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1 **The Effect of Vigorous Running and Cycling on Serum COMP, Lubricin and Femoral Cartilage**  
2 **Thickness: a Pilot Study**

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1 **Abstract**

2 *Purpose:* Our aim was to investigate lubricin, cartilage oligomeric matrix protein (COMP) and femoral  
3 cartilage deformation in response to different biomechanical loading of the knee joint (running *versus*  
4 cycling). *Methods:* Serum lubricin and COMP concentrations enzyme-linked immunosorbent assay  
5 (ELISA), and femoral cartilage thickness (supra-patellar transverse ultrasonography) were determined  
6 in 11 male runners (age: 40±6 years; weight: 76±8 kg) and 11 male cyclists (35±12 years; 75±5 kg) at  
7 baseline, immediately after, and 30 minutes after vigorous exercise (time trial: 10km run or 25km cycle).  
8 *Results:* At baseline, lubricin (runners: 104.0±19.8 ng/ml; cyclists: 119.1±23.9 ng/ml) and COMP  
9 (runners: 804.1±87.5 ng/ml; cyclists: 693.0±84.7 ng/ml) did not significantly differ, however, vigorous  
10 exercise was accompanied by an increase in lubricin (cyclists: 39.4%; p<0.05; runners: 56.9%; p<0.05)  
11 and COMP (cyclists: 32.1%; p<0.05; runners: 14.2%; p=0.14) that returned towards baseline following  
12 30 minutes of rest (p<0.05). No between-group differences were observed for baseline cartilage thickness  
13 at the intercondyle notch, medial condyle and lateral condyle, and vigorous exercise did not result in  
14 significant change for either group. *Conclusions:* In the absence of ultrasonographic knee cartilage  
15 deformation, the response of serum lubricin and COMP following acute vigorous exercise indicates an  
16 increase in joint lubrication and cartilage metabolism, respectively, which appears largely independent  
17 of exercise modality.

18 **Key words:** knee; joint loading; ultrasound; cartilage oligomeric matrix protein; proteoglycan-4

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1 **Abbreviations:**

2	COMP	Cartilage oligomeric matrix protein
3	ELISA	Enzyme-linked immunosorbent assay
4	MRI	Magnetic resonance imaging
5	OA	Osteoarthritis
6	PRG-4	Proteoglycan-4
7	US	Ultrasound
8	VO <sub>2max</sub>	Maximum oxygen uptake

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## 1 **Introduction**

2 Regular exercise has been shown to preserve knee cartilage volume and thickness (Racunica et al. 2007;  
3 Mosher et al. 2010) reduce cartilage defects (Racunica et al. 2007), increase proteoglycan content (Van  
4 Ginckel et al. 2010) as well as reduce knee disability and pain (O'Reilly et al. 1999). While regular  
5 moderate joint loading may be chondroprotective and promote healthy knee joint function (Urquhart et  
6 al. 2011), high levels of joint loading may result in negative adaptations (Driban et al. 2015). Weight  
7 bearing activities such as running are associated with much larger lower-body peak forces (D'Lima et  
8 al. 2008) and may be subject to an increased risk of osteoarthritis (OA) compared to non-weight bearing  
9 activities such as cycling (Vignon et al. 2006; Franciozi et al. 2013). To date, there remains a paucity of  
10 research regarding the effect of acute running and cycling exercise on overall joint function.

11 Articular cartilage is an avascular, aneural, and alymphatic tissue which has a crucial role in maintaining  
12 mechanical competence through its distribution of large joint loads in a relatively even manner from one  
13 subchondral bone plate to another (Mow and Guo 2002). Although mechanical loading is required for  
14 tissue maintenance and metabolism, chronic abnormal loading, exaggerated by obesity, joint  
15 malalignment or high levels of physical activity, have been implicated as extrinsic risk factors for  
16 cartilage damage (Guilak 2011). Articular cartilage is known to deform with exercise (deformational  
17 behaviour) before returning to pre-loading values with rest (Kessler et al. 2006). The magnitude of  
18 deformation differs between various types of exercise, is 'dose dependent', and may to some extent be  
19 related to mechanical differences associated with exercise (Eckstein et al. 2005). Deformational  
20 behaviour is also understood to provide unique information regarding the biochemical composition of  
21 the cartilage tissue (Eckstein et al. 2006) and has previously been correlated with an exercise-induced  
22 increase in cartilage oligomeric matrix protein (COMP) (Kersting et al. 2005).

