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Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study

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Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study

Robert D. Hyldahl, Alyssa Evans, Sunku Kwon, Sarah T. Ridge, Eric Robinson, J. Ty Hopkins, Matthew K. Seeley

Abstract

Introduction

Regular exercise protects against degenerative joint disorders, yet the mechanisms that underlie these benefits are poorly understood. Chronic, low-grade inflammation is widely implicated in the onset and progression of degenerative joint disease.

Purpose

To examine the effect of running on knee intra-articular and circulating markers of inflammation and cartilage turnover in healthy men and women.

Methods

Six recreational runners completed a running (30 min) and control (unloaded for 30 min) session in a counterbalanced order. Synovial fluid (SF) and serum samples were taken before and after each session. Cytokine concentration was measured in SF and serum using a multiplexed cytokine magnetic bead array. Ground reaction forces were measured during the run.

Results

There were no changes in serum or SF cytokine concentration in the control condition. The cytokine GM-CSF decreased from 10.7 ± 9.8 to 6.2 ± 5.9 pg/ml pre- to post-run ($p = 0.03$). IL-15 showed a trend for decreasing concentration pre- (6.7 ± 7.5 pg/ml) to post-run (4.3 ± 2.7 pg/ml) ($p = 0.06$). Changes in IL-15 concentration negatively correlated with the mean number of foot strikes during the run ($r^2 = 0.67$; $p = 0.047$). The control condition induced a decrease in serum COMP and an increase in SF COMP, while conversely the run induced an increase in serum COMP and a decrease in SF COMP. Changes in serum and SF COMP pre- to post-intervention were inversely correlated ($r^2 = 0.47$; $p = 0.01$).

Conclusions

Running appears to decrease knee intra-articular pro-inflammatory cytokine concentration and facilitates the movement of COMP from the joint space to the serum.

Keywords

Inflammation, Synovial fluid, Knee joint, Osteoarthritis, Exercise, Biomarker, COMP, Ground reaction force, GM-CSF, IL-15, IL-6

Abbreviations

ANOVA
Analysis of variance
COMP
Cartilage oligomeric matrix protein
GM-CSF
Granulocyte macrophage colony-stimulating factor
GRF
Ground reaction force
IFNa2
Interferon alpha-2
IL-10
Interleukin-10
IL-13
Interleukin-13
IL-15
Interleukin-15
IL-17
Interleukin-17
IL-1ra
Interleukin-1 receptor antagonist
IL1b
Interleukin-1 beta
IL1a
Interleukin-1 alpha
IL-4
Interleukin-4
IL-6
Interleukin-6
IL-8
Interleukin-8
IL-12p40
Interluekin-12 subunit beta
IP-10
Interferon-gamma-inducible protein-10
MCP-1
Monocyte chemoattractant protein-1
MIP-1a
Macrophage inflammatory protein-1 alpha
MIP-1b
Macrophage inflammatory protein-1 beta
OA
Osteoarthritis
RA
Rheumatoid arthritis
SF
Synovial fluid
Communicated by Olivier Seynnes.

Introduction

Engagement in regular exercise has well known and widespread salutary benefits for disease prevention and improvement of life quality. Among the benefits induced by regular, moderate intensity exercise is prevention of articular cartilage degenerative pathologies such as osteoarthritis (OA) (Mikesky et al. 2006; Semanik et al. 2012). Chronic physical exercise also improves measures of disability, pain and physical performance in patients with diagnosed knee and hip OA (Juhl et al. 2014; Ettinger et al. 1997; Zhang et al. 2010). In fact, physical exercise is the most recommended non-pharmacological intervention for OA patients (McAlindon et al. 2014). Despite the prevalence of data that support exercise as a successful symptomatic treatment for degenerative joint disorders, the mechanism(s) underlying its chondroprotective effects are unclear.

While a unifying model to explain the protective effect endowed by chronic exercise has not yet been established, researchers have identified possible factors that may contribute to its positive effect on joint health, including: greater knee stability, improved joint proprioception, and/or greater muscle strength (van der Esch et al. 2013; Knoop et al. 2013). An alternative, but not exclusive, hypothesis is that the joint loading associated with exercise promotes chondroprotection by altering the biochemical milieu of the intra-articular environment. Accumulating evidence suggests that a rise in chronic, low-grade synovial inflammation precedes structural degeneration that accompanies joint degenerative diseases (Benito et al. 2005; Sokolove and Lepus 2013; Haywood et al. 2003). Moreover, chronic intra-articular inflammation has been demonstrated to be a reliable predictive measure of disease development in early stage patients (Ayral et al. 2005). Importantly, long-term exercise is capable of attenuating the systemic low-grade inflammation associated with a number of pathologic conditions including cardiovascular disease, diabetes and obesity (Wärnberg et al. 2010). We, therefore, questioned whether an acute loading exercise (i.e., running) could generate an intra-articular anti-inflammatory environment that may contribute to the long-term benefits of chronic exercise on joint health.

Cyclic mechanical loading of the joint has also been shown to increase circulating markers representative of cartilage turnover, most notably the peptide cartilage oligomeric matrix protein (COMP). The magnitude of COMP changes, due to exercise, appears to be influenced by both the duration and intensity of the exercise (Denning et al. 2016; Niehoff et al. 2010), lending support to the theory that joint loading may induce cartilage adaptation by driving the remodeling response. Several specific characteristics of mechanical load (i.e., number of foot strikes, load rate, and impulse due to load) experienced during exercise, including running, have been related to measures associated with articular cartilage condition (Bennell et al. 2011; Pamukoff et al. 2016; Russell et al. 2010). Nevertheless, a relationship between joint loading and COMP has not been established. Further, because serum COMP also originates from anatomical sources outside of the knee joint capsule (e.g., ligaments and tendons of other load bearing joints) (Müller et al. 1998), it is difficult to know how accurately serum COMP concentration, before and after exercise, represents knee joint health changes due to exercise. Further, the relationship between serum COMP concentration and knee intra-articular COMP concentration during exercise, for healthy individuals, has not yet been studied.

