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The effect of aerobic walking and lower body resistance exercise on serum COMP and hyaluronan, in both males and females

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Abstract

Purpose: To compare the serum cartilage oligomeric matrix protein (COMP) and hyaluronan (HA) response to walking (high-repetition loading) and resistance training exercise (low-repetition loading) in males and females. *Methods*: 15 males (age: 28±6 years; BMI: 24±2; mean±SD) and 15 females (age: 26±4 years; BMI: 23±2) completed both a 40-minute walk at 80% of maximum heart rate and a 40-minute lower-body resistance training protocol, separated by a minimum of 48 hours. Serum COMP and HA were determined at rest, immediately post, and 30-minutes post exercise. Resting femoral cartilage thickness was also measured using ultrasonography. *Results*: COMP increased following walking (28.9%; P<0.001) and resistance training exercise (26.0%; P<0.001), remaining above baseline post-exercise following walking (mean difference: +28.3 ng/ml; 95% CI 3.8-52.8 ng/ml; P=0.02). Although the exercise response did not differ for gender, COMP concentrations were higher in males than in females at all time points (all, P<0.001). In contrast, HA concentrations did not change following either modality of exercise. However, females demonstrated higher HA pre-exercise (37.7±17.8 vs 26.2±12.8 ng/ml; P=0.006) and immediately post exercise (38.0±19.0 vs 28.2±15.5 ng/ml; P=0.033) compared to men. Finally, following adjustment for body size, femoral cartilage thickness was greater in men compared to women (notch: 2.66 vs 1.74 mm, P<0.001). *Conclusion***:** The effect of a single bout of lower body exercise on serum COMP and HA is independent of exercise modality in healthy men and women. Furthermore, having thicker femoral cartilage and higher baseline COMP in males does not appear to influence how the cartilage responds to exercise.

Key words: joint loading; ultrasound; femoral cartilage thickness; cartilage metabolism

Abbreviations:

BMI	Body mass index
COMP	Cartilage oligomeric matrix protein
ELISA	Enzyme-linked immunosorbent assay
HA	Hyaluronan
HRmax	Maximum heart rate
OA	Osteoarthritis
RM	Repetition maximum
US	Ultrasound
VO_{2max}	Maximum oxygen uptake

Introduction

Understanding the influence that physical exercise has on cartilage structure and function is important to improve knowledge of the potential benefits and / or risks that physical activity have in relation to development and progression of cartilage atrophy and degenerative joint disease. Serum biomarkers have the potential to be used to monitor the health of joint cartilage or detect underlying pathology and are understood to reflect the release of molecules or molecular fragments from the loaded joint (Bauer et al. 2006). For example, elevated serum COMP in response to exercise have been associated with decreases in cartilage volume in healthy trained runners (Kersting et al. 2005) and a long-term reduction in cartilage thickness in patients with OA (osteoarthritis) (Erhart-Hledik et al. 2012). We have previously demonstrated a comparable transient increase in cartilage oligomeric matrix protein (COMP) and lubricin, (biomarkers associated with cartilage catabolism/metabolism and lubrication, respectively) in response to a single bout of approximately 40 minutes of weight bearing exercise (running) and non-weight bearing exercise (cycling) (Roberts et al. 2016). Taken together, these findings suggest that an acute increase in serum COMP and lubricin is a normal healthy response to exercise, but that a minimal difference exists in the response to aerobic weight bearing and aerobic non-weight bearing exercise. This exercise-induced response is in accordance with several previous studies that have also shown an increase in serum COMP following activities such as walking and running, which involve joint loading that are high in loading frequency but relatively low in loading amplitude (Mündermann et al. 2005; Mündermann et al. 2009; Niehoff et al. 2010; Celik et al. 2013; Denning et al. 2015). The serum COMP response to exercise is typically greater and associated with longer recovery times, following prolonged bouts of exercise (Kim et al. 2009). The magnitude of increase has also been associated with certain joint mechanics and joint loading frequency (Denning et al. 2016). However, in contrast, a recent study found that mechanically increasing knee joint loading during running did not significantly change the response to a 30-minute run (Firner et al. 2018).