23 Clinical measures of early knee joint adaptation are elusive. However, easily accessible serum  
24 biomarkers are understood to reflect the release of molecules or molecular fragments from the loaded  
25 joint and are increasingly acknowledged as a method to monitor early joint adaptation, degenerative  
26 change, and OA (Bauer et al. 2006). COMP is a non-collagenous glycoprotein that binds to type II  
27 collagen and functions by assisting with the organisation and stabilisation of articular cartilage (Halász  
28 et al. 2007). COMP has previously been used a marker of cartilage metabolism / turnover (Saxne et al.  
29 1992), as well as to monitor cartilage degradation (Neidhart et al. 1997). When cartilage is broken down,

1 COMP is released into the synovial fluid and later into the blood serum (Sharif et al. 1995). Elevated  
2 baseline levels of serum COMP have been established following knee injury (Catterall et al. 2010), OA  
3 (Neidhart et al. 2000) and rheumatoid arthritis (Vilím et al. 2003; Law et al. 2015). Thus, it is understood  
4 that elevated serum COMP may reflect a shift towards increased cartilage degradation. Elevated baseline  
5 levels have also been reported in marathon runners (Neidhart et al. 2000). Previous studies have also  
6 demonstrated that exercise results in an acute ‘dose dependent’ increase in COMP from baseline  
7 (Neidhart et al. 2000; Mündermann et al. 2005; Kersting et al. 2005), which has been suggested to relate  
8 to increased cartilage turnover to acute tissue loading (Neidhart et al. 2000). Therefore, COMP may be  
9 a promising marker to evaluate the effect of acute running and cycling exercise on the knee joint.

10 A second novel and exciting marker is lubricin, a proteoglycan-4 (PRG-4) protein, encoded by the PRG-  
11 4 gene and understood to function by reducing the friction associated with joint movement (Jay et al.  
12 2007) and preventing cartilage wear (Rhee et al. 2005). PRG-4 includes the protein lubricin, which is a  
13 mucin-like o-linked glycosylated protein, as well as the homologous superficial zone protein,  
14 megakaryocyte stimulating factor and hemangiopoietin. These PRG-4 proteins are expressed in skeletal  
15 and non-skeletal tissues, with highest levels of expression in articular joints, bone, and liver (Ikegawa et  
16 al. 2000; Rhee et al. 2005). Although all PRG-4 proteins are implicated in articular joint protection,  
17 current understanding is that lubricin and superficial zone protein have an increased localization to the  
18 joint and play the greatest role (Rhee et al. 2005; Jay et al. 2007). In contrast, megakaryocyte stimulating  
19 factor and hemangiopoietin are understood to function in the regulation of megakaryopoiesis, and  
20 hematopoietic progenitor cell expansion, respectively (Novince et al. 2011); however, there remains less  
21 clarity regarding the localization of both megakaryocyte stimulating factor and hemangiopoietin.  
22 Importantly, mutations in the PRG-4 gene results in a recessive disorder known as camptodactyly-  
23 arthropathy-coxa vara-pericarditis syndrome, which is a characterized by early-onset joint failure, and  
24 thus supports the role of PRG-4 in articular protection. Given our interest in joint function we will refer  
25 to the PRG-4 proteins as lubricin, often known synonymously as superficial zone protein or PRG-4.  
26 Several *in vivo* animal studies have provided evidence to suggest that lubricin synthesis is down-  
27 regulated in degenerative joints (Abusara et al. 2013), following anterior cruciate ligament injury (Elsaid  
28 et al. 2012) and in a meniscectomy-induced OA model (Young et al. 2006). In contrast, low to moderate  
29 mechanical loading has been shown to significantly increase lubricin expression and is associated with

1 fewer degenerative changes (Ni et al. 2012). Whether exercise in humans has an effect on serum lubricin  
2 concentration may be a promising indicator of joint function and to date remains unknown.

3 In addition to serum biomarkers, ultrasonography (US) of the knee joint is a non-invasive, fast, and  
4 inexpensive imaging method that is increasingly used to assess early degenerative change. US is  
5 considered an excellent tool to assess several intra-articular abnormalities including knee effusions,  
6 synovitis and intra-articular bodies (Abraham et al. 2011; Kazam et al. 2011). US can also be utilised to  
7 provide a valid and reliable measure of femoral cartilage thickness at three identifiable locations, the  
8 intercondyle notch, medial condyle and lateral condyle (Naredo et al. 2009).

9 The sonographic evaluation of cartilage thickness, together with biomarkers lubricin and COMP, offer  
10 an attractive method to explore the physical and biochemical tissue response following an acute bout of  
11 running and cycling. Therefore, the aims of this study were to determine whether: 1) vigorous exercise  
12 will increase serum lubricin and serum COMP concentrations, 2) vigorous exercise will decrease  
13 cartilage thickness, and 3) trained runners completing a vigorous running protocol respond differently to  
14 trained cyclists completing a similarly matched vigorous cycling protocol.

## 15 **Methods**

### 16 *Participants*

17 A homogeneous group of healthy trained runners and cyclists were recruited for this study. In order to  
18 maintain a homogeneous sample the inclusion criteria for entry to the study included being male, aged  
19 between 18-59 years, and a body mass index of  $< 30 \text{ kg} / \text{m}^2$ . Runners were required to have recently  
20 completed a 10 km time trial in under 45 minutes and / or regularly complete  $> 25$  miles per week in  
21 training. In comparison, cyclists were required to have recently completed a 25 km time trial in under 45  
22 minutes and / or regularly complete  $> 80$  miles per week in training and / or regularly cycle  $> 4$  hours  
23 per week. Cyclists were also required to have a limited history of weight bearing activity and have not  
24 completed regular weight bearing sports training or running within the last 6 months. Exclusion criteria  
25 for both groups included: (i) diagnosed OA, rheumatoid arthritis, or other inflammatory disease, (ii)  
26 history of knee malalignment (varus / valgus) greater than  $15^\circ$ , (iii) previous knee injury (including  
27 meniscus tear or ligament damage or tear), (iv) recent fracture of lower extremity (within last 6 months),  
28 (v) current or prior use of lipid-lowering therapy (e.g. fibric acids, nicotinic acids, bile acid sequestrates,  
29 fish oils), corticosteroid injections, (vi) current or past (within last four weeks) glucosamine and / or

1 chondroitin supplementation use, (vii) additional exclusion factors included muscle weakness and  
2 musculoskeletal / orthopaedic problems prohibiting exercise participation. A control group was not  
3 included within this study design due to the difficulties of finding an equally well matched group of  
4 trained individuals who did not engage in weight bearing or non-weight bearing activities.