The primary purpose of this study was to explore intra-articular measures of inflammatory markers and COMP, before and after exercise, to better understand the potential salutary benefits of exercise on joint health. To accomplish this purpose, we formulated three aims: (1) to comprehensively examine the effect of an acute loading exercise (running) on intra-articular inflammation using a high throughput cytokine array in healthy, non-osteoarthritic individuals; (2) to determine the effect of the same acute loading exercise on intra-articular COMP concentration, and determine whether changes in knee intra-articular COMP, due to exercise, accurately represent changes observed in serum COMP; and (3) to evaluate

potential relationships between the aforementioned intra-articular measures of inflammatory markers and COMP, and three characteristics of mechanical load during running (number of foot strikes, load rate, and impulse due to vertical ground reaction force).

Methods

Subjects

A total of 15 healthy, recreational runners (11 men and 4 women, 24 ± 4 years; 72 ± 15 kg; and 175 ± 8 cm) were recruited to participate in this study. All subjects provided informed consent in accordance with the appropriate Institutional Review Board, and the Declaration of Helsinki. Each participant was (1) between the ages of 18 and 40, (2) had no history of lower-extremity surgery in their lifetime, (3) no history of knee articular cartilage injury, (4) no knee pain in the 3 months prior to data collection, and (5) able to run at 3.0, 3.5, or 4.0 m/s for 30 continuous minutes. Due to the difficulty of collecting synovial fluid (SF) from healthy joints, we were only able to collect sufficient SF at both time points, under both conditions (control and run), in 6 of the 15 subjects. We were successful in obtaining SF samples for two of the time points in two subjects and unsuccessful in collecting any SF from seven of the subjects. Analyses were performed only on the six subjects (five men and one woman, 26 ± 7 years; 71 ± 6 kg; and 174 ± 8 cm) with a full complement of SF samples.

Study design

This study was a randomized, cross-over trial. Each participant completed two experimental sessions (run and control) in a counterbalanced order 1 week apart. For both experimental conditions, baseline blood and synovial fluid samples were collected at a local healthcare facility, and then participants were immediately transported to a nearby university biomechanics laboratory. Participants remained in an unloaded (seated) position during this transport. For the run session, approximately 15 min after the baseline samples were collected; participants ran for 30 min at a self-selected speed on an AMTI (Watertown, MA) treadmill instrumented with two fore-aft force plates. Ground reaction forces (GRF) were measured for 30 s at the 9th (0:09:30), 19th (0:19:30), and 29th (0:29:30) minute. Immediately following the run, subjects were seated in a chair and a second blood sample was collected. Participants were then transported back to the local health care facility, in an unloaded position (wheelchair), where a third blood and second synovial fluid sample were taken approximately 15 min after the completion of the run. The control session was identical to the run session except, rather than running for 30 min, the participants rested in an unloaded, seated position in the laboratory for 30 min. All synovial fluid samples were taken from the right leg.

Mechanical load measures

Three-dimensional GRF data were collected using VICON Nexus (VICON, Centennial, CO, USA) software and then exported to MATLAB, where they were smoothed using a low-pass (50 Hz) 4th order Butterworth filter. We measured three variables that influence the way the right knee is loaded during running: (1) number of right foot strikes throughout the run, (2) mean load rate (i.e., the slope of the force × time curve) between heel strike and the impact transient peak (Fig. 1) for vertical GRF applied to the right leg, and (3) mean impulse (time integral) due to vertical GRF, for the right leg, during the stance phase of running. The second and third aforementioned variables were averaged across 60 stance phases, 20 from the 9th, 19th, and 29th minute of the run.

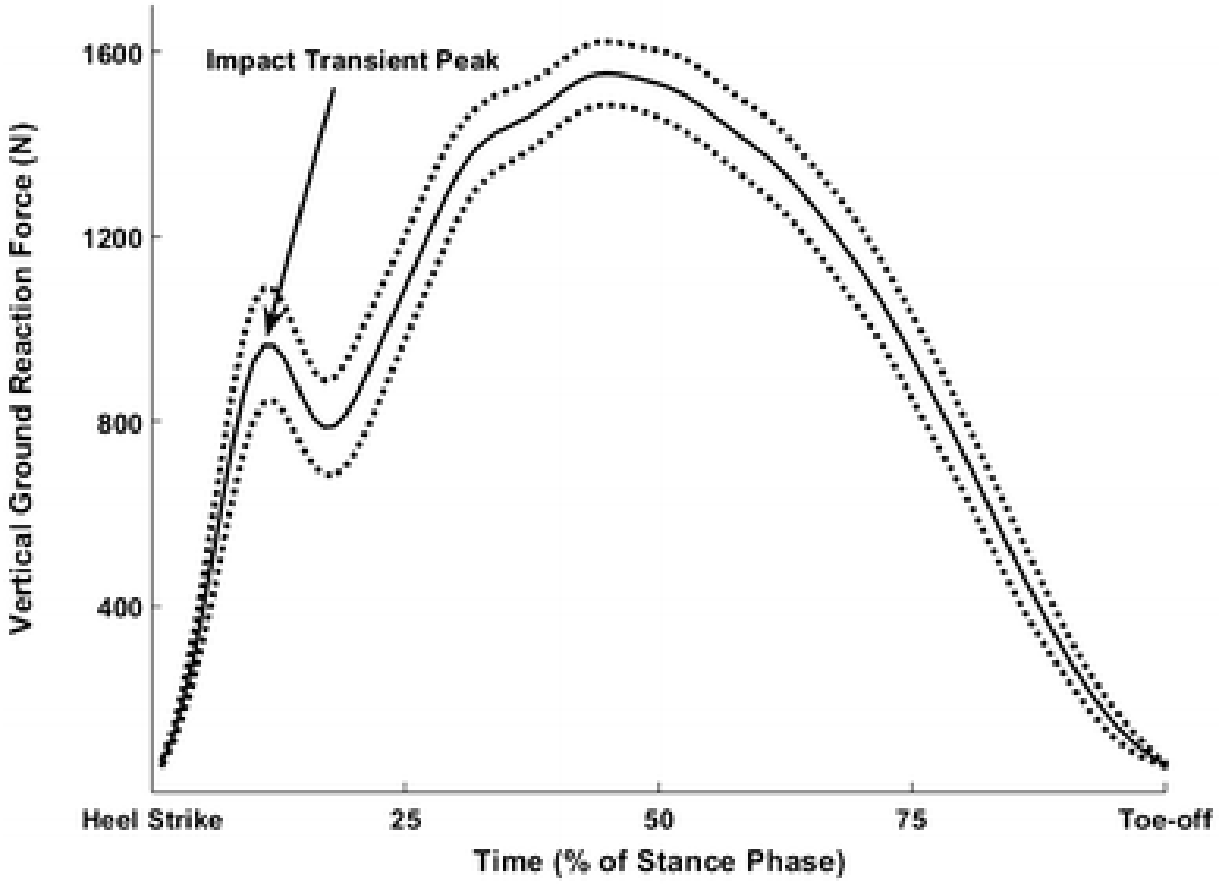

Fig. 1

Mean vertical ground reaction force (*solid line*) throughout the stance phase of running, for all of the trials observed during the present study, and the corresponding 95 % confidence intervals (*dotted lines*). For each trial, load rate was calculated as the average slope of the curve between the heel strike and impact transient peak