In contrast to walking or running, it is largely unknown whether knee joint loading through resistance training results in a similar response in serum biomarkers. This gap in knowledge is due to relatively few studies having explored the responses of serum biomarkers to high load and low repetition knee exercise, e.g. resistance exercise. Studies that have explored this report mixed findings. In healthy young people slow deep knee bends did not result in an acute increase serum COMP (Niehoff et al. 2010) and in rheumatoid arthritis patients acute lower body resistance exercise involving 3 sets of 8 repetitions did not result in a significant increase in serum COMP (Law et al. 2015). In contrast, drop jumps in healthy individuals have been shown to result in a

significant increase in serum COMP (Niehoff et al. 2011; Behringer et al. 2014). To date, no study has explored the serum biomarker response to a typical lower-body resistance exercise in healthy individuals, i.e. as part of a typical regime for prevention and treatment of a wide range of diseases, including those specific to knee joint.

Previous studies have mostly explored differences in joint loading protocols have been related to serum COMP only. Hyaluronan (HA), a high molecular weight glycosaminoglycan, composed of alternating subunits of glucosamine and glucuronic acid, is a major component of the connective tissue (Seebeck and Haima 2013) and is a promising biomarker. Serum HA has previously been associated with OA (Elliott et al. 2005) and has been linked with synovial inflammation and cartilage degradation (Garnero et al. 2001). Therefore, together with serum COMP, serum HA may provide an additional indicator of the status of the joint. However, there is currently limited research that has addressed serum HA concentrations and its relationship with joint loading. This is crucial to establish its reliability and future use as a clinical biomarker. Moreover, the potential relationship between changes in serum biomarkers and cartilage structure may assist in optimising knee joint health and preventing adverse knee joint damage.

Women have previously been shown to have both reduced femoral cartilage thickness (Ozcakar et al. 2014) and lower levels of serum COMP compared to men (Jordan et al. 2003; Mundermann et al. 2005; Verma and Dalal, 2013). It has been suggested that differences in cartilage between men and women relate to a smaller body size and reduced overall cartilage (Ding et al. 2003). Crucially, women have a greater risk of knee injuries in comparison to men, while older women also have a greater risk of developing OA compared to men (Arendt and Dick, 1995; Felson et al. 1987). Differences between men and women in neuromuscular and biomechanical loading patterns may possibly influencing their susceptibility to injury and OA (Russell et al. 2006). Consequently, determining whether the response of serum biomarkers following acute exercise is different in women is of interest, and to date, remains untested.

Therefore, the primary aim of this study was to compare the biomarker response to two commonly prescribed different types of exercise modalities i.e. resistance training exercise (high-load low frequency) and aerobic walking (low-load high frequency). Secondary aims of this study were to determine whether sex influences cartilage thicknesses and serum biomarkers.

It was hypothesised that acute loading exercise would result in a comparable increase in serum biomarkers, following a bout of 40 minutes of walking and following a bout of isolated lower body resistance exercise. We also hypothesised that women would demonstrate reduced baseline cartilage thickness and reduced baseline

levels of serum COMP and HA compared to men, but that differences at would not remain once body size was taken into consideration as a covariate. The final hypothesis was that sex would not alter the exercise response of serum biomarkers to acute loading.

Methodology

Participants

A group of healthy male and a group of healthy female individuals, which were well matched for age, body mass index (BMI) and physical activity history were recruited. Participants were targeted through word of mouth, poster advertisement, generic emails, and social media from the Bangor University community and the surrounding North Wales area. The inclusion for entry to the study included being: (i) male or female (ii) aged between 18-40 years (iii) BMI of < 30 kg / m^2. Exclusion criteria for both groups included: (i) diagnosed OA, rheumatoid arthritis, or other inflammatory disease, (ii) history of knee malalignment (varus / valgus) greater than 15°, (iii) previous knee injury (including meniscus tear or ligament damage or tear), (iv) recent fracture of lower extremity (within last 6 months), (v) current or prior use of lipid-lowering therapy (e.g. fibric acids, nicotinic acids, bile acid sequestrates, fish oils), corticosteroid injections, or high dose oral steroids (vi) current or past use (this includes single use in last week or daily use in last 3 months) of non-steroidal anti-inflammatory drugs (vii) current or past (within last four weeks) glucosamine and / or chondroitin supplementation use, (viii) additional exclusion factors included muscle weakness and musculoskeletal / orthopaedic problems prohibiting exercise participation. Exclusions specific to the female group included: (i) pregnancy (ii) menopausal.