5 Sample size calculation were conducted prior to commencing testing using G\*Power 3.1.3 (Heinrich-  
6 Heine-University) software. Assuming an alpha value of 0.05 and 80% power, a total sample size of 24  
7 participants was calculated in order to detect a moderate effect size (0.50). The local University ethics  
8 committee approved the study protocol and the study was carried out in compliance with the Helsinki  
9 declaration. Written informed consent was obtained from all participants.

#### 10 *Experimental protocol*

11 All participants were required to visit the physiology laboratories on two separate occasions with a  
12 minimum of 48 hours between visits and a maximum of 2 weeks. During the first visit height and body  
13 weight were assessed using a calibrated balance beam scale (SECA, California, USA) and wall mounted  
14 tape measure (SECA, California, USA), respectively. Subsequently, a venous blood sample (6 ml) was  
15 acquired following 15 minutes of seated rest (Mundermann et al 2005), prior to completing an  
16 incremental exercise protocol to determine maximum oxygen uptake ( $VO_{2max}$ ). During the second visit  
17 participants provided an additional venous blood sample (6 ml) and underwent ultrasound scanning of  
18 the femoral cartilage (to assess cartilage thickness) of the right leg, before, immediately post, and 30  
19 minutes post the vigorous time trial protocol. Participants were required to limit their training / racing  
20 prior to each visit and were unable to take part in the study if they had taken part in a marathon or ultra-  
21 distance event within 7 days of a testing day. Training within the 48 hours prior to testing was limited to  
22 1 hour of moderate intensity training and participants had to refrain from training in the 24 hours prior  
23 to testing. Participants also provided detailed information regarding their typical training (frequency,  
24 intensity, duration and type) and provided information regarding their training history (number of years  
25 of training).

#### 26 *Serum Lubricin and COMP analysis*

27 All blood samples were allowed to clot and subsequently centrifuged for 15 minutes (4°C, 1000g). Serum  
28 was immediately frozen to -80°C and stored until analysis. Serum lubricin (PRG-4) was analysed using

1 a commercially available sandwich ELISA (Human Proteoglycan 4 (PRG 4) ELISA kit CSB-E14124h,  
2 Cusabio Biotech Co, China). Similarly, serum COMP was analysed using a commercially available  
3 sandwich ELISA (Human COMP ELISA kit KA0021, Abnova Corporation, Taiwan) as previously  
4 described (Law et al. 2015). Intra-assay coefficient of variation was 6.1% and 6.0% for lubricin and  
5 COMP analysis, respectively, and the  $R^2$  curve was  $> 0.99$  for all curves.

#### 6 *Sonographic Assessment of Cartilage Thickness, Deformation and Recovery*

7 The ultrasound (US) assessment was performed using a 12 MHz linear-array probe (Esaote S.P.A.  
8 MyLab50 ultrasound, Firenze, Italy) and acoustic coupling gel (Aquasonic 100, Parker Laboratories, Inc,  
9 Fairfield, NJ). With participants lying in a supine position and with the right knee maximally flexed, the  
10 superior margin of the patellar was located and a line was marked on the skin using a washable marker  
11 at the point immediately above the superior margin of the patellar and at 1 cm intervals in a superior  
12 direction. The transducer was placed in a supra-patella transverse position, perpendicular to the bone  
13 surface and orientated to optimise the US image (Naredo et al. 2009; Özçakar et al. 2014). The location  
14 at which the cartilage thickness of the intercondyle notch appeared greatest was marked on the skin thus  
15 enabling the examiner to return the transducer to the exact location for all subsequent scans; transducer  
16 placement was  $2.0 \pm 0.5$  cm above the patellar for both right and left knees. The distance from the thin  
17 hyperechoic line formed at the synovial space-cartilage border to the line formed at the cartilage-bone  
18 border was used to measure minimal cartilage thickness at each location (Özçakar et al. 2014). Cartilage  
19 thickness deformation and recovery was calculated as the difference in cartilage thickness between the  
20 measure obtained pre-exercise and immediately-post the acute exercise protocol and the measure taken  
21 immediately-post and post-30 minutes of seated rest, respectively. The same researcher performed all  
22 ultrasonography scans following training by a consultant rheumatologist with expertise using this  
23 technique.

