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The Effect of a Lower Body Positive Pressure Supported Treadmill Exercise Regime on Systemic Biomarkers of Inflammation and Cartilage Degradation in Individuals with Knee Osteoarthritis: A Pilot Study

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ABSTRACT

Background: Knee osteoarthritis (OA) has been linked to a chronic low-grade inflammatory response and altered metabolic activity of articular cartilage. **Objective:** The purpose of this investigation was to evaluate the effectiveness of a 12-week (3 times/week) lower body positive pressure (LBPP) treadmill walking regime on knee pain and systemic biomarkers of inflammation and cartilage degradation. **Methods:** Sixteen overweight (BMI > 25 kg/m^2) knee OA patients were randomized to a LBPP treadmill walking exercise group (N = 7) or non-exercise control group (N = 9). Baseline and 12-week follow-up assessments evaluated the following dependent variables: acute knee pain during full weight bearing treadmill walking; inflammatory biomarkers (C-reactive protein, interleukin-1β, interleukin-6, s100A8/A9, and tumor necrosis factor-α), and catabolic metabolism of articular cartilage (sCOMP). **Results:** Knee pain at baseline and follow-up remained unchanged for the non-exercise control group ($P > 0.05$). However, knee pain for the LBPP exercise group was significantly decreased at follow-up ($P \leq 0.05$). No differences in the biomarkers of inflammation and cartilage degradation were observed for between and within group comparisons (all $P > 0.05$). **Conclusions:** Data suggested that the LBPP supported walking regime could be effectively used to promote regular weight bearing exercise without exacerbation of knee joint pain and did not increase levels of systemic inflammation or catabolic activity of articular cartilage in overweight knee OA patients. This pilot investigation offers important insight regarding the potential role that the LBPP technology could play in facilitating investigations examining the disease modifying effect of exercise on knee OA pathogenesis.

Key words: Cytokines, Osteoarthritis, Exercise, Pain, Articular Cartilage, Inflammation

INTRODUCTION

Osteoarthritis (OA) is a progressive joint degenerative disease that affects approximately 27 million North Americans, and occurs most frequently in the knee (Lawrence et al., 2008; Oliveria et al., 1995). The World Health Organization determined it to be the 4th and 8th most common source of disability among women and men, respectively (Jordan et al., 2003), with a life-time risk of nearly 1 in 2 for individuals over the age of 65 years. Knee OA patients experience progressive deterioration of articular cartilage, joint stiffness, quadriceps muscle weakness, and diminished joint range of motion which impacts their ability to perform normal activities of daily living. The consequences of knee OA include a decrease in overall health, quality of life, as well as loss of mobility, and a decrease in work productivity (Jinks et al., 2007). As such, developing effective treatment strategies that can be used to delay or arrest disease progression would have a significant impact on the overall health, well-being, and quality of life of individuals suffering with the disease.

Chronic low-grade inflammation has been linked to progressive knee OA and is believed to be one of the driving forces in disease pathogenesis (Stannus et al., 2010). A two to fourfold escalation in systemic levels of pro-inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) has been defined as chronic low grade inflammation (Zanchi et al., 2012). Additionally, the concentration of serum adipokines (cytokines emitted from adipose tissue) have been correlated with synovial joint inflammation in individuals diagnosed with knee OA (de Boer et al., 2012). It has been demonstrated that a change in concentrations of CRP and TNF-α over 2.7 years is related to an increase in total knee pain in an OA population, and that TNF-α and IL-6 predicted change in knee pain with standing after a 5 year follow-up (Attur et al., 2015). Recent evidence also indicates that the level of mRNA prostaglandin E2 (PGE2) in peripheral blood leukocytes and the plasma levels of PGE2 could be successfully used to delineate patients who had symptomatic knee OA from partici-

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pants who served as non-OA controls (Attur et al., 2015). High levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and cyclooxygenase-2 (COX-2) mRNA in peripheral blood leukocytes have also been used to predict a higher risk of joint space narrowing on radiographs of progressive knee OA (Attur et al., 2015). As such, a low-level, systemic inflammatory response may be one mechanism that results in progressive joint degradation and finding strategies to improve the inflammatory status of individuals with knee OA could help to delay disease progression, and improve the overall health and quality of life of affected individuals.