Experimental protocol

In this two group, randomised, crossover designed study, participants were required to visit the School of Sport, Health and Exercise Science at Bangor University on three separate occasions:

Visit 1

During this initial visit, participants were given a full verbal explanation of all procedures and given the opportunity to ask questions, prior to completing both medical and physical activity questionnaires, including the International Physical Activity Questionnaire (IPAQ) 7-day (long version) questionnaire (Craig et al. 2003) and a modified version of the Measurement of a Person's Habitual Physical Activity questionnaire (Baecke et al. 1982) as used and validated by Pols et al. (1995). Following a period of 30 minutes of seated rest, femoral

cartilage thickness was assessed using ultrasonography before the measurement of body weight and height. Participants subsequently completed a submaximal treadmill (HPcosmos Mercury 4 Med, Nussdorf-Traunstein, Germany) walking protocol to estimate maximum oxygen uptake (VO_{2max}) (Ebbeling et al. 1991). This protocol consisted of an initial 4-minute walk at a brisk but comfortable walking speed (3 and 4.5 mph) with heart rate within 50-70% of maximum heart rate (HRmax). If heart rate was not within the required range after the first minute of exercise the speed was adjusted accordingly. Following the initial 4-minute period, the gradient was increased to 5% for the subsequent 4 minutes. Heart rate and rate of perceived exertion was monitored throughout. In addition, participants who did not reach an intensity of 80% HRmax during this submaximal test were required to complete further incremental walking exercise bout using the treadmill until 80% HRmax or a rate of perceived exertion of 15 was achieved. This allowed the determination of the appropriate exercise intensity (walking speed and incline) for the walking exercise intervention. Finally, following a minimum of 15 minutes of recovery, participants completed an 8-repeitition maximum (RM) test of the leg press, leg extension and leg curl exercises (Whaley et al. 2006). This 8-RM test allowed the 1-RM to be accurately estimated using a regression equation (Brzycki 1993). The resistance training protocol followed the American College of Sports Medicine (ACSM) guidelines for muscle strength training by utilising 80% of the 1-RM for both the leg press, leg extension, and leg curl exercises. All exercises were performed in the departmental laboratory using commercially available leg press machine (HUR Main Line Leg Press 3540) and seated leg extension/curl weights machines (Powersport International Limited, 1986).

Visit 2 and 3

Visit 2 and 3 consisted of the exercise trials. Importantly, the order in which the exercise bouts were randomized. On arrival to the laboratory, participants were required to rest for 30 minutes before providing a baseline blood sample. Participants subsequently completed either an aerobic walking protocol, or a lower body resistance exercise protocol. Upon immediate completion of the exercise trial, a second blood sample was obtained. Lastly, following 30 minutes of seated rest post exercise a final blood sample was obtained. Blood samples (6 ml) were obtained from an antecubital vein, allowed to clot for a period of 60 minutes at room temperature, prior to being centrifuged for 15 min at 1000 × gravity as specified by the enzyme-linked immunosorbent assay (ELISA) kit inserts. Serum was subsequently aliquoted into eppendorf containers and immediately stored at -80°C until later analysis.

Serum COMP and HA analysis

Serum COMP was analysed using a commercially available sandwich ELISA (Human COMP ELISA kit KA0021, Abnova Corporation, Taiwan) as previously described (Law et al. 2015; Roberts et al. 2016). Likewise, serum hyaluronic acid was analysed using a commercially available competitive ELISA (Hyaluronic Acid (HA) ELISA Kit ABIN1873289, Cloud-Clone Corp, USA). Mean intra-assay coefficient of variation was 6.6% and 7.0% for serum COMP and HA, respectively, and the R^2 curve fit was > 0.99 across all analyses.

Ultrasonography

The ultrasound (US) assessment was performed using a 12 MHz linear-array probe (Esaote S.P.A. MyLab50 ultrasound, Firenze, Italy) and acoustic coupling gel (Aquasonic 100, Parker Laboratories, Inc, Fairfield, NJ, USA) following a period of between 15-30 minutes of seated rest. With participants lying in a supine position and with the knee maximally flexed, the superior margin of the patellar was located and a line was marked on the skin using a washable marker at the point immediately above the superior margin of the patellar and at 1 cm intervals in a superior direction. The transducer was placed in a supra-patella transverse position, perpendicular to the bone surface and orientated to optimise the US image (Naredo et al. 2009; Özçakar et al. 2014). The location at which the cartilage thickness of the intercondyle notch appeared greatest was marked on the skin and recorded to enable the examiner to return the transducer to the exact location for all subsequent scans. The same researcher performed all ultrasonography scans following training by a consultant rheumatologist with expertise using this technique.

