific biomarker concentration from baseline*
p<0.05 – significant increase in pain from baseline