Serum cartilage oligomeric matrix protein, or sCOMP (a non-collagenous glycoprotein that binds to type II collagen fibers and stabilizes the collagen fiber network of articular cartilage), is a biomarker commonly used to study articular cartilage metabolism (Lohmander et al., 1994). It provides reliable and accurate information for monitoring the effects of various exercise interventions on articular cartilage health (Denning et al., 2015; Roberts et al., 2019). Research suggests that sCOMP levels appear to respond in a "dose dependent" manner to both exercise duration and intensity, as well as to increased loads resulting from elevated body mass, occupational requirements (e.g. equipment worn by firemen, policemen, and military) and overload type fitness training (e.g. plyometrics) (Roberts et al., 2019). Data specific to an OA population indicates that elevated sCOMP concentrations are associated with both acute and chronic bouts of exercise (Erhart-Hledik et al., 2012; Mündermann et al., 2009). Research examining the effect of acute changes in body weight on sCOMP levels during walking exercise also suggest that catabolic activity occurs at a greater rate in loaded versus unloaded walking conditions (Denning et al., 2015). A more recent investigation examining healthy participants, also reports that increasing body weight by 20% (using a weighted vest) resulted in a significant increase in sCOMP levels after 30 minutes of walking exercise, as compared to exercise completed using 20% unweighted or normal body weight conditions (Herger et al., 2019).

Regular exercise training has been shown to have an anti-inflammatory affect and may play a role in joint health (Roberts et al., 2019; Woods et al., 2012). Aerobic and resistance training exercise have both shown efficacy in lowering markers of systemic inflammation (Woods et al., 2012), and regular participation in exercise is widely recognized as a central component of knee OA disease prevention and management strategies (Fernandes et al., 2013; Zhang et al., 2008). Unfortunately, many forms of exercise exacerbate joint symptoms in knee OA patients (especially over-weight individuals), resulting in poor exercise adherence and lead to an over-reliance on medications that place the patient at increased risk of associated co-morbidities. This poses a significant challenge to research that is designed to examine the effect of exercise on inflammation associated with OA disease progression. The introduction of a novel technology called Lower Body Positive Pressure (LBPP) now facilitates safe and individualized exercise prescription for patients at significant risk (such as those with knee OA) of exacerbation of knee joint symptoms during regular exercise (Peeler et al., 2015; Peeler & Ripat, 2018; Takacs et al., 2011, 2013). Previous research indicates that LBPP technology can be successfully used during walking exercise to control knee pain and symptoms, and helps promote long-term walking exercise adherence by knee OA patients at significant risk of exacerbation of joint symptoms (Peeler et al., 2015, 2018; Peeler & Ripat, 2018). However, previous work has not assessed the effects of LBPP supported walking exercise on objective biomarkers of cartilage degradation or inflammatory cytokines in overweight knee OA patients. Therefore, the purpose of this research was to evaluate the effect of participation in a 12-week LBPP-supported walking exercise program by patients with mild – moderate knee OA on knee pain during full-weight bearing walking, and systemic biomarkers of inflammation and cartilage degradation. We hypothesized that the LBPP-supported treadmill exercise regime could be completed by patients without exacerbation of knee joint pain or increase in pro-inflammatory biomarkers and the catabolic activity of articular cartilage (from baseline concentrations).

METHOD

Participants and Experimental Research Design

In a parallel group and randomized fashion, this small pilot project recruited a total of 16 participants (n = 7 LBPP group and n = 9 control group) from the general community population using local poster and newspaper advertisements. Inclusion criteria included: (1) Age 45-65 years; (4) Body mass index (BMI) over 25 kg/m^2 (defined as overweight); (3) Knee pain when performing normal activities of daily living (walking, squatting, or kneeling); (4) Mild to moderate knee osteoarthritis in one or both knees as confirmed by radiographic evaluation (i.e., Kellgren & Lawrence grades II & III). Exclusion criteria included: (1) History of traumatic injury or surgery in the lower extremity within the last year; (2) Radiographic evidence of severe knee OA (i.e., Kellgren & Lawrence grade IV); (3) Require the use of a walking aid or crutches to ambulate; (3) History of a medical condition that precludes regular participation in an exercise program; (4) Diagnosed with diabetes, cardiovascular disease, or screen positive for ankylosing spondylitis, psoriatic arthritis, chronic reactive arthritis, or renal problems requiring peritoneal dialysis or hemodialysis; (5) Incapable of providing informed consent due to language barrier or mental status; (6) Unable or unwilling to return for follow-up appointments. For patients currently on medication, initiation of the testing protocols was delayed 4-weeks to allow medications to adequately clear their systems and they were asked to discontinue use over the course of the 12-week investigation.