US images were analysed by 'Image J' software (Image J, National Institute of Health, Bethesda, MD, USA) to determine the minimal cartilage thickness. The distance from the thin hyperechoic line formed at the synovial space-cartilage border to the line formed at the cartilage-bone border was used to measure minimal cartilage thickness at the lateral condyle, medial condyle and intercondylar notch (Özçakar et al. 2014). Anatomic reference points used in the present study corresponded to the midpoint of the intercondyle notch and 1 cm apart in the medial and lateral directions were used as an estimate of the medial and lateral condyle cartilage thickness, respectively (Roberts et al. 2016). Naredo and colleagues previously demonstrated good reproducibility in femoral cartilage thickness measurement (ICC = 0.832, 0.701 and 0.696 for the intercondylar notch, medial condyle and lateral condyle, respectively) when using comparable anatomical reference points (Naredo et al. 2009). Prior to analysis, all images were de-identified by second researcher for blinded analysis. Based on the pixel resolution (15.8 pixels /mm) of the images captured by ultrasonography, the ImageJ software allowed images to be measured to an accuracy of greater than one-tenth off a mm, or more specifically, one

pixel was equal to 0.06 mm. The cartilage thickness of each image was measured in triplicate and an average of the three measurements was used for all data analysis. As required, the image contrast was adjusted to assist in appropriately identifying the hyperechoic line formed at the synovial space-cartilage border to the line formed at the cartilage-bone border.

Exercise intervention

The exercise protocols were designed to offer an aerobic and resistance training stimulus that was matched for time. Importantly, this study adopted a pragmatic approach that aimed to assess the impact of 'real-world' exercise sessions on markers associated with knee joint cartilage. The aerobic walking protocol was designed to offer a low load, high frequency modality. While in contrast, the resistance training protocols offered a high load, low frequency modality. Additionally, heart rate was assessed at regular intervals throughout both exercise protocols, Blood lactate was also assessed at rest and following completion of each exercise intervention. Heart rate and blood lactate were used to monitor the stress associated with the activity and to aid the comparison of each activity. Blood lactate was assessed via capillary blood sampling (5 ul), collected from the fingertip and immediately analysed using a portable lactate analyser (LactatePro, Arkray, Japan).

Walking protocol

The walking protocol consisted of 40 minutes of treadmill walking exercise. The exercise intensity was derived from the walking protocol conducted during the first visit to the department. As appropriate, the speed and incline were adjusted throughout to ensure all participants maintained an intensity as close to 80% HRmax as possible.

Resistance training protocol

This session included 40 minutes of lower-body resistance training. This training aimed to specifically target muscles around the knee joint, optimising high load, low frequency loading of the knee. In total, five exercises including leg press, leg extension, leg curls, squats and alternate lunges were utilised. Each resistance machine exercises (leg press, leg extension, and leg curl) consisted of one set of 15 repetitions with half-load, prior to completing three sets of eight repetitions at 80% 1-RM. Similarly, both the squat and alternate lunge exercises, involved completing one set of 15 body weight repetitions, prior to completing three sets of eight repetitions using dumbbells of 10% body weight. A minimum of one minute of rest was provided between sets. All

participants were supervised throughout the session and informed to complete the exercises in a controlled manner, with correct exercise form, and with an emphasis on limiting the aerobic exercise response.

Statistical analysis

Statistical analyses were performed utilising statistical analysis software [SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA)]. A three-factor mixed design was used to assess the effect of exercise intervention (walking vs resistance training), sex (male vs female) and time (pre, immediately post exercise, and 30 minutes post exercise), on each dependent variable (serum COMP and serum HA). Significant interactions and/or main effects were analysed post hoc using Bonferroni-corrected t-tests where appropriate. Independent sample t-tests were used to assess differences between males and females. Independent sample t-tests were also conducted to determine whether differences in mean cartilage thickness exists between male and female participants at each location (right intercondyle notch, lateral condyle, medial condyle). As appropriate analysis of covariance (ANCOVA) analyses was subsequently used to adjust for differences in body size. For this analysis, a composite variable reduced from weight and height (weight x height: Blazek et al. 2014) was used. Normality of data was explored by visual inspection of Q–Q plots and through analysis of the model's residuals and outliers were removed as necessary. All figures and tables are presented as mean ± SD, with statistical significance set as ($P < 0.05$).