24 'Image J' (Image J, National Institute of Health, Bethesda, MD, USA) software was used to measure the  
25 minimal cartilage thickness at the lateral condyle, medial condyle and intercondylar notch. Anatomic  
26 reference points used in the present study corresponded to the midpoint of the intercondyle notch and 1  
27 cm apart in the medial and lateral direction were used as an estimate of the medial and lateral condyle  
28 cartilage thickness (Figure 1); Naredo and colleagues previously demonstrated good reproducibility in  
29 femoral cartilage thickness measurement when using comparable anatomical reference points (Naredo

1 et al. 2009). In addition, our own repeatability study demonstrated a moderate to high intra class  
2 correlations for resting femoral cartilage thickness measurements conducted on two separate days (ICC  
3 between 0.635 – 0.807 for all locations and across age groups; with ICC between 0.800 - 0.934 in young  
4 individuals and between 0.719 - 0.813 in middle aged individuals). Prior to analysis, all images were de-  
5 identified by second researcher for blinded analysis. Based on the pixel resolution (15.8 pixels /mm) of  
6 the images captured by ultrasonography, the ImageJ software allowed images to be measured to an  
7 accuracy of greater than one-tenth off a mm, or more specifically, one pixel was equal to 0.06 mm. The  
8 cartilage thickness of each image was measured in triplicate and an average of the three measurements  
9 was used for all data analysis. As required, the image contrast was adjusted to assist in appropriately  
10 identifying the hyperechoic line formed at the synovial space-cartilage border to the line formed at the  
11 cartilage-bone border.

#### 12 *Maximum Oxygen Uptake ( $VO_{2max}$ )*

13 Breath-by-breath analysis using an online gas analyser (Cortex, Leipzig, Germany) was used to  
14 determine cardiorespiratory fitness. Participants in the running group performed an incremental exercise  
15 test using the treadmill (HPCosmos Mercury 4 Med, Nussdorf-Traunstein, Germany). In contrast,  
16 participants in the cycling group performed an incremental exercise test using the cycle ergometer (Lode  
17 Excalibur Sport, Groningen, Netherlands). Heart rate was monitored continuously using a heart rate  
18 monitor (Polar Electro, Kempele, Finland) and the rating of perceived exertion was measured using the  
19 Borg scale every 3 minutes.  $VO_{2max}$  was defined as the highest 30-s average in  $VO_2$  and was accepted if  
20 two of the following were met; a plateau in  $VO_2$  despite the continuation of exercise, a respiratory  
21 exchange ratio  $\geq 1.05$ , or participants reached  $> 95\%$  of predicted maximum heart rate.

#### 22 *Time-trial exercise protocol*

23 The vigorous exercise protocol was specific to the participant's activity: trained runners completed a 10  
24 km running time trial using the treadmill ergometer and trained cyclists completed a 25 km cycling time  
25 trial using a cycle ergometer. Runners self-regulated their speed using the treadmill monitor, while the  
26 gradient remained standardised at 1% to account for the lack of wind resistance. Similarly, cyclists also  
27 adopted a self-selected workload and cadence. Although participants self-regulated their workload,  
28 travelling speed and exercise-time were not provided. Importantly, participants were instructed to  
29 complete the time-trial protocol as intensely as possible in order to mimic actual competition. Continuous

1 verbal encouragement was provided throughout. Both heart rate and rating of perceived exertion  
2 measurements were taken at several time points throughout the trial to ensure the protocol replicated  
3 competitive conditions.

#### 4 *Data analysis*

5 Statistical analyses were performed utilising statistical analysis software (SPSS for Windows version  
6 20.0 [SPSS, Chicago, IL, USA]). A two-factor (treatment x time) repeated-measures analysis of variance  
7 was used to assess the within groups difference (baseline vs. immediately post-exercise vs 30 minutes  
8 post-exercise), between group difference (runners vs. cyclists) and time by treatment interactions (group  
9 differences over time) for serum lubricin, serum COMP, and femoral cartilage thickness. A single  
10 baseline value (average of visit 1 and visit 2) was used for within group analysis of serum lubricin and  
11 COMP in order to determine a robust baseline value and the coefficient of variation was calculated.  
12 Significant interactions and / or main effects were analysed post-hoc using Bonferroni-corrected t-tests  
13 where appropriate. Independent sample t-tests were used to assess differences between runners and  
14 cyclists. Pearson correlations (parametric data) and Spearman's rank correlations (non-parametric data)  
15 were performed to examine the relationships between all baseline continuous variables. As appropriate,  
16 sensitivity analysis was completed to determine the effect of outliers and to strengthen the conclusions  
17 drawn from the analyses. Effect sizes were calculated using the Cohen d equation; 0.2, 0.5 and 0.8 were  
18 considered as small, moderate and large effect size, respectively. Normality of data was explored by  
19 visual inspection of Q-Q plots and through analysis of the models residuals. All figures and tables are  
20 presented as mean  $\pm$  standard deviation, with statistical significance set as ( $p < 0.05$ ).

#### 21 **Results**

22 Of the twenty-four participants who completed the study, two cyclists revealed a history of weight-  
23 bearing sporting activity and were consequently removed from study. Therefore, in total, twenty-two  
24 participants (running group:  $n = 11$ ; cycling group:  $n = 11$ ) were included within the analyses. Both groups  
25 were comparable for the number of training years, average number of days and average number of hours  
26 of training completed per week (Table 1). Overall, participants within the running and cycling groups  
27 can be described as well-trained athletes and provide a good opportunity for comparison between groups.