Synovial fluid and blood sampling

Synovial fluid was aspirated (without lavage) from the lateral suprapatellar space of the participant's right knee using a 10-mL syringe and 18-gauge 1.5″ hypodermic needle. Once aspirated, samples were stored on ice, centrifuged at 4,000 rpm at 4 °C for 10 min to remove cell debris and stored at −80 °C. Blood samples were taken from a vein in the antecubital region and centrifuged at 4,000 rpm. Serum was then aliquoted into cryo-vials and stored at −80 °C until analysis.

COMP analysis

COMP was quantitatively assessed in all serum and synovial fluid samples using a commercially available ELISA kit (R&D systems, Minneapolis, MN, USA) according to the manufacturer's recommendations. All samples were run in duplicate.

Cytokine analysis

A Luminex Magpix multiplexing platform was used for multianalyte profiling of synovial fluid and serum samples. Cytokines were measured using a 17-plex cytokine kit in compliance with manufacturer's

parameters (EDM Millipore, Billerica, MA). Cytokines included: GM-CSF, IFNa2, IL-10, IL-12p40, IL-13, IL-15, IL-1ra, IL1b, IL1a, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1a, MIP-1b and IL-17. Briefly, 25 µl of serum or synovial fluid was incubated overnight at 4 °C with antibody-conjugated magnetic beads. The bead-complex was then washed, followed by 30 min incubation at room temperature on a plate shaker in biotinylated detection antibody. Streptavidin–phycoerythrin was subsequently added and samples were incubated for an additional 30 min on a plate shaker at RT. A Magpix (Luminex Corporation, Austin, TX, USA) system was used to quantify bead-complexes. All samples were run in duplicate. Data analysis was based on a minimum of 80 beads using median fluorescence values. Cytokines that measured below the lower limit of quantification in greater than half of the samples among the 6 subjects were considered undetectable and not included in the analysis. Cytokines that measured below the lower limit of quantification in less than half of the samples among the 6 subjects were included in the analysis. This was done to ensure that cytokines that were undetectable in a subject under one condition (i.e., pre-run) and became detectable in another condition (i.e., post-run) could be included in the analysis. In this case, cytokines that measured below the detectable limit were indicated as the value of the lowest point on the calibration curve divided by 2.

Statistical analysis

Both serum and synovial fluid COMP data were analyzed independently using a two-way repeated measures ANOVA with factors for time and condition (control vs. run). COMP data are presented as mean ± SD. Correlation analyses were used to evaluate potential relationships between changes in serum COMP and synovial fluid COMP within each condition. Correlational analyses were done using a mixed models linear regression. Due to non-normal data distribution, differences in synovial fluid and serum cytokines within each condition were determined using a Mann–Whitney U test. Cytokine data are presented as median ± interquartile range (IqR). Effect sizes were calculated using Cohen's d. Mixed model linear regression analyses were used to evaluate changes in cytokine concentrations, due to the run, and the three variables related to knee load, as well as subject demographics (height, mass, body mass index and age) and knee load variables. A $p < 0.05$ was accepted as a significant difference.

Results

Synovial fluid and serum cytokine concentration

Table 1 presents cytokine concentrations from synovial fluid samples pre- and 15 min post-intervention for both the control and running conditions. The control condition (unloading) did not alter the concentration of any cytokine. However, the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF) displayed a trend ($p = 0.06$) for increasing following the unloaded period (Fig. 2a). In the exercise condition, synovial fluid GM-CSF decreased in all subjects (Fig. 2c) and median GM-CSF decreased by 41 % (from 6.8 ± 10.8 to 4.0 ± 5.1 pg/ml; $p = 0.06$; effect size = 0.55) pre- to post-exercise. Additionally, median interleukin-15 (IL-15) decreased 48 % (17.8 ± 11.7 to 9.6 ± 15.1 pg/ml) as a result of the run, and demonstrated a trend for statistical significance and a large effect size ($p = 0.06$; effect size = 0.96)(Fig. 2d). As shown in Fig. 2d, IL-15 decreased in five of the six subjects. The remaining subject had IL-15 levels that were below the detectable limit in both the pre- and post-exercise sample. Changes in IL-15 were highly variable in the control condition and were not significantly different pre- to post-unloading (Fig. 2b). There were no changes in any cytokine measured in the serum pre- to post-unloading in the control condition. In the exercise condition, interleukin-6 increased in the serum from a median of 14.0 pg/ml pre-run to 24.9 pg/ml post-run ($p = 0.02$).

Table 1 Overview of synovial fluid cytokine concentration (pg/ml) in the control and exercise condition before and 15 min after each intervention