Sample size calculations were performed using G*Power 3.1.3 (Heinrich- Heine-University) software (Faul et al. 2007). Sample size calculations were completed using serum COMP as the primary outcome variable. To establish whether an exercise-induced increase exists, a minimum sample size of 14 participants will be required (5% alpha, 80% beta) to detect an exercise-induced increase in serum COMP. This data was based on the expected magnitude of change of serum COMP following a drop jump intervention (Behringer et al. 2014). To test for differences between sex, a minimum of 4 participants per group (calculated by *priori* analysis using G-Power software [5% alpha, 80% beta] was required. This data is based on baseline differences in serum COMP previously observed between men and women (Mundermann et al. 2005). To strengthen conclusions, this study aimed to recruit a well-matched sample of 15 healthy males and 15 healthy females, aged between 18-40 years.

Results

Thirty participants (male n = 15; female n = 15) matched for age and BMI were included within the analyses. Anthropometric, physical characteristics training habits for both groups are shown in Table 1. Males were significantly taller and heavier than female participants. Familiarisation tests also identified that males had both a greater estimated VO_{2max} and absolute lower-body muscle strength. Training habits were comparable between groups for the number of exercise training years, average number of days, average number of hours completed per week, physical activity over the last 7 days (7 day IPAQ) and physical activity over the last 12 months. Overall, participants studied can be described as healthy, recreationally active males and females that provide a good opportunity for comparison between groups.

Heart Rate and Lactate Responses

The average heart rate (as a percentage of age-predicted maximum) for the resistance training exercise and the walking exercise was, 55 ± 5% and 76 ± 6%, respectively. Blood lactate concentrations significantly increased following resistance training exercise (pre: 1.7 ± 0.7 vs post: 4.3 ± 2.0 mmol/L, $P < 0.001$). In contrast, despite an increase following the aerobic walking exercise protocol (pre: 1.6 ± 0.6 vs 2.2 ± 1.5 mmol/L) this did not reach significance ($P = 0.07$). No difference was observed between sexes.

Serum COMP

Mean serum COMP significantly increased from baseline following both modalities of exercise. Following walking, serum COMP concentration increased by 28.9% (baseline: 490.3 ± 200.2 ng/ml; immediately post exercise: 631.8 ± 223.4 ng/ml) and following resistance training, serum COMP concentrations increased by 26.0% (baseline: 501.8 ± 180.0 ng/ml; immediately post exercise: 632.5 ± 196.0 ng/ml). Following a period of 30 minutes of seated rest, serum COMP concentrations returned towards baseline (walking: 518.6 ± 210.8 ng/ml; resistance training group: 473.3 ± 169.1 ng/ml). Post hoc analyses revealed that following walking, serum COMP concentrations remained elevated compared to baseline (mean difference: 28.3 ng/ml; 95% CI 3.8 to 52.8 ng/ml; $P = 0.02$). In contrast, following resistance training, serum COMP dropped below baseline concentrations (mean difference: 28.4 ng/ml; 95% CI 0.8 – 56.0; $P = 0.04$). However, absolute concentrations (30-minute post exercise) between the walking group and resistance training group did not significantly differ (518.6 ± 210.2 vs 473.3 ± 169.1 ng/ml; $P = 0.39$). Likewise, absolute serum COMP concentration did not differ between modalities at baseline, or immediately post exercise (Figure 1). The change in serum COMP concentration over time was comparable between males and females (Figure 1). However, serum COMP concentrations were higher in males than in females at baseline (595.0 ± 138.7 vs 395.4 ± 174.1 ng/ml),

immediately post exercise (751.6 ± 167.0 vs 517.6 ± 204.8 ng/ml) and 30-minutes post exercise (591.2 ± 143.4 vs 400.7 ± 185.5 ng/ml) (all, $P < 0.001$).