#### 28 *Serum Lubricin*

1 Following exercise serum lubricin increased significantly irrespective of the exercise modality (Figure  
2 2a); concentrations increased by 31.3% following cycling (baseline:  $114.0 \pm 41.8$  ng/ml; immediately  
3 post-exercise:  $166.0 \pm 61.9$  ng/ml,  $p < 0.01$ ) and by 35.7% following running (baseline:  $105.0 \pm 29.6$   
4 ng/ml; immediately post-exercise:  $163.1 \pm 58.0$  ng/ml,  $p < 0.01$ ). Thirty minutes of seated rest resulted  
5 in a significant decrease towards pre-exercise values in both groups (cycling group:  $112.0 \pm 47.1$  ng/ml;  
6 running group:  $122.6 \pm 49.1$  ng/ml, both  $p < 0.01$ ). The impact of exercise on serum lubricin is reinforced  
7 by the large effect size observed by the cycling (0.99) and running groups (1.27). There was no significant  
8 difference and only a small effect size (0.25) in baseline serum lubricin between cyclists ( $114.0 \pm 41.8$   
9 ng/ml) and runners ( $105.0 \pm 29.6$  ng/ml). Sensitivity analysis did not alter the primary findings and  
10 indicated that any difference between baseline lubricin of cyclists and runners may be minimal.

#### 11 *Serum COMP*

12 Serum COMP concentration increased in both groups following exercise (Figure 2b); however, this  
13 increase was significant following cycling only (baseline:  $693.9 \pm 189.0$  ng/ml; immediately post-  
14 exercise:  $916.4 \pm 228.1$  ng/ml,  $p < 0.01$ ). Although non-significant ( $p = 0.14$ ), the increase in serum  
15 COMP following running (baseline:  $804.1 \pm 259.3$  ng/ml; immediately post-exercise:  $918.1 \pm 290.7$   
16 ng/ml) represented a moderate effect size (0.47). In a similar manner to lubricin, both groups  
17 demonstrated a significant return toward pre-exercise values following 30 minutes of seated rest (cycling  
18 group:  $649.5 \pm 176.2$  ng/ml;  $p < 0.01$ , running group  $752.6 \pm 244.8$  ng/ml;  $p < 0.05$ ). No significant  
19 differences at baseline between the cycling and running group were revealed. However, mean baseline  
20 COMP tended to be 14% greater (moderate effect size, 0.48) in runners compared with cyclists ( $p =$   
21  $0.27$ ). Sensitivity analysis found serum COMP to be significantly increased following running ( $p < 0.05$ )  
22 as well as cycling ( $p < 0.01$ ); this also indicated that differences in baseline serum COMP between  
23 runners and cyclists may actually be minimal.

#### 24 *Sonographic Assessment of Cartilage Thickness, Deformation and Recovery*

25 Cartilage thickness following running did not significantly change (all  $p > 0.05$ ) at the intercondyle notch  
26 (baseline:  $2.02 \pm 0.47$  mm; immediately post-exercise:  $2.14 \pm 0.48$  mm), medial condyle (baseline:  $2.15$   
27  $\pm 0.23$  mm; immediately post-exercise:  $2.09 \pm 0.49$  mm) or lateral condyle (baseline:  $2.05 \pm 0.21$  mm;  
28 immediately post-exercise:  $1.94 \pm 0.34$  mm). Likewise, there was no significant change (all  $p > 0.05$ ) in  
29 cartilage thickness following cycling at the intercondyle notch, (baseline:  $2.27 \pm 0.38$  mm; immediately

1 post-exercise:  $2.38 \pm 0.63$  mm) medial condyle (baseline:  $2.04 \pm 0.32$  mm; immediately post-exercise:  
2  $2.02 \pm 0.27$  mm) or lateral condyle (baseline:  $2.15 \pm 0.31$  mm; immediately post-exercise:  $2.21 \pm 0.28$   
3 mm) (Figure 3). In addition, following running and cycling the 30 minutes of seated rest provided did  
4 not significantly change intercondyle, medial or lateral femoral cartilage thickness. There was also no  
5 significant difference between baseline femoral cartilage thickness of runners and cyclists at the  
6 intercondyle notch, medial condyle and lateral condyle cartilage thickness (all  $p > 0.05$ ). The greatest  
7 between group difference in baseline cartilage thickness was observed at the intercondyle notch (running  
8 group:  $2.02 \pm 0.47$  mm: cycling group  $2.27 \pm 0.38$  mm) which represented a moderate effect size (0.58).

### 9 *Correlation analysis*

10 Correlation analysis was performed for the full sample ( $n=22$ ), with very few correlations observed  
11 between outcome measures (Table 2). Advancing age was associated with baseline reduced cartilage  
12 thickness at the lateral condyle ( $r = -0.650$ ,  $p < 0.01$ ), while the number of previous training years was  
13 associated with increased cartilage thickness at the intercondyle notch ( $r = 0.481$ ,  $p < 0.05$ ).