	Control condition					Exercise condition				
	Pre-unloading		Post-unloading		*p* value	Pre-exercise		Post-exercise		*p* value
	median ± IqR	CV (%)	median ± IqR	CV (%)		median ± IqR	CV (%)	median ± IqR	CV (%)	
GM-CSF	4.5 ± 8.6	127.5	7.5 ± 12.0	94.3	0.06	6.8 ± 10.8	64.3	4.0 ± 5.1*	37.3	0.03
IL-10	1.9 ± 6.1	139.2	2.5 ± 20.9	127.3	0.63	2.5 ± 13.4	112.4	4.0 ± 4.4	63.0	0.81
IL-15	21.6 ± 15.3	60.1	17.7 ± 8.6	40.3	0.69	17.8 ± 11.7	93.6	9.6 ± 15.1	53.4	0.06
IL-1ra	46.5 ± 411.9	156.2	12.3 ± 244.0	229.1	0.63	16.7 ± 258.6	213.0	8.7 ± 210.2	701.2	0.5
IL-1α	6.9 ± 112.0	217.6	35.9 ± 349.0	141.0	0.256	8.5 ± 89.0	139.7	19.2 ± 35.5	112.0	0.56
IL-6	3.5 ± 8.7	115.6	6.7 ± 20.0	104.4	0.13	3.7 ± 10.3	34.2	5.9 ± 6.8	39.3	0.99
IL-8	7.6 ± 27.7	132.3	3.8 ± 24.3	180.0	0.99	5.7 ± 20.3	83.1	6.7 ± 13.2	67.9	0.99
IP-10	595 ± 578.3	65.4	544.1 ± 480.4	59.2	0.69	532.0 ± 640.2	78.0	536.2 ± 639.1	74.3	0.31
MCP-1	1055 ± 760	53.9	859.7 ± 308.2	22.0	0.63	859.9 ± 680.0	58.6	781.5 ± 767.8	81.1	0.84
MIP-1a	2.8 ± 19.3	152.8	1.0 ± 17.9	185.2	0.99	7.7 ± 16.2	70.0	4.1 ± 14.5	54.3	0.5
MIP-1b	39.1 ± 57.4	87.6	20.0 ± 50.6	147.1	0.59	23.5 ± 96.5	117.2	18 ± 54.8	125.1	0.13

For the control condition, subjects were unloaded for 30 min. For the exercise condition, subjects ran on a treadmill for 30 min. Data are presented as median ± interquartile range (IqR) and the coefficient of variation (CV). Data were analyzed using the non-parametric Mann–Whitney U test; * $p < 0.05$ relative to the pre-exercise value. IFNa2, IL-12p40, IL-13, IL-4, MIP-1a, and IL-17 could not be detected

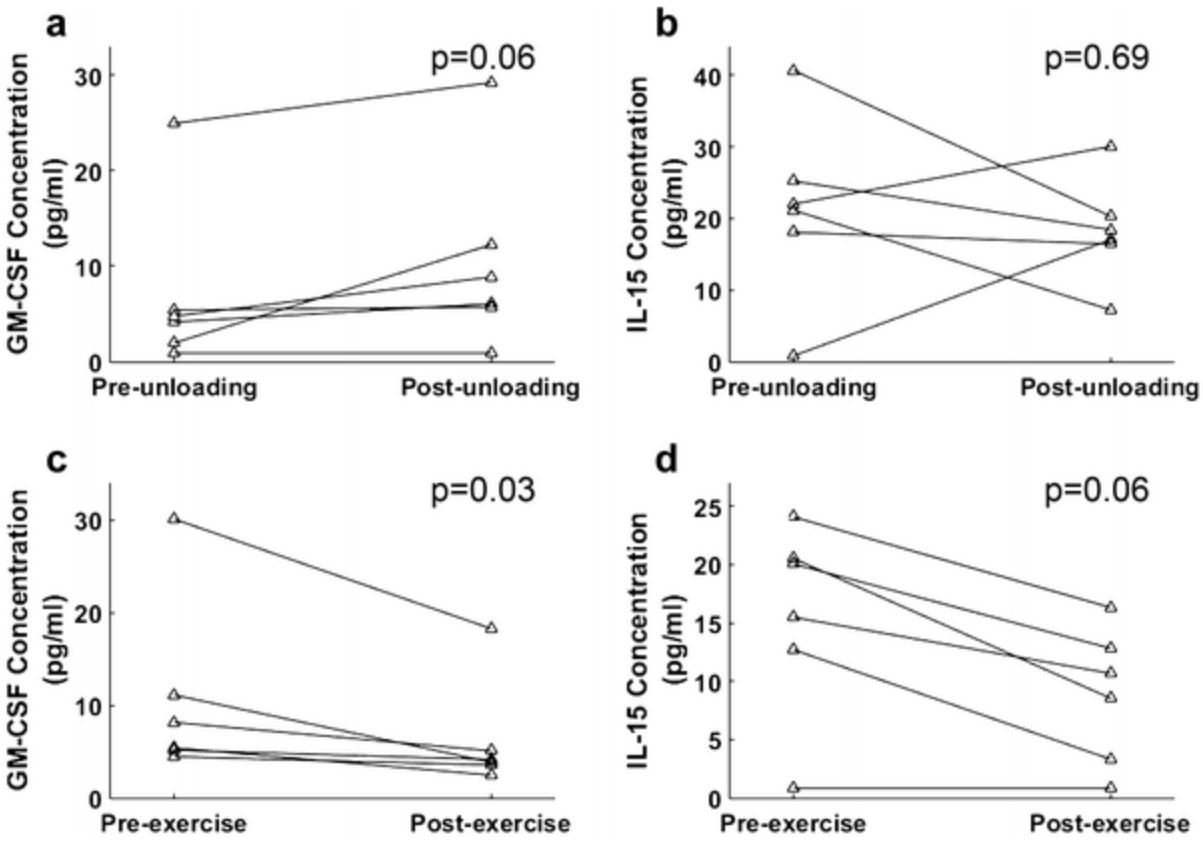

Fig. 2

Synovial fluid cytokine concentrations decrease following a 30-min run. GM-CSF and IL-15 concentration (pg/ml) in the control condition (**a**, **b**) before and after 30 min of unloading. GM-CSF and IL-15 concentration in the exercise condition (**c**, **d**) before and after 30 min of running. Data for all six subjects are shown

Cytokines and mechanical load

Subjects ran at an average running speed of 3.25 ± 0.21 m/s. Mean vertical GRF loading rate, impulse due to vertical GRF, and number of right foot strikes per 30-min run were 44.4 ± 10.0 KN/s, 256 ± 39 Ns, and 2490 ± 236 strikes, respectively. We observed one significant correlation between absolute change in synovial fluid cytokine concentration during the run and the three measures of mechanical load. As number of foot strikes increased, so did decrease for IL-15 ($r^2 = 0.67$; $p = 0.05$). This indicates that subjects who took more steps during the run experienced a greater decrease in IL-15 concentration during the run. Finally, we observed no significant correlations between subject demographics (height, mass, body mass index and age) and the three variables related to knee load.