Serum HA

Mean serum HA did not significantly change following either walking or resistance exercise (all, $P > 0.05$). Furthermore, there was no difference over time between males and females. However, mean serum HA concentrations were higher in females compared to males at baseline (37.7 ± 17.8 vs 26.2 ± 12.8 ng/ml, $P = 0.006$), immediately post exercise (38.0 ± 19.0 vs 28.2 ± 15.5 ng/ml, $P = 0.033$) and at 30-minutes post exercise (36.0 ± 19.4 vs 28.2 ± 15.9 ng/ml, $P = 0.107$) (Figure 2).

Cartilage thickness

The assessment of cartilage thickness revealed that males had significantly thicker cartilage compared to females at the intercondyle notch, medial condyle and lateral condyle (Table 2). The greatest mean difference in cartilage thickness was at the intercondyle notch, followed by the medial and lateral condyles (Table 2). Furthermore, there was a significant difference between males and females in mean cartilage thickness at the intercondyle notch [(2.66 (95% CI 2.44 to 2.87) vs 1.74 mm (95% CI 1.52 to 1.97), $P = 0.001$] whilst adjusting for body size using a composite variable that considered both the height and weight of participants. ANCOVA analyses were not completed for the lateral and medial condyle due to violations in key test assumptions.

Discussion

This study demonstrated for the first time that acute walking and resistance exercise result in a similar temporary increase in serum COMP. This study is also the first to directly establish that the serum COMP response to exercise is unaffected by sex. However, in contrast to our hypotheses, serum HA remained unaffected by either bout of exercise. Moreover, although sex was found to be unrelated to the exercise response, men were found to have higher level of serum COMP, lower levels of serum HA and revealed thicker femoral cartilage at all locations compared to women.

Several studies have previously demonstrated that walking results in an acute increase in serum COMP (Mündermann et al. 2005; Celik et al. 2013; Denning et al. 2016). The exact mechanism contributing to the increase in serum COMP is unknown, however it is understood to be a physiological response that reflects increased healthy cartilage turnover or metabolism, rather than cartilage degradation. It would seem

unreasonable to expect cartilage damage from acute walking or resistance exercise in a group of healthy individuals. The increase in serum COMP following walking in the present study (+28.9%) was generally greater than previously reported by Mündermann et al. (2005) and Denning et al. (2016) following walking, +9.7% and +5.27%, respectively. This difference may be due to the exercise duration being shorter (Mündermann et al. 2005), or due to the self-paced nature of the walking exercise (Denning et al. 2016). Furthermore, the accumulative load on the knee joint between walking studies may be influenced through increases in walking speed, which have previously been associated with increased serum COMP response (Denning et al. 2015). Although all the participants were healthy and of similar age, differences between studies may also relate to variations in the study cohorts and in training status. The increase in serum COMP following walking in the present study (+28.9%) was comparable to the increase in serum COMP following a similar duration (approx. 40 minutes) bout of vigorous cycling (+32.1%) and higher than vigorous bout of running (+14.2%) in trained individuals (Roberts et al. 2016). A greater increase following walking compared to vigorous running was somewhat surprising given that the overall load following running was higher (exercise time: 40 min vs a 46 min (average); intensity of exercise: 76% vs 90.4% HRmax; distance covered: 4.2 vs 10 km). Another plausible possibility for this finding relates to training status. For example, the participants in the present study were generally less trained than the individuals who participated in our previous work (Roberts et al. 2016). Others have also indicated that exercise training may lessen the acute serum COMP response to acute walking exercise (Celik et al. 2013; Firner et al. 2018), potentially by consolidating the cartilage matrix and consequently reducing release of COMP from the extracellular matrix and eventually into the circulation.

The present study was the first to demonstrate that resistance exercise and walking, which were matched for exercise duration, result in a very similar increase in serum COMP concentration. This suggests that cartilage responds in a similar manner to activities that vary in the type, frequency and in the region of loading in healthy individuals. Moreover, this supports previous research that demonstrated a similar increase in serum COMP when comparing running and drop jumps (Niehoff et al. 2011). Despite returning toward baseline concentrations, serum COMP remained significantly elevated 30 minutes post exercise following walking. This finding supports previous research that suggests that loading frequency and kinematics may be an important factor in COMP release and duration (Piscoya et al. 2005; Denning et al. 2016; Firner et al. 2018). A higher relative post exercise response has previously been associated with increased future cartilage loss (Erhart-Hledik et al. 2012). One possibility is that walking has a greater impact on cartilage metabolism via the high frequency loading and may be less beneficial compared to resistance training for future cartilage health. However, since

walking is a low impact activity, and that the participants were young and healthy, it would seem unreasonable to suggest that this type of activity was causing any damage. It would instead appear more likely that differences in the post exercise response relate to variations in the triggering mechanisms of cartilage turnover / metabolism. Further studies to determine the timeframe for post-exercise recovery to baseline COMP levels and how meaningful this is to future cartilage health is still warranted.