### 14 **Discussion**

15 To our knowledge this is the first study that has explored the effect of acute exercise on serum lubricin  
16 in humans. This study demonstrated that an acute bout of vigorous exercise results in an increase in both  
17 serum lubricin and COMP concentrations. In contrast to the serum biomarkers, femoral cartilage  
18 thickness was unaffected by vigorous exercise. In relation to the modality of exercise, the increase in  
19 serum lubricin following vigorous exercise was comparable for both runners and cyclists. However,  
20 unlike serum lubricin, the magnitude of change in serum COMP was less following running compared  
21 to cycling. The current study also demonstrates that serum lubricin, serum COMP, and femoral cartilage  
22 thickness do not significantly differ at baseline between runners and cyclists. Furthermore, there were no  
23 significant correlations between femoral cartilage thickness and either serum biomarker.

24 The increase in serum lubricin and COMP following acute vigorous cycling and running exercise in  
25 healthy humans in the current study are consistent with reports of increased PRG-4 and COMP  
26 production in response to mechanical load in cartilage explants and animal models (Piscoya et al. 2005;  
27 Nugent-Derfus et al. 2007; Abusara et al. 2013). The increase in serum COMP in the present study is  
28 also consistent with several studies that have previously demonstrated that serum COMP increases  
29 following exercise (Neidhart et al. 2000; Kersting et al. 2005; Niehoff et al. 2011). The increases in

1 serum lubricin and COMP observed in the present study may reflect the release into the synovial fluid  
2 and circulation as a result of exercise-induced loading of the knee articular cartilage. However, the  
3 release from other joint sources such as the synovium, tendons, ligaments and bone, as well as non-joint  
4 sources cannot be discounted.

5 The elevation in serum lubricin and COMP concentration may be a result of the release of cartilage  
6 components into the synovial fluid and circulation in response to exercise. However, the exact  
7 mechanisms contributing to the increase in biomarker concentrations and the movement from the extra-  
8 cellular matrix into the bloodstream remains unclear. With regard to serum COMP, elevated levels within  
9 the circulation in response to loading may reflect increased cartilage turnover (Saxne et al. 1992),  
10 potential tissue damage (Neidhart et al. 2000), or an exercise-induced increase in the transport/removal  
11 from the joint into the blood (Helmark et al. 2012). In addition, acute increases in serum biomarkers  
12 conceivably may reflect the integrity of the joint. Importantly, joint movement is considered an  
13 important factor in increasing intra-articular pressure and possibly contributes to the movement of  
14 cartilage constituents into the circulation (Levick and McDonald 1995), thus supporting the exercise-  
15 induced increase observed in the present study.

16 Given the role of lubricin in reducing the coefficient of friction within the joint (Jay et al. 2007), the  
17 acute increase in serum lubricin may reflect a temporary increase in joint lubrication following exercise.  
18 This may also explain why exercise has previously been shown to improve symptoms such as knee joint  
19 stiffness and pain among individuals with joint disease (O'Reilly et al. 1999). Interestingly, several *in*  
20 *vivo* animal studies have recently demonstrated that lubricin expression is attenuated under conditions  
21 associated with high levels of joint loading, including repeated high intensity exercise and with abnormal  
22 loading (Ni et al. 2012; Elsaid et al. 2012; Abusara et al. 2013). Therefore, due to the high impacted  
23 loading associated with running loads compared to cyclists we postulated that runners might demonstrate  
24 reduced resting lubricin concentrations. However, in contrast to our working hypothesis, no differences  
25 were observed between exercise modalities. In addition, there was no correlation between baseline  
26 lubricin and any parameter of training history, including miles, frequency or duration of training;  
27 however, this could be related to our homogenous sample, which was similar in age, fitness level and  
28 training habits.

1 In terms of serum COMP, increases are typically associated with a ‘load-dependent’ change, with the  
2 greatest increase following extensive running (Neidhart et al. 2000; Niehoff et al. 2011). In the present  
3 study serum COMP in the running group increased by +14.2% with acute exercise and was comparable  
4 to the +14.7% increases reported following a 1 hour training run (Kersting et al. 2005), and +15.0%  
5 increase following 31 km of marathon running in trained endurance runners (Neidhart et al. 2000).  
6 However, our finding that serum COMP increased more following cycling versus running (+32.1% vs  
7 +14.2%) is somewhat surprising given that cycling is associated with lower tibiofemoral forces than  
8 running (Kutzner et al. 2012). Besides the magnitude of joint load, loading frequency has been suggested  
9 as an important factor in COMP release (Piscoya et al. 2005). However, given that the average cadence  
10 of cyclists and the typical stepping rate of recreational runners are similar, it appears unlikely that loading  
11 frequency was responsible for the results in the present study. Furthermore, the impact of loading  
12 frequency on the acute increase of serum COMP is not a universal finding. Thirty minutes of two  
13 different impact-loading conditions, i.e. running (high frequency, low amplitude) and drop landing (low  
14 frequency, high amplitude), have also been shown to result in a very similar serum COMP response  
15 (Niehoff et al. 2011).