Ethics approval (E2014:117) was provided by the institutional Research Ethics Board at the University of Manitoba. All participants provided written informed consent, completed baseline demographic forms, a Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire (Roos & Lohmander, 2003) and were radiographically evaluated by the same musculoskeletal radiologist for mild to moderate knee OA using the Kellgren & Lawrence grading system (Kohn et al., 2016). Anthropometric measurements

(including leg alignment classification) were collected in the same out-patient orthopedic clinic using previously described methodologies (Peeler et al., 2015). Body mass and height were directly measured and used to calculate BMI (body mass [kg/height squared (m^2)]. With participants positioned in a supine lying position on a treatment table, leg circumferences (expressed as the average of 3 measurements to the nearest cm) were taken at knee joint line (KJL); 15 and 8 cm above KJL; and 15 cm below KJL. Participant leg alignment was determined by measuring the space between the knees with the individual standing, with the feet together and knees fully extended. If the knees contacted each other the leg alignment was categorized as valgus; if the distance between the knees was >1 cm the alignment was categorized as varus; a distance of <1 cm was considered normal alignment (Witvrouw et al., 2000). As part of the baseline evaluation, a 30 minute full-weight bearing (FWB) treadmill walk at 5.0 km/h (0º incline) was completed according to previously described methodologies (Peeler et al., 2015, 2018). During this FWB treadmill walking session, participants used a Visual Analog Scale (VAS) tool to numerically rate their knee pain severity on a scale of 0 – 10 at 5-minute intervals. The average of the 6 VAS measures taken over the 30 minutes was used as the representative VAS score for that FWB walking session. Participants were then randomly allocated in a 1:1 blocked fashion to either the LBPP walking group or the non-exercise control group using computer randomization found at: http://www.randomizer.org/. Baseline measures were repeated on all participants (regardless of group assignment) following the completion of the 12-week intervention. The same assessor was used to complete each participant's baseline and follow-up evaluations, and that researcher was blinded to each subject's group assignment, previous test scores, and intake results (i.e., anthropometry, KOOS results, radiographic evaluation).

LBPP Supported Exercise Intervention

Participants randomized to the exercise group completed 30 minutes of LBPP supported low-load walking 3 times per week for 12 consecutive weeks on an Alter-G treadmill (Alter-G Inc – Menlo Park, CA). All walking was done at a comfortable treadmill speed of 5.0 km/h and 0º incline (see Figure 1), which our previous research suggests is safe and effective for use with OA patients (Peeler et al., 2015; Takacs et al., 2013). The first five minutes of each treadmill walking session was used as a warm-up and allowed participants to reach their target heart rate and adjust to walking on the Alter-G treadmill's belt surface. This time period also facilitated adjustment of the LBPP within the treadmill's air chamber, with the amount of LBPP being systematically increased in 5% increments until the participant reported no further reduction in knee pain (i.e., the goal was to select a LBPP pressure that was high enough to eliminate or substantially reduce a participant's VAS scoring of acute knee pain for the duration of the 30-minute walking session). The target heart rate for the participants was tracked using a Garmin Forerunner (405CX, Schaffhausen, Switzerland) heart rate monitor system to maintain heart rate between 60-70% of their age-predicted maximum heart rate (220-age). Participants in the exercise group were blinded to the amount of LBPP that was used during each Alter-G treadmill walking session. Compliance in the LBPP walking group was monitored by participant adherence to their scheduled walking sessions.

Figure 1: Knee OA patient walking on the Alter-G treadmill

Participants in the non-exercise control group were asked to refrain from engaging in any form of structured exercise training beyond their normal activities of daily living for the duration of the 12-week investigation. Compliance by the non-exercise control group was monitored through "phone check-ins" which were conducted by study personnel every two weeks throughout the duration of the intervention to confirm that participants were not engaging in any form of structured exercise training.

