

## **Changes in cartilage biomarker levels during a transcontinental multistage footrace over 4486 km**

Annegret Mündermann<sup>1,2</sup>, Christopher Klenk<sup>3</sup>, Christian Billich<sup>4</sup>, Corina Nüesch<sup>1,2</sup>, Geert Pagenstert<sup>1</sup>, Arno Schmidt-Trucksäss<sup>3</sup>, Uwe Schütz<sup>4,5</sup>

<sup>1</sup>Orthopaedics and Traumatology Hospital, University of Basel, Basel, Switzerland

<sup>2</sup>Department of Biomedical Engineering, University of Basel, Basel, Switzerland

<sup>3</sup>Division of Sports and Exercise Medicine, Department of Sport, Exercise and Health, University of Basel, Basel, Switzerland <sup>4</sup>Department of Diagnostic and Interventional Radiology, University Hospital Ulm, Ulm, Germany

<sup>5</sup>Orthopaedic and Pain Outpatient Center “Am Grünen Turm”, Lake Constance-Oberschwaben, Ravensburg, Germany

### *Original Investigation*

Mündermann A., C. Klenk, C. Billich, C. Nüesch, G. Pagenstert, A. Schmidt-Trucksäss, and U. Schütz (2017) Changes in cartilage biomarkers during a transcontinental multistage footrace over 4486 km. *American Journal of Sports Medicine* 45(11):2630-2636. Copyright Ó 2017 The Authors. Reprinted by permission of SAGE Publications.  
<http://journals.sagepub.com/doi/10.1177/0363546517712945>

Address of correspondence: PD Dr. Annegret Mündermann  
Head Functional Biomechanics  
Orthopaedics and Traumatology Hospital  
University of Basel  
Spitalstrasse 21  
4031 Basel, Switzerland  
Tel. +41 61 3285445  
Email [annegret.muendermann@unibas.ch](mailto:annegret.muendermann@unibas.ch)

### *Acknowledgements*

This study was funded by grants of the German Science Foundation (DFG SCHU 2514/1-1 and SCHU 2514/1-2), the Swiss National Science Foundation (SNSF #32003B\_159871/1) and the Department Sport, Exercise and Health, University of Basel.

*Word count:* 3395 words

Running title: Multistage ultramarathon and cartilage

1 **Abstract**

2 **Background:** Cartilage turnover and load-induced tissue changes are frequently assessed by  
3 quantifying concentrations of cartilage biomarkers in serum. To date information on the  
4 effects of ultramarathon running on articular cartilage is scarce.

5 **Hypothesis:** Serum concentrations of cartilage oligomeric matrix protein (COMP), matrix  
6 metalloproteinases (MMP)-1, 3, and 9, collagen COL2-3/4Clong mono (C2C), collagen II C-  
7 propeptide (CPII) and C2C:CPII will increase throughout a multistage ultramarathon.

8 **Study Design:** Cross-sectional study.

9 **Methods:** Five blood samples were collected from 38 runners (4 female; age,  $49.0 \pm 10.7$   
10 years; body mass index, start:  $23.1 \pm 2.3 \text{ kg/m}^2$ , finish:  $21.4 \pm 1.9 \text{ kg/m}^2$ ) before ( $t_0$ ) and  
11 during ( $t_1$ : 1002 km;  $t_2$ : 2132 km;  $t_3$ : 3234 km;  $t_4$ : 4039 km) a 4486 km multistage  
12 ultramarathon. Serum COMP, MMP-1, 3, and 9, C2C and CPII levels were assessed using  
13 commercial enzyme-linked immunosorbent assay. Linear mixed models were used to detect  
14 significant changes in serum biomarker levels over time with time-varying covariates body  
15 mass, running speed, and daily running time.

16 **Results:** Serum concentrations of COMP, MMP-9 and MMP-3 changed significantly  
17 throughout the multistage ultramarathon. On average, concentrations increased during the  
18 first measurement interval (MI1:  $t_1-t_0$ ) by 22.5% (change MI1 [95% confidence interval],  
19 COMP: [0.29;0.71] ng/mL), 22.3% (MMP-3: [0.24;15.37] ng/mL), and 95.6% (MMP-9:  
20 [81.7;414.5] ng/mL), and remained stable throughout MI2, MI3 and MI4. Serum  
21 concentrations of MMP-1, C2C, CPII, and C2C:CPII did not change significantly throughout  
22 the multistage ultramarathon. Changes in MMP-3 were statistically associated with changes  
23 in COMP throughout the ultramarathon race (MMP-3: Wald  $Z=3.476$ ,  $P=.001$ ).