16 Baseline serum COMP concentrations did not significantly differ between runners and cyclists, and both  
17 groups were well within previously reported upper normal limits (<5000 ng/ml) (Saxne et al. 1992).  
18 Previously, baseline concentrations in marathon runners have been shown to be greater than the normal  
19 limit of 5000 ng/ml and comparable to the elevated baseline levels previously reported in individuals  
20 following joint injury and in patients with OA (Neidhart et al. 2000; Catterall et al. 2010). The baseline  
21 serum COMP concentrations found in our healthy cohort are lower than previously reported in clinical  
22 populations, such as OA and rheumatoid arthritis (Neidhart et al. 2000; Law et al. 2015). This difference  
23 suggests that the trained runners and cyclists have a healthy cartilage turnover and limited, if any,  
24 cartilage degeneration. The greater levels of serum COMP previously reported in marathon runners  
25 (Neidhart et al. 2000) may result from differences such as increased training history and / or the onset of  
26 early degenerative joint change, or perhaps methodological differences such as the inclusion criteria and  
27 exercise restrictions before the blood sampling. The large degree of variation that exists in the reported  
28 baseline values indicates caution is warranted when comparing between studies.

29 **To our knowledge the present study is the first to utilize ultrasonography to assess femoral cartilage**  
30 **thickness at baseline and following exercise in a group of healthy well-trained runners and cyclists. In**

1 contrast to our initial hypothesis, the results of the current study demonstrate that neither vigorous  
2 running nor vigorous cycling resulted in significant deformation of the femoral cartilage. In the present  
3 study there was no significant deformation in cartilage thickness following either acute running or  
4 cycling. In contrast, running for a period of 30 minutes has previously resulted in cartilage thickness  
5 decreases at the femur (4%-8%) and tibia (0%-12%) in both marathon runners and sedentary controls  
6 (Mosher et al. 2010). Significant cartilage thickness deformation has also been demonstrated at the  
7 medial femoral condyle (-2.6%) following 30 minute of running in a sample of healthy young adults  
8 (Niehoff et al. 2011). The difference between the exercise-induced decrease in cartilage thickness  
9 observed in the aforementioned studies and the present study might relate to the use of ultrasound to  
10 measure cartilage thickness compared to magnetic resonance imaging (MRI). Although ultrasonography  
11 is valid, reliable (Naredo et al. 2009) and reduces the time-delay in measurement following exercise,  
12 MRI not only increases the ability to detect small changes following exercise due to a greater  
13 measurement precision, but also provides the opportunity to measure individual cartilage plates as well  
14 as total cartilage volume compared femoral cartilage thickness alone. Nonetheless, differences in the  
15 frequency and amplitude of mechanical loading may also influence cartilage deformation (Niehoff et al.  
16 2011). Furthermore, it is also possible that differences relate to potential adaptations in the biomechanical  
17 and/or mechanical properties of articular cartilage in response to chronic mechanical loading (Van  
18 Ginckel et al. 2010); this may explain potential differences between trained and less trained individuals.

19 The assessment of baseline femoral cartilage thickness using ultrasound has been largely limited to  
20 patient cohorts, including for example, individuals with OA (Tarhan et al. 2003), Meniscal injury  
21 (Akkaya et al. 2013), spinal cord injury (Kara et al. 2013), and pes planus (or flatfoot) (Öztürk et al.  
22 2015). Furthermore, there remains a paucity of studies using ultrasonography to assess femoral cartilage  
23 thickness in healthy cohorts. Despite this, one recent study using ultrasonography demonstrated that  
24 healthy men whom regularly engage in exercise have thicker femoral cartilage than individuals who do  
25 not (Özçakar et al. 2014). Interestingly, in the present study, baseline cartilage thickness did not  
26 significantly differ between exercise modalities, though a moderate effect size indicated that runners may  
27 perhaps have reduced cartilage thickness at the intercondyle notch compared to cyclists. The effect of 3  
28 months (Cotofana et al. 2010) and 6 months (Hinterwimmer et al. 2013) running training on patellar,  
29 femoral and tibial cartilage volume and thickness found limited change, thus suggesting that running  
30 training is well tolerated. Furthermore, marathon runners have previously been shown to have

1 significantly greater femoral cartilage thickness compared to sedentary controls (Mosher et al. 2010).  
2 Therefore, weight-bearing exercise, such as running, appears to be both well tolerated and potentially  
3 positive for cartilage morphology compared to sedentary behaviour. However, the slightly greater  
4 cartilage thickness observed in the cycling group suggests that low impact non-weight bearing exercise,  
5 such as cycling, could perhaps provide a slightly superior loading stimulus for cartilage thickness at the  
6 weight-bearing femoral intercondyle notch. A larger sized study is required to determine whether cycling  
7 is more favourable for the knee joint compared with running. Interestingly, the current study also  
8 demonstrated a positive correlation between cartilage thickness and the number of training years across  
9 both runners and cyclists. **This finding seemingly provides further evidence for the benefits of regular  
10 training on cartilage morphology and supports the previous work of Özçakar and colleagues (2014).**