Blood Draw Procedure

Blood draws were taken from all participants at baseline and following the 12-week intervention. Participants came in a rested (no moderate-vigorous exercise for 48 hours prior) and fasted (a minimum of 8 hours) state for the blood collection procedure. All blood draws were completed immediately prior to each participant completing a full-weight bearing (FWB) treadmill walking session for 30-minutes at a set speed of 5.0 km/h and 0º incline. Most research to date has examined baseline sCOMP levels after a 30-minute passive (seated) rest period (Denning et al., 2015; Herger et al., 2019) to allow for sCOMP to return to resting levels; however, our study chose to test the participants without a 30-minute passive rest before the blood draw. Thus, the walk into the laboratory could have elevated participants' sCOMP levels, but this would have been similar both times each participant came for testing (i.e., baseline and 12-weeks). Blood from the antecubital vein (approximately 25 mL) was drawn by a certified phlebotomist into 4 vacutainer tubes containing EDTA and 1 serum separator (SST) vacutainer tube. Blood in the EDTA vacutainer tubes was then centrifuged at 2500 rpm for 15 minutes to separate the plasma. The blood in the SST vacutainer tubes was allowed to clot for 30 minutes at room temperature prior to being centri-

fuged for 15 minutes at 2500 rpm. Immediately following centrifuge, the blood was aliquoted into microtubes (approximately 10 x 1 mL per tube), labelled, and stored in a -80°C freezer until analysis.

Blood Assays

Blood assays were completed using enzyme-linked immunosorbent assay (ELISA) techniques. All the ELISA kits were purchased from R&D Systems, Minneapolis, MN, USA. Blood assays included the following biomarkers: high sensitivity C-reactive protein (hsCRP), high sensitivity interleukin-6 (IL-6), high sensitivity tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), s100A8/A9, and serum cartilage oligomeric matrix protein (sCOMP). We used plasma to analyze hsCRP, IL-6, TNF-α, IL-1β, and s100A8/A9, while we used serum to analyze sCOMP.

Each biomarker was measured in triplicate according to the manufacturer's instructions. Briefly, the ELISA assay was based on a double antibody "sandwich" technique where a 96 well microplate was coated with the capture antibody of interest. Samples, controls, and standards were pipetted into the microplate and incubated for the manufacturers recommended time period to allow for antibody-sample interaction and binding. Once this initial incubation period was complete, each microplate was washed with buffer to remove excess debris. An enzyme conjugated to a secondary antibody was then introduced into each microplate well, allowing the molecule of interest to bind to the secondary antibody during a second incubation period. Once this process was complete, a chromogenic substrate was added to each microplate well which causes a color change in the assay. A microplate reader (EPOCH, BioTek Instruments, Winooski, Vt, USA) that was set for absorbance at various wavelengths was then utilized to quantify the degree of color change. For the IL-6, IL-1β, and TNF-α assays, the absorbance was set at 490 nm and corrected for absorbance at 650 nm. For the sCOMP, s100A8/A9, and CRP assays, the absorbance was set at 450 nm and corrected for absorbance at 540 nm.

Statistical Analyses

A repeated measures analysis of variance (ANOVA) was used to analyze the effects of time (baseline and 12-weeks) by group (LBPP exercise vs. no exercise) on all the dependent variables. The significance level for the study was set at $P \leq 0.05$. A Fisher's LSD post hoc test was performed to determine where differences were located if a significant interaction was found. All data are presented as means ± SD. All data was analyzed using Statistica version 13.0.

RESULTS AND DISCUSSION

Knee Pain and Systemic Biomarkers of Inflammation and Cartilage Degradation.

Demographic and KOOS questionnaire data for all participants is presented in Table 1. The sample was representative of an aging and obese OA patient population with normal leg alignment, long onset and duration of symptoms, and KOOS scoring that was consistent with data for patients with mild to moderate knee OA (Vina & Kwoh, 2018). No significant differences were observed between the exercise and control group when comparing demographic and KOOS data.

Table 1. Patient Demographic Information at Baseline - Mean ± SD

Variable	Exercise N=7	Control N=9
Gender (Male/Female)	2 / 5	5 / 4
Age (years)	58.8 ± 6.8	56.7 ± 6.8
Leg Alignment (Varus/Normal/Valgus)	0 / 7 / 0	1 / 8 / 0
Affected Knee (unilateral/bilateral)	5 / 2	5 / 4
Duration of Symptoms (months)	110.0 ± 132.6	164.0 ± 130.0
KOOS total score	60 ± 7	64 ± 12

*P ≤ 0.05

Anthropometric data organized by group are presented in Table 2. Anthropometric scores for each group remained stable throughout the duration of the 12-week investigation – there were no significant changes observed when comparing baseline versus follow-up data for either group. No significant differences were observed in the anthropometric data when comparing groups at either baseline or following the 12-week walking exercise intervention.