Synovial fluid and serum COMP

For serum COMP, we observed a significant main effect of time ($p < 0.001$) and a significant time x condition (control vs. exercise) interaction ($p < 0.001$). In the control (unloaded) condition, mean serum COMP concentration decreased from 150.7 ± 73.7 ng/ml to 126.6 ± 67.0 ng/ml at the immediately post-unloading time point (Fig. 3a), and to 111.3 ± 50.2 ng/ml at the 15-minute post-unloading time point. During the 15 min between blood draws, the subjects remained in an unloaded seated position. In contrast, exercise increased mean serum COMP concentration from 134 ± 60.0 ng/ml in the pre-exercise

sample to 180.8 ± 60.6 ng/ml at the immediately post-exercise time point (Fig. 3a). By 15 min post-exercise, serum COMP concentrations were 117 ± 50.1 ng/ml, which was not significantly different than the pre-exercise measurement. For knee synovial fluid COMP concentrations, we likewise observed a significant time × condition (control vs. exercise) interaction ($p = 0.004$). Synovial fluid COMP increased significantly in the control (unloaded) condition from 1001.2 ± 743.8 to 1823.1 ± 1209.2 ng/ml at the 15-min post-unloading time point (Fig. 3b). Conversely, exercise resulted in a decrease in synovial fluid COMP from 1529.7 ± 859.8 to 914 ± 923.5 ng/ml pre- to 15-min post-run, respectively (Fig. 3b). Similar to the control condition, subjects were placed in an unloaded seated position for the 15 min between finishing the run and the synovial fluid draw. Mixed models linear regression showed no significant relationship between the change in serum and SF COMP 15 min post-exercise ($r^2 = -0.03$; $p = 0.61$). However, when changes in SF COMP concentrations (15-minutes post-exercise) were compared to the change in serum COMP immediately post-exercise, there was a significant inverse relationship ($r^2 = -0.48$; $p = 0.013$) (Fig. 3c).

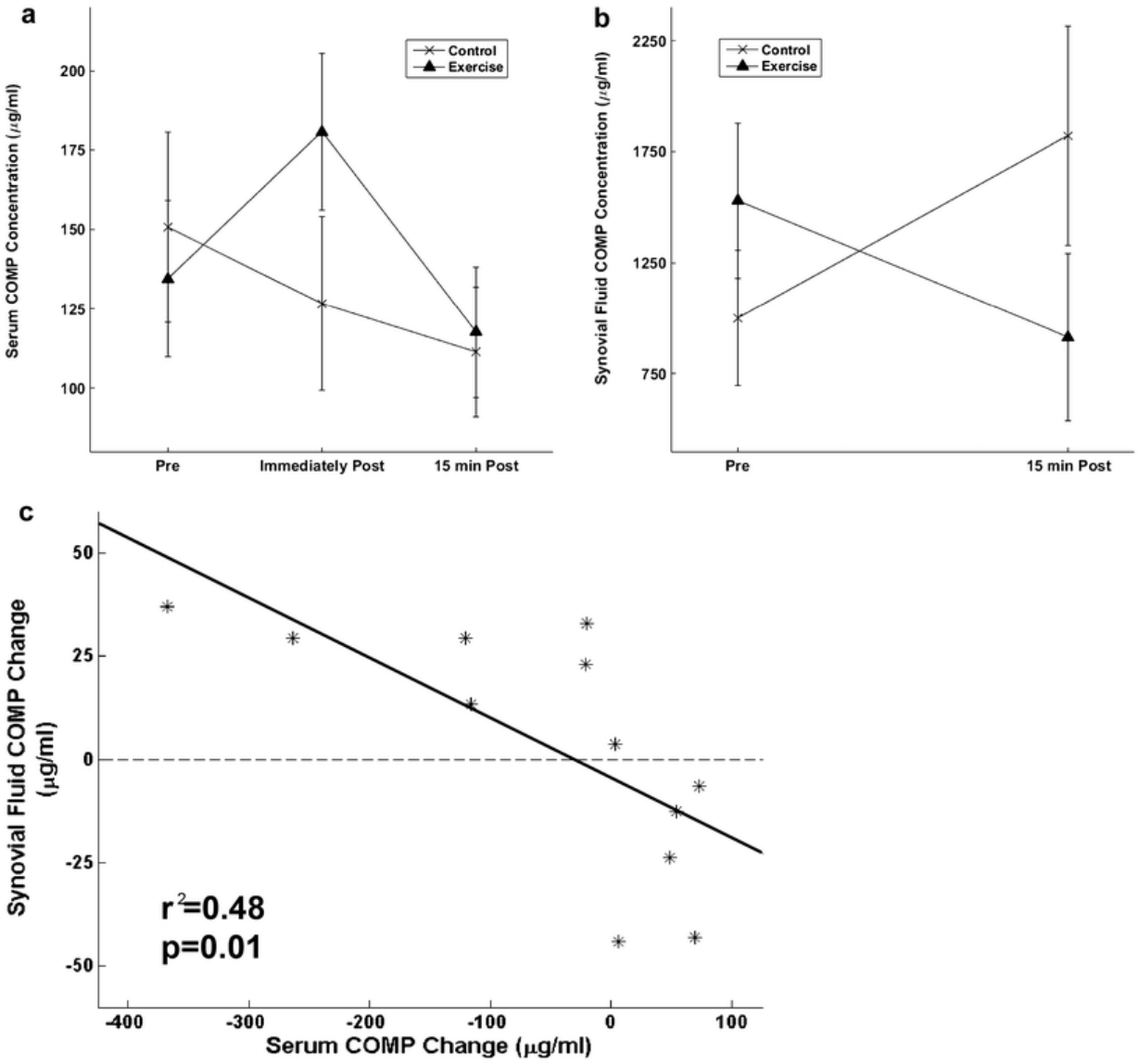

Fig. 3

Synovial fluid and serum COMP concentrations in the control and exercise condition. **a**Concentration of serum COMP (μg/ml) in the control (unloading) and exercise condition before, immediately after and 15 min after each intervention. **b** Concentration of synovial fluid COMP (μg/ml) in the control (unloading) and exercise condition before and 15 min after each intervention. **c** Association between changes in serum COMP (pre-exercise vs. immediately post-exercise) and synovial fluid COMP (pre-exercise vs. 15 min post-exercise) when data from exercise and control condition are pooled

Discussion

Physical activity has been associated with a reduced risk for development of joint degenerative diseases such as OA, and exercise is a widely prescribed and effective treatment modality for OA (McAlindon et al. 2014; Zhang et al. 2010). To better understand the underlying chondroprotective nature of exercise, we screened SF and serum samples from healthy subjects for markers of inflammation and cartilage turnover following a common joint loading exercise. Our most notable finding was that 30 min of running was sufficient to lower the intra-articular concentration of two pro-inflammatory cytokines (GM-CSF and IL-15), both with characterized roles in the pathogenesis of joint disease. Further, we confirmed that running invariably increases circulating levels of COMP; and show for the first time a significant inverse relationship between SF and serum COMP concentrations. Regrettably, we were only able to obtain paired samples under both the control and exercise conditions for 6 subjects. Nevertheless, we feel that the data are of value because, to our knowledge, this is the first study to comprehensively assess a large panel of pro- and anti-inflammatory mediators in the knee joint of healthy men and women following exercise.