11 Despite our encouraging research we must acknowledge that this study does have some limitations.  
12 Although joint structures are understood to be a primary source of lubricin and COMP, both are also  
13 expressed in several different tissues and neither are produced exclusively within the knee joint. Further  
14 work is therefore required to determine whether serum markers, particularly with exercise, reflect a  
15 functional or structural change at the knee joint. Research is also warranted in order to understand the  
16 movement of these biomarkers from the joint cavity into the circulating blood. Moreover, the ELISA  
17 assay used in the present study to represent serum lubricin detects not only the lubricin protein, but also  
18 several post-translational modifications of the PRG-4 gene including, superficial zone protein,  
19 megakaryocyte stimulating factor and hemangiopoietin. Therefore, we must not discount that exercise-  
20 induced increases may also reflect an increase from other organs as a consequence of metabolic exercise-  
21 induced stressors. Also, as mentioned previously, we must also acknowledge the shortcomings of US  
22 compared to MRI. **A study utilising greater variation in athletic ability and the type of exercise training,  
23 as well as including a control group may also help establish whether or not differences exist between  
24 weight bearing and non-weight bearing exercise.**

## 25 **Conclusion**

26 Our study suggests that an acute bout of either running or cycling stimulates an increase in cartilage  
27 metabolism and may also offer joint protection through lubricin provoked joint lubrication. Given the  
28 role of lubricin in maintaining joint function, we suggest that the increase in serum lubricin may indicate  
29 a potentially therapeutic and protective benefit at the joint level. Finally, serum lubricin, serum COMP,

1 and femoral cartilage thickness does not significantly differ between healthy trained runners and cyclists;  
2 the reported values would seemingly indicate a natural variation in a group of healthy trained individuals.  
3 Future studies should progress these encouraging and novel findings by investigating a larger, less  
4 homogenous sample, as well as developing a greater mechanistic understanding regarding the effect of  
5 exercise on serum biomarkers in humans.

## 6 **Conflicts of interest**

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

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**Table 1.** Participant training habits and time trial performance

Variable	10-km Run ( <i>n</i> = 11)		25-km Cycle ( <i>n</i> = 11)	
	Mean ± SD	Range	Mean ± SD	Range
VO <sub>2max</sub> (L.min <sup>-1</sup> )	4.3 ± 0.3	(3.6 – 5.0)	4.4 ± 0.6	(3.1 – 5.0)
Training experience (years)	7.7 ± 6.5	(1 - 22)	5.0 ± 4.9	(1 - 15)
Weekly distance (miles)	36.8 ± 11.1	(25 - 60)	114.5 ± 41.8**	(60 - 170)
Weekly frequency (days)	4.9 ± 1.4	(3 - 7)	4.0 ± 1.4	(2 - 6)
Training duration (hours)	7.1 ± 2.4	(4.0 - 10.5)	7.7 ± 2.1	(5.5 - 12)
TT times (min:sec)	46:15 ± 5:38	(39:53 - 49:02)	37:39 ± 2:30**	(34:03 - 42:16)

Significant difference between groups (\* *p* < 0.05; \*\* *p* < 0.01). Values are the mean ± SD unless otherwise stated

**Table 2.** Correlations between physical / exercise / training related parameters and parameters of joint function

Parameter	COMP		Lubricin		Baseline cartilage thickness		
	baseline	$\Delta$	baseline	$\Delta$	Notch	Medial	Lateral
		COMP		Lubricin			
Age	0.177	-0.130	0.010	0.271	-0.249	-0.064	-0.650**
Body mass	0.325	-0.292	0.079	-0.064	0.272	0.012	0.114
BMI	0.268	0.134	-0.022	0.150	0.286	-0.066	-0.124
Body fat %	0.163	0.193	0.049	0.136	0.039	-0.205	-0.259
VO <sub>2max</sub>	-0.198	-0.140	-0.169	-0.202	0.158	0.326	0.298
TT-time	0.104	-0.141	-0.176	0.062	-0.236	0.112	-0.255
Training years	0.316	-0.207	0.021	0.350	0.481*	0.323	0.093
Training miles	-0.008	0.160	0.011	0.128	0.242	-0.230	0.225
Training frq / wk	0.138	-0.182	-0.151	-0.386	-0.165	0.037	0.088

$\Delta$ , change from baseline to immediately post exercise; (\*  $p < 0.05$ ; \*\*  $p < 0.01$ )

**Figure captions:**

**Fig. 1** Images to show ultrasound assessment methodology. a) Demonstrating supra patellar transverse placement of the US probe and b) anatomical marking to ensure correct placement and re-placement of US probe. c) US transverse image of the femoral articular cartilage; M, represents location of medial condyle; N, intercondyle notch; L, lateral condyle

**Fig. 2** Mean a) serum lubricin and b) COMP concentrations, pre-exercise, immediately post, and 30 minutes post a 10 km running or a 25 km cycling time trial. \* and \*\* = significant difference over time at  $p < 0.05$  level and  $p < 0.01$  level, respectively. Significance marked above data line represents cycling group and below represents running group. Data are means  $\pm$  standard deviation

**Fig. 3** Mean cartilage thickness (mm) pre-exercise, immediately post, and 30 minutes post a 10 km running or a 25 km cycling time trial for a) intercondyle notch, b) medial condyle, and c) lateral condyle. Data are means  $\pm$  standard deviation