No significant differences in acute knee pain were observed between groups when performing full-weight bearing (FWB) walking during baseline evaluation. Lower Body Positive Pressure data for the exercise group suggested that the amount of LBPP support required by participants to exercise pain-free (i.e. visual analogue scale [VAS] equal to 0) significantly diminished ($p \leq 0.05$) over the duration of the 12-week intervention (1st LBPP supported session: mean of 19.2% ± 10.8 of body mass; Last LBPP supported session: mean of 4.9% ± 4.0 of body mass). VAS data from FWB treadmill walking sessions completed at baseline and follow-up (Table 3) also indicated that participants in the exercise group experienced a significant decrease ($p \leq 0.05$) in acute knee pain following completion of the 12-week LBPP supported exercise intervention. No differences were noted when comparing baseline and 12-week follow-up FWB walking data for the control group.

Biomarker data for inflammation and articular cartilage catabolism are presented in Table 4. Results indicated that there were no significant group × time interactions for the inflammatory biomarkers (CRP: $F(1, 14) = 0.515$, $P = 0.485$; IL-6: $F(1, 14) = 0.075$, $P = 0.789$; IL-1β: $F(1, 14) = 0.904$, $P = 0.358$; TNF-α: $F(1, 14) = 0.273$, $P = 0.609$; S100A8/A9: $F(1, 14) = .002$, $P = 0.964$), suggesting that the 12-week exercise intervention had no effect on levels of systemic inflammation within our knee OA patient population. Also, sCOMP data indicated that there was no significant group × time interaction ($F(1, 14) = 1.041$, $P = 0.325$), suggesting

Table 2. Anthropometric Measurements - Mean ± SD

Variable	Exercise (N= 7)		Control (N= 9)	
	Baseline	Follow-up	Baseline	Follow-up
Body mass (kg)	89.7 ± 20.9	89.3 ± 22.9	99.6 ± 19.0	98.6 ± 18.8
Height (m)	1.71 ± 0.11	1.68 ± 0.08	1.74 ± 0.09	1.76 ± 0.10
BMI (kg/m^2)	30.6 ± 5.6	31.2 ± 5.4	32.7 ± 4.4	31.4 ± 4.8
KJL Circumference (cm)	39.0 ± 3.0	40.1 ± 3.1	40.9 ± 3.6	41.2 ± 3.6
Thigh Circumference (cm) - 15 cm Above KJL	50.1 ± 4.3	52.5 ± 5.2	52.6 ± 3.9	49.2 ± 5.5
Thigh Circumference (cm) - 8 cm Above KJL	44.2 ± 5.2	44.6 ± 4.5	45.3 ± 4.0	45.7 ± 3.9
Leg Circumference (cm) - 15 cm Below KJL	38.7 ± 3.6	38.2 ± 3.3	40.8 ± 1.8	40.4 ± 2.0

KJL – Knee Joint Line, *P ≤ 0.05

Table 3. Acute knee pain (0 - 10-point VAS) during full weight bearing treadmill walking sessions completed at baseline and follow-up - Mean ± SD

Group	Baseline VAS	Follow-up VAS
LBPP (n = 7)	2.1 ± 1.2	0.7 ± 0.7*
Control (n = 9)	2.8 ± 1.7	2.0 ± 1.9

*P ≤ 0.05, follow-up was significantly less than baseline in the exercise group. VAS: visual analogue scale; LBPP: lower body positive pressure.

that articular cartilage metabolism for the exercise group remained stable throughout the duration of the 12-week intervention.

Values are mean ± SD. LBPP: lower body positive pressure; CRP: C-reactive protein; IL-6: interleukin-6; IL-1β: interleukin-1 beta; TNF-α: tumor necrosis factor-alpha; s100 A8/A9: calprotectin; sCOMP: serum cartilage oligomeric matrix protein

DISCUSSION

The results of this pilot project supported our central hypothesis that a 12-week LBPP supported treadmill walking regime could be completed by overweight patients with progressive knee OA without exacerbation of knee joint pain, or an increase in pro-inflammatory biomarkers and catabolic activity of articular cartilage. This is significant because our results contradict previous reports which suggest a negative correlation between participation in aerobic exercise and levels of systemic inflammation in a variety of pathologies, as well as research which suggests that increased levels of sCOMP occur as a result of participation in prolonged weight bearing exercise regimes (Gleeson et al., 2011; Roberts et al., 2019; Walsh et al., 2011). Our data contribute to an emerging body of literature about inflammatory and articular cartilage biomarkers that are specific to osteoarthritis, and offer important insight regarding the potential role that "unloading" technologies such as LBPP might play in facilitating long-term investigations into the effect that regular exercise may have on the presence of biomarkers associated with OA pathogenesis.