24 **Conclusions:** Elevated COMP levels indicate increased COMP turnover in response to  
25 extreme running, and the association between load-induced changes in MMP-3 and changes  
26 in COMP suggests the possibility that MMP-3 may be involved in the degradation of COMP.

27 **Clinical Relevance:** These results suggest that articular cartilage is able to adapt even to  
28 extreme physical activity possibly explaining why the risk of degenerative joint disease is not  
29 elevated in the running population.

30 **Key Terms:** Cartilage biomarkers, articular cartilage, tissue metabolism, extreme running

31 **What is known about the subject:** The effect of extreme running on articular cartilage  
32 metabolism is poorly understood.

33 **What this study adds to the existing knowledge:** Compared to single stage ultramarathons,  
34 COMP levels leveled off during the multistage ultramarathon suggesting that regular short  
35 recovery periods throughout ultra exercises in highly adapted ultra-endurance athletes may be  
36 sufficient for reaching a steady-state. Although the regulation of COMP is poorly understood,  
37 the statistical association between load-induced changes in MMP-3 and load-induced changes  
38 in COMP suggest that MMP-3 may be involved in the degradation of COMP.

## 1 **Introduction**

2 While in recent years, marathon running has become increasingly popular with more  
3 than 700 races per year worldwide and up to 50,000 participants per event<sup>34</sup>, single stage  
4 ultramarathons (distances >42 km without break) and multistage ultramarathons (distances  
5 >42 km per day over multiple days) are performed by fewer athletes per event with races of  
6 varying distances. Ultramarathons represent extreme stress for the human body not only  
7 because of the duration of the physical activity but also due to environmental conditions such  
8 as weather and terrain.

9 The effects of multistage ultramarathon on health have received scientific interest,  
10 although the literature is largely limited to effects on the cardiovascular system<sup>18</sup>,  
11 respiratory<sup>43</sup> and skeletal muscle<sup>38</sup>, and the gastrointestinal system<sup>38</sup>. Interestingly, to date  
12 information on the effects of ultramarathon running on articular cartilage is scarce. A  
13 previous study<sup>37</sup> on a transcontinental multistage footrace over 4486 km reported an initial  
14 T2\*-signal increase during the first 1000 km followed by a slight decrease throughout the  
15 remainder of the race (with medium to high effect sizes) without any morphological or  
16 cartilage thickness changes in the ankle joints. These changes were interpreted as an increase  
17 in glucosaminoglycan as observed by Roos and Dahlberg<sup>31</sup> in the weight-bearing posterior  
18 medial femoral condyle following moderate exercise. While these results provide an  
19 indication for the ability of the normal cartilage matrix to partially regenerate under ongoing  
20 multistage ultramarathon burden in the ankle joints<sup>37</sup>, detailed knowledge on cartilage  
21 metabolism in response to extreme running exercise—especially with intermittent brief  
22 recovery periods such as during a multistage ultramarathon—is not available.

23 Cartilage turnover and load-induced tissue changes are frequently assessed by  
24 quantifying concentrations of cartilage biomarkers in serum. Potential cartilage biomarkers  
25 include structural proteins or enzymes reflecting cartilage metabolism. For instance, elevated

26 levels of cartilage oligomeric matrix protein (COMP) are associated with a higher incidence  
27 risk of knee osteoarthritis (OA)<sup>33</sup>, and load-induced changes in serum COMP predict  
28 cartilage thickness changes in patients with knee OA.<sup>6</sup> COMP levels are sensitive to exercise  
29 bouts of walking (30 minutes<sup>20</sup>; 4000 steps<sup>5</sup>) and running (30 minutes<sup>25, 26</sup>; marathon (42  
30 km)<sup>22</sup>) but not to deep knee bends (120 in 30 minutes<sup>25</sup>). Previous studies have shown that  
31 COMP levels continue to increase throughout ultramarathon running races<sup>14, 38</sup> in runners  
32 without osteoarthritis. Hence, load-induced changes in COMP appear to be sensitive to load  
33 magnitude and number of loading cycles during exercise bouts.

34 Matrix metalloproteinases (MMPs) are a multi-member family of proteinases with a  
35 wide range of substrates including extracellular components, cytokines, receptors, and cell  
36 motility factors.<sup>19, 44</sup> For instance, interstitial collagenase (MMP-1) is produced by  
37 chondrocytes, osteoblasts and synovial cells that degrades collagen types I, II, and III in the  
38 extracellular matrix and mediates cartilage destruction<sup>2, 40</sup>, and is expressed at higher levels  
39 by OA chondrocytes than by normal chondrocytes suggesting a predominant role of MMP-1  
40 in OA pathogenesis.<sup>7, 39</sup> Stromelysin-1 (MMP-3) is in part responsible for the degradation of  
41 non-collagen matrix proteins in cartilage in rheumatoid arthritis and OA<sup>2</sup>, and increased  
42 levels of MMP-3 and stromelysin-2 (MMP-10) are found in articular cartilage and synovium  
43 of these patients.<sup>10, 27, 42</sup> Gelatinase B (MMP-9) and collagenase-3 (MMP-13) coordinate  
44 cartilage collagen and aggrecan breakdown. Native collagen 2 is degraded by MMP-1, -8, -13,  
45 and -14, and partially degraded collagen 2 is then further degraded by MMP-2, MMP-9, and  
46 stromelysin-1 (MMP-3).<sup>4</sup>