The present study also provides evidence that differences exist in baseline concentrations of serum COMP as well as between femoral cartilage thickness in males and females. Baseline serum COMP concentrations have previously been shown to be lower in females compared to males (Jordan et al. 2003). This may be related to an increased joint size, or to increased total cartilage, meniscal and tendon size in men compared to women (Jordan et al. 2003). Likewise, smaller knee articular cartilage size in women may also relate to their smaller body and joint size in comparison to men (Ding et al. 2003). The present study provides further normative data of the differences that exist between sexes in femoral cartilage thickness of healthy knee joints. The comparable response of serum COMP to exercise between males and females, as well as previous work that has demonstrated a similar cartilage deformation behaviour (Hudelmaier et al. 2001), indicate that differences in baseline thickness are unlikely to relate to any functional difference in young healthy individuals who are matched for aged and BMI, and whom have very similar levels of training history and fitness. However, longitudinal research is still required to investigate whether a reduction in baseline femoral cartilage thickness play a role in the future incidence of injury and / or OA, particularly among women.

The present study found no evidence of any exercise-induced change in serum HA in healthy individuals. A previous report by Engström-Laurent and Hällgren (1987) also found no evidence of a change in HA following moderate intensity cycling, although a bout of heavy cycling exercise resulted in a modest increase in HA. In contrast, moderate acute exercise in patients with rheumatoid arthritis elicited a large increase in serum HA (Engström-Laurent and Hällgren 1987). A greater exercise-related increase in rheumatoid arthritis patients was related to synovitis mass, suggesting that joint inflammation may be key in the synthesis and accumulation of serum HA. In a separate study, plasma HA has been shown to rise with exercise time and demonstrate an exponential increase with increasing exercise intensity in healthy individuals (Hinghofer-Szalkay et al. 2002). As with serum COMP, any exercise-induced change in serum HA in healthy individuals is understood to be due to a physiological response rather than a change in structure. Based on the available literature, it is possible that the HA did not change due to no, or limited, fragments in the knee joint, or that the exercise duration and intensity used in the present study was simply insufficient to increase serum HA in healthy men and women.

Moreover, it is possible that difference in the exercise-response between serum COMP and HA relate to either a greater release of COMP from the joint, differences in the transport across the joint membrane and into the systemic circulation, and/or differences in the clearance of biomarkers by the liver and kidney. Moreover, the present study found that baseline concentrations were like previously reported values in healthy individuals and lower than those reported in individuals with joint disease, including OA (Criscione et al. 2005; Wakitani et al. 2007) and rheumatoid arthritis patients (Engström-Laurent and Hällgren 1987). Surprisingly, the present study found higher serum concentrations of HA in women compared to men. Serum HA has previously been shown to be influenced by various individual factors, including sex, with higher serum HA concentrations typically found in men compared to women (Elliott et al. 2005). There is no clear explanation for the higher HA concentrations observed in this female population.

It is essential to acknowledge that it remains to be determined whether increases in serum COMP following exercise reflect cartilage turnover (Saxne and Heinegard 1992), tissue damage (Neidhart et al. 2000), or an increase in the transport/removal from the joint into the blood (Helmark et al. 2012). Moreover, a recent study found that increased serum COMP following exercise corresponded with a decrease in synovial fluid COMP (Hyldahl et al. 2016). This supports previous findings indicating that exercise facilitates the movement of COMP from within the joint into the circulation (Helmark et al. 2012), possibly due to an increase in intra-articular pressure (Levick and McDonald 1995). Moreover, in relation to serum HA, the unaffected serum concentration may indicate that HA remained within the joint despite an increase in exercise. Given that HA is used as a therapeutic intervention for OA (Shimizu et al. 2010) and considered an important joint lubricant (Schmidt et al. 2007), this may be a positive finding. Although the knowledge within the area of biomarkers is constantly advancing, further studies are required to determine how the response of serum biomarkers to loading reflects changes at the joint level.