Our sample size was representative of a sedentary, aging, and overweight patient population who have been struggling with pain and symptoms associated with progressive knee OA for an extended period of time (Michael et al., 2010; Paradowski et al., 2005). KOOS data suggested that participants experienced significant and progressive knee pain and symptoms with typical activities of daily living, and VAS data obtained from FWB treadmill walking sessions confirmed that participants experienced acute knee pain during 30 minutes of walking. Data gathered from this small patient sample is thought to be generalizable to a larger population of symptomatic knee OA patients who are experiencing progressive joint degeneration and are at risk for exacerbation of knee pain and joint symptoms during exercise. As such, these results provide new insight and clarity about the impact that regular exercise may have on biomarkers of inflammation and cartilage degradation which are theorized to be associated with disease pathogenesis and progression.

Data for the study's exercise group confirms the results of earlier investigations involving the use of the LBPP technology with an overweight knee OA population and indicate that LBPP can be effectively used to facilitate regular and prolonged exercise participation by an overweight knee OA population without exacerbation of joint symptoms (Peeler et al., 2015, 2018; Peeler & Ripat, 2018; Takacs et al., 2013). Beyond this, results again emphasize reductions in body load facilitated by the LBPP technology support the prescription of an individualized exercise regime that can be customized to each patient's specific level of knee pain, stage of knee OA, and body weight.

The current literature suggests that regular exercise is effective for reducing inflammation for a variety of inflammatory-based pathologies (Gleeson et al., 2011; Walsh et al., 2011). Unfortunately, the extent to which this is true for individuals living with knee OA is unclear. In part, this may be due to the fact that studies which are designed to examine the long-term effect of exercise on inflammation frequently result in exacerbation of joint symptoms in overweight and mostly sedentary knee OA patients, and thus are confounded by poor exercise adherence and high study drop-out rates. Previous research examining the influence of isokinetic strength training or aerobic training suggests that

Table 4. Baseline (pre) and 12-week (post) follow-up of blood biomarkers as assessed after a LBPP walking intervention or control condition in individuals diagnosed with mild to moderate knee osteoarthritis.

Variable	Exercise (N = 7)		Control (N = 9)		*P-values*		
	Pre	Post	Pre	Post	Time	Group	Time x Group
CRP (ng/mL)	30.5±17.1	21.0±18.5	26.3±19.7	23.1±17.7	0.169	0.901	0.485
IL-6 (pg/mL)	1.6±1.4	1.1±0.6	1.9±2.2	1.1±0.6	0.225	0.802	0.789
IL-1β (pg/mL)	0.15±0.09	0.13±0.08	0.11±0.06	0.13±0.05	0.943	0.533	0.358
TNF-α (pg/mL)	0.92±0.54	0.93±0.45	0.79±0.32	0.74±0.32	0.778	0.432	0.609
s100 A8/A9 (ng/mL)	470.5±165.4	487.8±242.7	516.8±413.5	539.0±511.5	0.713	0.793	0.964
sCOMP (ng/mL)	239.2±78.3	236.2±57.5	198.9±63.4	214.3±54.6	0.501	0.328	0.325

Values are mean ± SD. LBPP: lower body positive pressure; CRP: C-reactive protein; IL-6: interleukin-6; IL-1β: interleukin-1 beta; TNF-α: tumor necrosis factor-alpha; s100 A8/A9: calprotectin; sCOMP: serum cartilage oligomeric matrix protein

serum IL-6 or TNF-α levels was unaffected in knee OA patients who completed 6 weeks of exercise training (3x/week) (Samut et al., 2015). Our study was underpowered to detect a change in CRP concentrations with a post-hoc power calculation indicating only 14.5% power with the current CRP data. To reach 80% power with these same data would have required a sample size of n = 52. Some researchers have hypothesized that an exercise training threshold may exist for OA patients, and must be reached in order for a long-term decrease in systemic inflammatory markers to be observed (Zanchi et al., 2012). The results of our 12-week investigation (3x/week) neither support nor deny this assertion, as our data indicated that regular exercise participation by knee OA patients had little effect on systemic concentrations of IL-6, TNF-α, or CRP.

