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

3

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23

1 **Abstract**

2 *Purpose:* To compare the serum cartilage oligomeric matrix protein (COMP) and hyaluronan (HA) response to
3 walking (high-repetition loading) and resistance training exercise (low-repetition loading) in males and females.
4 *Methods:* 15 males (age: 28±6 years; BMI: 24±2; mean±SD) and 15 females (age: 26±4 years; BMI: 23±2)
5 completed both a 40-minute walk at 80% of maximum heart rate and a 40-minute lower-body resistance training
6 protocol, separated by a minimum of 48 hours. Serum COMP and HA were determined at rest, immediately
7 post, and 30-minutes post exercise. Resting femoral cartilage thickness was also measured using
8 ultrasonography. *Results:* COMP increased following walking (28.9%; P<0.001) and resistance training exercise
9 (26.0%; P<0.001), remaining above baseline post-exercise following walking (mean difference: +28.3 ng/ml;
10 95% CI 3.8-52.8 ng/ml; P=0.02). Although the exercise response did not differ for gender, COMP
11 concentrations were higher in males than in females at all time points (all, P<0.001). In contrast, HA
12 concentrations did not change following either modality of exercise. However, females demonstrated higher HA
13 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
14 ng/ml; P=0.033) compared to men. Finally, following adjustment for body size, femoral cartilage thickness was
15 greater in men compared to women (notch: 2.66 vs 1.74 mm, P<0.001). *Conclusion:* The effect of a single bout
16 of lower body exercise on serum COMP and HA is independent of exercise modality in healthy men and
17 women. Furthermore, having thicker femoral cartilage and higher baseline COMP in males does not appear to
18 influence how the cartilage responds to exercise.

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

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1 **Abbreviations:**

2	BMI	Body mass index
3	COMP	Cartilage oligomeric matrix protein
4	ELISA	Enzyme-linked immunosorbent assay
5	HA	Hyaluronan
6	HRmax	Maximum heart rate
7	OA	Osteoarthritis
8	RM	Repetition maximum
9	US	Ultrasound
10	VO _{2max}	Maximum oxygen uptake

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1 **Introduction**

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

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

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

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

14 Women have previously been shown to have both reduced femoral cartilage thickness (Ozcakar et al. 2014) and
15 lower levels of serum COMP compared to men (Jordan et al. 2003; Mundermann et al. 2005; Verma and Dalal,
16 2013). It has been suggested that differences in cartilage between men and women relate to a smaller body size
17 and reduced overall cartilage (Ding et al. 2003). Crucially, women have a greater risk of knee injuries in
18 comparison to men, while older women also have a greater risk of developing OA compared to men (Arendt and
19 Dick, 1995; Felson et al. 1987). Differences between men and women in neuromuscular and biomechanical
20 loading patterns may possibly influencing their susceptibility to injury and OA (Russell et al. 2006).
21 Consequently, determining whether the response of serum biomarkers following acute exercise is different in
22 women is of interest, and to date, remains untested.

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

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

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

4 **Methodology**

5 Participants

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

20 Experimental protocol

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

23 *Visit 1*

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

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

19 *Visit 2 and 3*

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

29 *Serum COMP and HA analysis*

1 Serum COMP was analysed using a commercially available sandwich ELISA (Human COMP ELISA kit
2 KA0021, Abnova Corporation, Taiwan) as previously described (Law et al. 2015; Roberts et al. 2016).
3 Likewise, serum hyaluronic acid was analysed using a commercially available competitive ELISA (Hyaluronic
4 Acid (HA) ELISA Kit ABIN1873289, Cloud-Clone Corp, USA). Mean intra-assay coefficient of variation was
5 6.6% and 7.0% for serum COMP and HA, respectively, and the R^2 curve fit was > 0.99 across all analyses.

6 Ultrasonography

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

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

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

5 *Exercise intervention*

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

15 *Walking protocol*

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

20 *Resistance training protocol*

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

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

3

4 Statistical analysis

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

18 Sample size calculations were performed using G*Power 3.1.3 (Heinrich-Heine-University) software (Faul et
19 al. 2007). Sample size calculations were completed using serum COMP as the primary outcome variable. To
20 establish whether an exercise-induced increase exists, a minimum sample size of 14 participants will be required
21 (5% alpha, 80% beta) to detect an exercise-induced increase in serum COMP. This data was based on the
22 expected magnitude of change of serum COMP following a drop jump intervention (Behringer et al. 2014). To
23 test for differences between sex, a minimum of 4 participants per group (calculated by *priori* analysis using G-
24 Power software [5% alpha, 80% beta] was required. This data is based on baseline differences in serum COMP
25 previously observed between men and women (Mundermann et al. 2005). To strengthen conclusions, this study
26 aimed to recruit a well-matched sample of 15 healthy males and 15 healthy females, aged between 18-40 years.