The present study provides some new insights into the effect of exercise modality and sex on several cartilage biomarkers. However, it must also be acknowledged that this study does have some limitations. Objective measures of load on the knee joints during each of the exercise modalities were not undertaken. Despite attempting to provide a comparable exercise bout in relation to exercise time and intensity, resistance training result did result in a significant increase in blood lactate concentration, which was not observed following walking. This suggests that the metabolic stress associated with 40 minutes of resistance training may be higher than 40 minutes of walking. Moreover, although joint structures are understood to be a key source of COMP and HA, we must recognise that neither COMP or HA are produced exclusively within the knee joint and other

tissues may contribute to the concentrations observed in this study. Furthermore, in relation to the sex-differences observed in both serum COMP and serum HA, we must recognise that to date it remains unknown whether menstrual cycle phase, or use of oral contraceptives, significantly influences the serum concentration of these biomarkers, both of which were not controlled for in the present study. Furthermore, while we asked participants about comorbidities, we do not have objective data on liver and kidney function, both of which may particularly affect serum HA levels. Crucially, despite strict methodological standardisation in line with previous research, caution is required when comparing concentrations between experimental studies and when comparing absolute values with other published research studies.

Conclusion

The current study suggests that an acute bout of either walking or resistance exercise stimulates an increase in cartilage metabolism. This study also provides evidence to suggest that these exercise modalities, which comprise of markedly different loading patterns, effect the cartilage in a similar manner and do not differ between sexes. However, the post exercise response of serum COMP following walking suggests that loading frequency may be an important factor in COMP release in healthy individuals. To progress current understanding further, longitudinal studies should attempt to determine how cartilage is affected by regular long-term acute increases in serum biomarkers and whether the response to exercise changes with training. In addition, future studies should also attempt to provide additional detail of biomarker kinetics between synovial fluid and serum concentrations, particularly in relation to HA.

Conflicts of interest

The authors disclose that no funding was received for this work and have no conflicts of interest to declare.

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Table 1. Baseline anthropometric, physical characteristics and exercise habits of participants in the two groups

	Male		**Female**	
Variable	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Age (years)	28±6	19-40	26±4	20-33
Height (metres)	1.77±0.04	1.72-1.84	1.67±0.07**	1.51-1.78
Body mass (kg)	77±7	62-88	64±9**	40-82
BMI (kg/m^2)	24±2	20-27	23±2	18-26
Estimated VO_{2max}	56±4	50-65	48±3**	44-54
Leg press (8RM)	199 ± 32	150-250	150±31**	110-230
Leg extension (8RM) (kg)	36±13	20-65	21±7**	10-40
Leg curl (8RM) (kg)	17±7	5-35	10±5**	5-25
Lifetime training experience (years)	11±7	2-29	11±7	2-20
Weekly frequency (day/week)	3±2	0-7	4±2	0-6
Training duration (hr/week)	3±3	0-10	5±3	0-12
7 day IPAQ (MET min/week)	4096±3701	777-14838	2952±2005	1152-8748
12 month physical activity index	8.4±1.0	6.9-10.1	7.7±1.4	5.1-9.3

MET = metabolic equivalent; Significant difference between groups (* P < 0.05; **P < 0.01). Data are means ± standard deviation

Table 2. Mean cartilage thickness (mm) in both males and females

	Male		**Female**	
Variable	**Mean +/- SD**	**Range**	**Mean +/- SD**	**Range**
Cartilage thickness (mm)				
Notch	2.50±0.25	2.04-2.98	1.91±0.25 **	1.57-2.47
Lateral	2.18±0.22	1.79-2.49	1.82±0.28 **	1.31-2.29
Medial	2.18±0.37	1.52-2.73	1.74±0.13 **	1.55-1.93

Significant difference between groups (* P < 0.05; ** P < 0.01). Data are means ± standard deviation

Figure captions:

Fig. 1 Mean serum COMP concentration, pre-exercise, immediately post, and at 30 min post 40 min of walking or 40 mins of resistance training exercise in a) males and b) females. * and ** = significant difference over time at $P < 0.05$ level and $P < 0.01$ level, respectively. Significance marked above data line represents walking group and below represents resistance training group. Data are means ± standard deviation

Fig. 2 Mean serum HA concentration, pre-exercise, immediately post, and at 30 min post 40 min of walking or 40 mins of resistance training exercise in a) males and b) females. Data are means ± standard deviation