Research targeting overweight and obese knee OA patients which used diet, exercise, or a combination of both, has reported that individuals who lost 5% of their total body weight and fat mass demonstrated a clinically significant reduction in levels of CRP and IL-6 in the systemic circulation (Beavers et al., 2015). Further research from the same lab targeting overweight/obese knee OA patients also revealed that a combination of intensive diet and exercise resulted in more weight loss than exercise alone and resulted in a greater decrease in IL-6 systemically (Messier et al., 2013). Research targeting older, obese adults with self-reported knee OA also indicates that an intensive diet and exercise regime which produced an average weight loss of 8.7% was associated with a significant decrease in soluble tumor necrosis factor receptor-1 (sTNFR1) when compared to the control group (Miller et al., 2008). Interestingly, this same study also indicated that there were no significant decreases in the other cytokines (IL-6, TNF-α, or sTNFR2) or the acute phase protein CRP (Miller et al., 2008). Additionally, research comparing the impact of exercise versus diet-induced weight loss on inflammatory biomarkers in a cohort of older, obese adults with knee OA, showed a significant decrease in levels of CRP, IL-6 and sTNFR for the diet group, but no effect associated with exercise training (Nicklas et al., 2004). The results of our study are in agreement with the results of these earlier investigations. Data indicated that CRP, IL-6, IL-1β, TNF-α, and s100A8/A9 were unaffected by participation in a 12-week (3x/week) LBPP supported exercise regime, and that inflammatory biomarker levels for the exercise group were similar to levels for the control group at both baseline and follow-up. These findings help to clarify the results of previous investigations which examined the effect of both diet and exercise on inflammatory biomarkers. In the current study, participants in the exercise group required an average of 13% of LBPP support over the 12-week intervention, which simulated an average change in body mass of 11.8 kg for participants. Data suggested that this acute change in body mass during exercise had no significant effect on the levels of inflammatory biomarkers. This finding would seem to confirm that prolonged weight loss (rather than prolonged exercise participation) that occurs as a result of either diet or exercise participation is the critical factor for significantly lowering levels of inflammatory biomarkers that have been associated with knee OA pathogenesis and progression. It may be that in order to significantly reduce inflammation, there needs to be a significant decrease in body fat mass as well. Previous research has suggested that adipokines (cytokines released from adipose tissue) play an endocrine role in metabolism and thus, without a significant change in fat mass there is unlikely to be a significant change in the amount of adipokines/cytokines released into the systemic circulation from adipose tissue (Chilibeck et al., 2014). Our 12-week LBPP supported walking regime was not designed to decrease body weight, as we only exercised the participants for a total of 90 minutes/week. A previous systematic review has concluded that for moderate intensity aerobic exercise, weight loss will only modestly occur when total duration of aerobic exercise is a minimum of 120 minutes per week (Thorogood et al., 2011). Thus, our intervention did not have the weekly duration necessary to result in a significant decrease in body fat mass.

The sCOMP results for this investigation are very interesting because they contradict previous exercise-based data which indicate that prolonged exercise or increased joint loading results in an increase in sCOMP levels (Hyldahl et al., 2016; Roberts et al., 2018). In the present study, data indicated that there were no significant differences in sCOMP levels (either at baseline or follow-up) when comparing LBPP exercise and control group participants. Previous investigations examining collagen turnover indicate that exercise load (Denning et al., 2015; Leiter et al., 2012) and body weight (Hoch et al., 2011; Lotz et al., 2013) both