27 Results

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

9 Heart Rate and Lactate Responses

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

15 Serum COMP

16 Mean serum COMP significantly increased from baseline following both modalities of exercise. Following
17 walking, serum COMP concentration increased by 28.9% (baseline: 490.3 ± 200.2 ng/ml; immediately post
18 exercise: 631.8 ± 223.4 ng/ml) and following resistance training, serum COMP concentrations increased by
19 26.0% (baseline: 501.8 ± 180.0 ng/ml; immediately post exercise: 632.5 ± 196.0 ng/ml). Following a period of
20 30 minutes of seated rest, serum COMP concentrations returned towards baseline (walking: 518.6 ± 210.8
21 ng/ml; resistance training group: 473.3 ± 169.1 ng/ml). Post hoc analyses revealed that following walking,
22 serum COMP concentrations remained elevated compared to baseline (mean difference: 28.3 ng/ml; 95% CI 3.8
23 to 52.8 ng/ml; $P = 0.02$). In contrast, following resistance training, serum COMP dropped below baseline
24 concentrations (mean difference: 28.4 ng/ml; 95% CI 0.8 – 56.0; $P = 0.04$). However, absolute concentrations
25 (30-minute post exercise) between the walking group and resistance training group did not significantly differ
26 (518.6 ± 210.2 vs 473.3 ± 169.1 ng/ml; $P = 0.39$). Likewise, absolute serum COMP concentration did not differ
27 between modalities at baseline, or immediately post exercise (Figure 1). The change in serum COMP
28 concentration over time was comparable between males and females (Figure 1). However, serum COMP
29 concentrations were higher in males than in females at baseline (595.0 ± 138.7 vs 395.4 ± 174.1 ng/ml),

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

3 Serum HA

4 Mean serum HA did not significantly change following either walking or resistance exercise (all, $P > 0.05$).
5 Furthermore, there was no difference over time between males and females. However, mean serum HA
6 concentrations were higher in females compared to males at baseline (37.7 ± 17.8 vs 26.2 ± 12.8 ng/ml, $P =$
7 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
8 (36.0 ± 19.4 vs 28.2 ± 15.9 ng/ml, $P = 0.107$) (Figure 2).

9 Cartilage thickness

10 The assessment of cartilage thickness revealed that males had significantly thicker cartilage compared to
11 females at the intercondyle notch, medial condyle and lateral condyle (Table 2). The greatest mean difference in
12 cartilage thickness was at the intercondyle notch, followed by the medial and lateral condyles (Table 2).
13 Furthermore, there was a significant difference between males and females in mean cartilage thickness at the
14 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
15 for body size using a composite variable that considered both the height and weight of participants. ANCOVA
16 analyses were not completed for the lateral and medial condyle due to violations in key test assumptions.

17 Discussion

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

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

1 unreasonable to expect cartilage damage from acute walking or resistance exercise in a group of healthy
2 individuals. The increase in serum COMP following walking in the present study (+28.9%) was generally
3 greater than previously reported by Mündermann et al. (2005) and Denning et al. (2016) following walking,
4 +9.7% and +5.27%, respectively. This difference may be due to the exercise duration being shorter
5 (Mündermann et al. 2005), or due to the self-paced nature of the walking exercise (Denning et al. 2016).
6 Furthermore, the accumulative load on the knee joint between walking studies may be influenced through
7 increases in walking speed, which have previously been associated with increased serum COMP response
8 (Denning et al. 2015). Although all the participants were healthy and of similar age, differences between studies
9 may also relate to variations in the study cohorts and in training status. The increase in serum COMP following
10 walking in the present study (+28.9%) was comparable to the increase in serum COMP following a similar
11 duration (approx. 40 minutes) bout of vigorous cycling (+32.1%) and higher than vigorous bout of running
12 (+14.2%) in trained individuals (Roberts et al. 2016). A greater increase following walking compared to
13 vigorous running was somewhat surprising given that the overall load following running was higher (exercise
14 time: 40 min vs a 46 min (average); intensity of exercise: 76% vs 90.4% HR_{max}; distance covered: 4.2 vs 10
15 km). Another plausible possibility for this finding relates to training status. For example, the participants in the
16 present study were generally less trained than the individuals who participated in our previous work (Roberts et
17 al. 2016). Others have also indicated that exercise training may lessen the acute serum COMP response to acute
18 walking exercise (Celik et al. 2013; Firner et al. 2018), potentially by consolidating the cartilage matrix and
19 consequently reducing release of COMP from the extracellular matrix and eventually into the circulation.

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

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

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

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

1 Moreover, it is possible that difference in the exercise-response between serum COMP and HA relate to either a
2 greater release of COMP from the joint, differences in the transport across the joint membrane and into the
3 systemic circulation, and/or differences in the clearance of biomarkers by the liver and kidney. Moreover, the
4 present study found that baseline concentrations were like previously reported values in healthy individuals and
5 lower than those reported in individuals with joint disease, including OA (Criscione et al. 2005; Wakitani et al.
6 2007) and rheumatoid arthritis patients (Engström-Laurent and Hällgren 1987). Surprisingly, the present study
7 found higher serum concentrations of HA in women compared to men. Serum HA has previously been shown to
8 be influenced by various individual factors, including sex, with higher serum HA concentrations typically found
9 in men compared to women (Elliott et al. 2005). There is no clear explanation for the higher HA concentrations
10 observed in this female population.

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

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

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

9 **Conclusion**

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

19 **Conflicts of interest**

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

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

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

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

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

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

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

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1 **Figure captions:**

2

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

7 **Fig. 2** Mean serum HA concentration, pre-exercise, immediately post, and at 30 min post 40 min of walking or
8 40 mins of resistance training exercise in a) males and b) females. Data are means \pm standard deviation

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