Though the effect of exercise on intra-articular inflammation has not previously been investigated in healthy subjects, data in OA patients suggest a similar anti-inflammatory effect. Helmark et al. (2010) used a micro-dialysis method to assess the changes in intra-articular inflammatory markers in OA patients following 25 sets of one-legged knee extension exercises. Their most remarkable finding was a significant increase in interleukin-10 (IL-10), attributable to the exercise intervention. IL-10 is widely known to possess anti-inflammatory properties, and has been found to have chondroprotective properties via its inhibition of pro-inflammatory cytokine production by macrophages in diseased knee joints (Hart et al. 1995). In the current study, IL-10 was detected at very low levels and was not statistically different pre- to post-exercise. The IL-10 response was variable, and our inability to detect changes in IL-10 was likely influenced by low sample size and low levels of detection in our non-pathologic subject population. Similar to acute bouts of exercise, chronic exercise also appears to decrease intra-articular markers of inflammation in OA patients. Specifically, 4 weeks of exercise therapy, combined with anti-inflammatory medication in OA patients, decreased SF tumor necrosis factor-α (TNF-α) concentration to a greater extent than anti-inflammatory medication alone (Zhang et al. 2013). Unfortunately, in our sample of healthy subjects, TNF-α was undetectable in all conditions, indicating that its intra-articular accumulation may be a feature of diseased knees. Taken together with the OA studies, the results of the present study suggest that joint loading in the form of purposeful exercise has an intra-articular anti-inflammatory effect, and that the effect is present in both diseased and non-diseased knee capsules.

One of the most remarkable findings of the present study was the decrease in concentration of the cytokine GM-CSF in knee joint synovial fluid of all subjects following the run. This result is particularly interesting because synovial fluid GM-CSF is known to be significantly elevated in cases of knee OA and rheumatoid arthritis (RA) (Cameron et al. 1997; Arend and Dayer 1990), as well as in cases of knee injury that precede knee OA (Stone et al. 2015). Further, it is believed that neutralization of GM-CSF might effectively control OA pain and progression (Cook et al. 2012). In recent studies, GM-CSF antagonists have been shown to be a promising therapeutic avenue for the treatment of both RA and OA (Greven et al. 2014). Also interesting was the statistical trend towards increasing synovial fluid GM-CSF concentrations in the control condition. This suggests that unloading the joint, even for short periods, may be sufficient to initiate changes in the intra-articular biochemical environment. Our findings fall in line with evidence that sedentary behavior (i.e., reduced knee joint load) is generally detrimental to joint health and results in morphologic cartilage changes in both healthy and osteoarthritic subjects (Lee et al. 2015; Owman et al. 2014). However, to our knowledge, it seems that neither the acute nor chronic

effect of unloading on markers of joint disease has been investigated. Taken together, reduction of GM-CSF following loading exercise may represent a potential mechanism of the joint protection provided by exercise and should be investigated more extensively in future studies. The other notable cytokine finding was the trend for reduction in IL-15 concentration attributable to the exercise. In our cohort, five out of six subjects showed a decrease in IL-15 following the run, and it was undetectable in the pre- and post-exercise sample of the other subject. Due to our low sample size, the undetectable sample in one subject might have contributed to the statistically non-significant (0.06) result. In light of this, along with a reasonably large effect size (0.96), we feel that the finding is compelling, particularly insomuch as IL-15 has been associated with OA pathogenesis and severity (Miller et al. 2014; Tao et al. 2015).

The only significant correlation between mechanical variables and changes in cytokine concentration pre- to post-exercise was found for IL-15 and the number of foot strikes during the run. As number of foot strikes increased, so did a decrease for IL-15, indicating that subjects who took more steps during the run experienced a greater decrease in IL-15. The fact that the frequency of GRF application (i.e., number of foot strikes) was associated with an intra-articular biomarker change supports the idea that frequency of load application is as important as some other mechanical characteristics of load (e.g., peak knee joint contact force or peak external knee adduction moment). Denning et al. (2016) observed changes in concentration for an OA-associated biomarker (COMP) due to running, and these changes also appeared to be dependent upon step count. Further, Maly et al. (2013) observed that cumulative knee adductor load, defined as the product of the knee adductor impulse and daily step count, was twice as high for OA patients relative to healthy controls. The present results in combination with this previous work indicate that, in addition to observing peak force or peak torque magnitudes, it is important that researchers who study effect(s) of movement mechanics on knee joint health observe a variety of load characteristics, including frequency of load application. Regarding changes of serum cytokines, the only significant finding was an increase in IL-6 following the run. This result was expected, as increased circulating IL-6 is a remarkably consistent finding following exercise interventions (Fischer 2006). The lack of change in intra-articular IL-6 indicates that the knee joint is likely not a significant source of circulating IL-6. This assertion is further supported by previous studies, which have shown quite definitively that circulating IL-6 is primarily derived from working muscles following exercise (Steensberg et al. 2000).