significantly influence the physiological activity of articular cartilage. To date, research has been unable to quantify how changes in joint load (Arokoski et al., 2000; Franz et al., 2007; Messier et al., 2013) may directly influence the metabolic activity of articular cartilage in weight bearing joints (i.e., how does each kilogram of weight loss/gain influence articular cartilage metabolism). The sCOMP data from the current investigation seem to suggest that a modulation of body mass by an average of 13% among exercise group participants over the duration of the 12-week intervention was enough to mitigate exercise-induced changes in cartilage metabolism observed during other acute exercise–based investigations. Beyond this, it would appear that the 90 minutes/week of LBPP supported exercise was a duration which avoided a spike in the catabolic activity of the articular cartilage of weight bearing joints. Caution must be used when interpreting these results which are from a small sample size. However, these sCOMP data would seem to agree with subjective and qualitative results from previous knee OA investigations completed by our research group which indicated that LBPP-based walking exercise could be used to decrease acute knee pain while performing regular walking exercise, and was effective for reducing chronic knee pain and joint symptoms, increasing thigh muscle strength, and overall joint function during normal activities of daily living over the long term. Further research is clearly needed to examine the impact of acute changes in body mass on articular cartilage metabolism within a knee OA patient population at significant risk of exacerbation and progression of knee joint degeneration because of regular participation in weight bearing exercise regimes.

This research is not without limitation. Our protocol measured only systemic blood (rather than knee joint specific) biomarker concentrations of inflammation and cartilage metabolism in knee OA patients. While this is the most common methodological approach currently being utilized in OA based investigations [in order to limit the invasiveness of procedures about the affected joint(s)] and provides objective insight into how systemic inflammation may be affecting function of the musculoskeletal system as a whole, it does not provide a direct joint-specific measure of cartilage health or metabolism within the affected knee joint(s). As such, it is possible that other patient comorbidities may have influenced the results of our investigation. The total duration of exercise (90 minutes) completed per week by the LBPP exercise group participants may also be of concern as it is below the current American Heart Association recommendation of 150 minutes/week. However, the goal of the current investigation was to examine the impact of a 12-week LBPP-supported exercise regime on knee joint pain, and biomarkers of inflammation and cartilage metabolism in overweight knee OA patients. As such, we sought to avoid the prescription of an exercise intervention which could induce a change in body mass among sedentary individuals, and instead used an exercise protocol which our previous research indicates is well tolerated by knee OA patients (i.e., avoids exacerbation of joint symptoms), but does not induce a change in body mass/fat mass that would confound interpretation of our primary outcome measures. Finally, data from this pilot investigation are from a very limited sample size. While participant demographic, anthropometric and KOOS data suggest that the sample was representative of our target population, caution should still be used when interpreting these preliminary results. Further research involving a larger number of knee OA participants would help to confirm the data presented in this preliminary report.

Strengths and Practical Implication

This pilot investigation offers important insight regarding the potential role that the LBPP technology could play in facilitating investigations designed to examine the long-term impact of exercise on knee OA pathogeneses and progression. While exercise is widely recognized as a critical component in knee OA prevention, research to date has been unable to quantify the disease modifying effect of long-term exercise participation by patients with knee OA. Beyond this, it is currently unclear what constitutes an optimal exercise dosage and there is a lack of scientific evidence available to guide individualized exercise prescription based on an individual's joint pain, functional capacity, or stage of knee joint degeneration. As such, ineffective exercise frequently aggravates knee joint symptoms, leads to poor exercise adherence and high drop-out rates, and promotes reliance on medications that can result in other co-morbidities. The results of this 12-week pilot investigation suggest that the LBPP technology can be effectively used to promote weight bearing exercise among overweight knee OA patients without fear of exacerbating joint symptoms or systemic biomarkers of inflammation and cartilage degradation. We believe that the LBPP technology will assist the development of more effective evidence-based exercise strategies that can be used in the management of day-to-day joint symptoms and tracking of disease progression and pathogeneses, ultimately leading to improvements in the physical health, quality of life and social well-being of individuals diagnosed with knee OA.

CONCLUSIONS

The results of this investigation suggest that systemic biomarkers of inflammation and cartilage degradation within a knee OA patient population are unaffected by participation in a 12-week LBPP supported treadmill walking exercise intervention. Data serve to clarify previous knee OA based research and appear to suggest that systemic biomarkers of inflammation are influenced more by weight loss than exercise. Data also confirm that the LBPP "unweighting" technology can be effectively used to promote regular weight bearing walking exercise without exacerbation of joint pain, or inducing catabolic activity of articular cartilage which could negatively affect the progression of mild to moderate knee OA in over-weight patients at significant risk for rapid disease progression. Future research should evaluate the effects of the LBPP exercise intervention in a larger number of participants to increase the statistical power of the research and to confirm its effect on cartilage metabolism in overweight and sedentary knee OA patients at substantial risk

of exacerbation of joint symptoms and rapid progression of disease.

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