In accordance with several previous studies, we found that mechanical loading of the knee joint, due to exercise, increases circulating levels of COMP immediately post-exercise (Denning et al. 2016; Niehoff et al. 2010). We also show that 15 min of unloading following the exercise is sufficient to reduce COMP levels back to baseline. Previous work in our laboratory has shown that COMP concentration returns to baseline no later than 30 min post-exercise (Denning et al. 2016), while others have shown a return to baseline after 60 min using a similar 30 min run (Niehoff et al. 2011), and 30 min following a walking exercise (Mündermann et al. 2005). Interestingly, Mündermann et al. (2005) showed that, although serum COMP initially returns to baseline 30 min following loading, it appears to increase again after 5.5 h of unloading, suggesting the possibility of a delayed cartilage metabolic response. The physiological significance of elevated serum COMP in response to exercise is not fully understood. However, a hypothesis implicated in the delayed increase in serum COMP up to 5 h post-loading is that late appearing COMP fragments in the serum reflect cartilage turnover, indicative of a remodeling process induced by joint loading. A possible explanation for the immediate changes in serum COMP following loading (15 min to 5 h) is that the cyclic loading of exercise enhances diffusion of the COMP fragments (Mündermann et al. 2005). While no study has directly assessed the diffusion properties of COMP fragments from the cartilage to the blood in knee joints, our data are consistent with this idea. We show here that under unloading conditions (the control session) COMP concentration increases in the SF, while concomitantly decreasing in the serum. This result is consistent with the findings of Mündermann et al.

(2005), who showed significant declines in serum COMP over a 6 h unloading period in healthy adults. In a likewise and counter manner, loading the knee joint reduces COMP SF concentration, while increasing COMP in the serum. To determine if there was a significant inverse relationship between COMP changes in the two compartments (SF and serum), we performed regression analysis on data points from both the control and exercise conditions. Ideally, we would have preferred to compare SF and serum data points at the same time point immediately post-exercise. Unfortunately, due to the constraints of having to travel a short distance to collect SF samples, our first SF draw occurred 15 min post-exercise. There was no correlation between the two compartments when compared 15 min post-exercise—the lack of relationship being driven primarily by the fact that serum COMP had already returned to baseline levels 15-min post-exercise. However, when we compared changes in SF COMP 15-min post-exercise to changes in serum COMP immediately post-exercise, we found a significant inverse relationship. This relationship may provide evidence that joint loading facilitates the diffusion of COMP. However, a definitive conclusion is not possible, as we were not able to collect SF immediately post-exercise, when serum COMP remained elevated in the serum. Nevertheless, the data are sufficient to emphasize the critical nature of controlling physical activity prior to serum biomarker tests and may suggest that the increased serum COMP following lower body loading is derived primarily from the knee joint. This latter observation is important, as serum COMP is an oft-utilized biomarker for the diagnosis of knee-related cartilage pathology (Bay-Jensen et al. 2016). Whether running induces greater COMP release from the articular cartilage into the synovial fluid, or simply facilitates its exit into the blood serum cannot be determined by our data and is an interesting question for future research.

We again acknowledge that our conclusions are based on a relatively small sample of human subjects. Unfortunately, we were only able to collect sufficient SF samples under both conditions pre- and post-exercise in 6 of the 15 recruited subjects. The difficulty of collecting adequate SF from non-effused joints has been acknowledged previously by others (Helmark et al. 2012). Thus, it appears uncertain whether sampling a large number of subjects with healthy knees is possible using the present aspiration technique. Perhaps the micro-dialysis method used by Helmark et al. (2010) to assess cytokine concentration in knees of OA patients may be more appropriate for intra-articular sampling of healthy joints. Notwithstanding the small sample size, the strength of the present study is bolstered by a more robust crossover, repeated measures design, in which subjects randomly completed both the exercise and control session. However, it will be important to confirm these data in future studies using a larger sample size.

Conclusion

In summary, this is the first study to evaluate a wide panel of inflammatory mediators in the knee joints of healthy subjects following running. Our results suggest that running decreases intra-articular inflammation and brings to light a novel potential mechanism for the chondroprotective nature of exercise in non-pathologic knees. Interestingly, this benefit appears to be related to frequency of load application. We also provide additional detail on the kinetics of COMP between the serum and SF compartments and reveal an interesting relationship indicating that serum COMP following lower body exercise may be derived, in large part, from the knee joint.

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Compliance with ethical standards

Conflict of interest

The authors report no conflict of interest.

References

Arend WP, Dayer JM (1990) Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheum 33:305–315CrossRefPubMedGoogle Scholar

Ayral X, Pickering E, Woodworth T, Mackillop N, Dougados M (2005) Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis—results of a 1 year longitudinal arthroscopic study in 422 patients. Osteoarthr Cartil 13:361–367CrossRefPubMedGoogle Scholar

Bay-Jensen AC et al (2016) Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria. Osteoarthr Cartil 24:9–20. doi:10.1016/j.joca.2015.10.014CrossRefPubMedGoogle Scholar

Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B (2005) Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis 64:1263–1267. doi:10.1136/ard.2004.025270CrossRefPubMedPubMedCentralGoogle Scholar

Bennell KL, Bowles K-A, Wang Y, Cicuttini F, Davies-Tuck M, Hinman RS (2011) Higher dynamic medial knee load predicts greater cartilage loss over 12 months in medial knee osteoarthrities. Ann Rheum Dis 70:1770–1774CrossRefPubMedGoogle Scholar

Cameron M, Buchgraber A, Passler H, Vogt M, Thonar E, Fu F, Evans CH (1997) The natural history of the anterior cruciate ligament-deficient knee changes in synovial fluid cytokine and keratan sulfate concentrations. Am J Sport Med 25:751–754CrossRefGoogle Scholar

Cook AD et al (2012) Granulocyte-macrophage colony-stimulating factor is a key mediator in experimental osteoarthritis pain and disease development. Arthritis Res Ther 14:R199CrossRefPubMedPubMedCentralGoogle Scholar

Denning WM et al (2016) Ambulation speed and corresponding mechanics are associated with changes in serum cartilage oligomeric matrix protein. Gait Posture 44:131–136CrossRefPubMedGoogle Scholar

Ettinger WH et al (1997) A randomized trial comparing aerobic exercise and resistance exercise with a health education program in older adults with knee osteoarthritis: the fitness arthritis and seniors trial (FAST). JAMA 277:25–31CrossRefPubMedGoogle Scholar

Fischer CP (2006) Interleukin-6 in acute exercise and training: what is the biological relevance? Exerc Immunol Rev 12:6–33PubMedGoogle Scholar

Greven D et al (2014) Preclinical characterisation of the GM-CSF receptor as a therapeutic target in rheumatoid arthritis. Ann Rheum Dis Annrheumdis 74(10):1924–1930CrossRefGoogle Scholar

Hart P, Ahern M, Smith M, Finlay-Jones J (1995) Comparison of the suppressive effects of interleukin-10 and interleukin-4 on synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis. Immunology 84:536PubMedPubMedCentralGoogle Scholar

Haywood L, McWilliams D, Pearson C, Gill S, Ganesan A, Wilson D, Walsh D (2003) Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum 48:2173–2177. doi:10.1002/art.11094CrossRefPubMedGoogle Scholar

Helmark IC et al (2010) Exercise increases interleukin-10 levels both intraarticularly and peri-synovially in patients with knee osteoarthritis: a randomized controlled trial. Arthritis Res Ther 12:R126. doi:10.1186/ar3064CrossRefPubMedPubMedCentralGoogle Scholar

Helmark IC, Petersen MC, Christensen HE, Kjaer M, Langberg H (2012) Moderate loading of the human osteoarthritic knee joint leads to lowering of intraarticular cartilage oligomeric matrix protein. Rheumatol Int 32:1009–1014. doi:10.1007/s00296-010-1716-7CrossRefPubMedGoogle Scholar

Juhl C, Christensen R, Roos EM, Zhang W, Lund H (2014) Impact of exercise type and dose on pain and disability in knee osteoarthritis: a systematic review and meta-regression analysis of randomized controlled trials. Arthritis Rheum 66:622–636. doi:10.1002/art.38290CrossRefGoogle Scholar

Knoop J et al (2013) Knee joint stabilization therapy in patients with osteoarthritis of the knee: a randomized, controlled trial. Osteoarthr Cartilage 21:1025–1034CrossRefGoogle Scholar

Lee J et al (2015) Sedentary behavior and physical function: objective evidence from the osteoarthritis initiative. Arthritis Care Res (Hoboken) 67:366–373CrossRefGoogle Scholar

Maly MR, Robbins SM, Stratford PW, Birmingham TB, Callaghan JP (2013) Cumulative knee adductor load distinguishes between healthy and osteoarthritic knees-A proof of principle study. Gait Posture 37:397–401. doi:10.1016/j.gaitpost.2012.08.013CrossRefPubMedGoogle Scholar

McAlindon T et al (2014) OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthr Cartil 22:363–388. doi:10.1016/jjoca201401003CrossRefPubMedGoogle Scholar

Mikesky AE, Mazzuca SA, Brandt KD, Perkins SM, Damush T, Lane KA (2006) Effects of strength training on the incidence and progression of knee osteoarthritis. Arthritis Care Res (Hoboken) 55:690–699. doi:10.1002/art.22245CrossRefGoogle Scholar

Miller RE, Miller RJ, Malfait A-M (2014) Osteoarthritis joint pain: the cytokine connection. Cytokine 70:185–193CrossRefPubMedPubMedCentralGoogle Scholar

Müller G, Michel A, Altenburg E (1998) COMP (cartilage oligomeric matrix protein) is synthesized in ligament, tendon, meniscus, and articular cartilage. Connect Tissue Res 39:233–244CrossRefPubMedGoogle Scholar

Mündermann A, Dyrby CO, Andriacchi TP, King KB (2005) Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. Osteoarthr Cartil 13:34–38CrossRefPubMed

Niehoff A, Kersting UG, Helling S, Dargel J, Maurer J, Thevis M, Brüggemann G-P (2010) Different mechanical loading protocols influence serum cartilage oligomeric matrix protein levels in young healthy humans. Eur J Appl Physiol 110:651–657CrossRefPubMedGoogle Scholar

Niehoff A et al (2011) Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. Osteoarthr Cartil 19:1003–1010CrossRefPubMedGoogle Scholar

Owman H, Tiderius CJ, Ericsson YB, Dahlberg LE (2014) Long-term effect of removal of knee joint loading on cartilage quality evaluated by delayed gadolinium-enhanced magnetic resonance imaging of cartilage. Osteoarthr Cartil 22(7):928–932CrossRefPubMedGoogle Scholar

Pamukoff DN, Lwewk MD, Blackburn JT (2016) Greater vertical loading rate in obese compared to normal weight young adults. Clin Biomech 33:61–65CrossRefGoogle Scholar

Russell EM, Braun B, Hamill J (2010) Does stride length influence metabolic cost and biomechanical risk factors for knee osteoarthritis in obese women? Clin Biomech 25:438–443CrossRefGoogle Scholar

Semanik PA, Chang RW, Dunlop DD (2012) Aerobic activity in prevention and symptom control of osteoarthritis. PM&R 4:S37–S44. doi:10.1016/j.pmrj.2012.02.015CrossRefGoogle Scholar

Sokolove J, Lepus CM (2013) Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. Ther Adv Musculoskelet Dis 5:77–94. doi:10.1177/1759720x12467868CrossRefPubMedPubMedCentralGoogle Scholar

Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B (2000) Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6 levels. J Physiol 1:237–242CrossRefGoogle Scholar

Stone A et al (2015) Osteoarthritic changes in vervet monkey knees correlate with meniscus degradation and increased matrix metalloproteinase and cytokine secretion. Osteoarthr Cartil 23:1780–1789CrossRefPubMedPubMedCentralGoogle Scholar

Tao Y, Qiu X, Xu C, Sun B, Shi C (2015) Expression and correlation of matrix metalloproteinase-7 and interleukin-15 in human osteoarthritis. Int J Clin Exp Pathol 8:9112–9118PubMedPubMedCentralGoogle Scholar

van der Esch M et al (2013) The association between reduced knee joint proprioception and medial meniscal abnormalities using MRI in knee osteoarthritis: results from the Amsterdam osteoarthritis cohort. Osteoarthr Cartilage 21:676–681CrossRefGoogle Scholar

Wärnberg J, Cunningham K, Romeo J, Marcos A (2010) Physical activity, exercise and low-grade systemic inflammation. Proc Nutr Soc 69:400–406. doi:10.1017/s0029665110001928CrossRefPubMedGoogle Scholar

Zhang W et al (2010) OARSI recommendations for the management of hip and knee osteoarthritis: part III: changes in evidence following systematic cumulative update of research published through January 2009. Osteoarthr Cartil 18:476–499. doi:10.1016/j.joca.2010.01.013CrossRefPubMedGoogle Scholar

Zhang S-L, Liu H-Q, Xu X-Z, Zhi J, Geng J-J, Chen J (2013) Effects of exercise therapy on knee joint function and synovial fluid cytokine levels in patients with knee osteoarthritis. Mol Med Report 7:183–186