47 Another important cartilage component—and hence relevant in the context of  
48 cartilage mechanosensitivity—is type II collagen. In the process of collagen fibril formation,  
49 the C-propeptide is removed from the procollagen extracellularly and directly reflects the rate

50 of type II procollagen synthesis (CPII).<sup>24</sup> Cleavage of type II collagen by collagenases yields  
51 fragments, such as the C2C epitope (COL2-3/4Clong mono)<sup>29</sup>, reflecting degradation.

52 The purpose of this study was to determine serum changes in cartilage biomarkers  
53 during a multistage ultramarathon race. We hypothesized that serum concentrations of  
54 COMP, MMP-1, 3, and 9, C2C, CPII, and C2C:CPII will increase throughout a multistage  
55 ultramarathon.

56

## 57 **Materials and Methods**

58 Of the 67 participants of a 4486 km multistage ultramarathon from the South of Italy  
59 to the North Cape taking place from April 19 to June 21<sup>36</sup>, 36 runners (4 female; mean  $\pm$  1  
60 standard deviation; age,  $49.0 \pm 10.7$  years; height,  $174 \pm 8$  cm; body mass start,  $70.2 \pm 10.2$   
61 kg, body mass finish,  $65.2 \pm 8.5$  kg; body mass index, start:  $23.1 \pm 2.3$  kg/m<sup>2</sup>, finish:  $21.4 \pm$   
62  $1.9$  kg/m<sup>2</sup>) volunteered for this study after providing informed consent. This study was  
63 approved by the institutional review board and complied with the Declaration of Helsinki.

64 The race comprised 64 running days without any rest days with a mean distance per stage of  
65 70.1 km (range, 44.0 to 95.1 km). All runners arrived at the same predetermined daily  
66 intermediate finish where they stayed overnight. Because of the season (late spring to early  
67 summer) and the route from South to North, temperatures stayed relatively constant  
68 throughout the race.<sup>36</sup> All runners were official race participants meeting the ultramarathon  
69 registration requirements:  $\geq 18$  years; medical health certificate; and proof of appropriate  
70 ultramarathon running performance. In the 12 months prior to the race, participants spent an  
71 average 7 to 20 hours per week to run an average of 50 to 220 km per week. Five participants  
72 had a unilateral focal chondral defect in the patellofemoral joint (femur) and one participant  
73 in the tibiofemoral joint (tibia) without any symptoms diagnosed by magnetic resonance

74 imaging MRI performed as part of an associated MRI study on these runners.<sup>35-37</sup> The MR  
75 signal of these defects did not change throughout the ultramarathon.

76 Serum samples were collected within 4 days prior to the race ( $t_0$ ) and on days 15 ( $t_1$ :  
77 1002 km), 31 ( $t_2$ : 2132 km), 47 ( $t_3$ : 3234 km), and 58 ( $t_4$ : 4039 km) of the 64-day race.  
78 Average running speed and daily running time for each of the four measurement intervals  
79 (MI; MI1:  $t_1-t_0$ ; MI2:  $t_2-t_1$ ; MI3:  $t_3-t_2$ ; MI4:  $t_4-t_3$ ) between blood sampling was calculated and  
80 body mass measured for each runner. Blood samples were taken from the cubital vein after  
81 the daily running stage. The samples were immediately centrifuged, aliquoted, frozen (below  
82  $-20^\circ\text{C}$ ), and transferred to  $-80^\circ\text{C}$  after the race. Serum biomarker levels were determined in  
83 duplicates using commercial enzyme-linked immunosorbent assays: (COMP: Wieslab®  
84 hCOMP quantitative kit (Euro Diagnostica AB, Malmö, Sweden); MMP-1: RayBio® Human  
85 MMP-1 ELISA kit (RayBiotech Inc., Norcross, GA, USA); MMP-3 and MMP-9: Human  
86 MMP-3 Quantikine Kit and Human MMP-9 Quantikine Kit (Bio-Techne Ltd., Abingdon,  
87 UK); C2C and CPII: Collagen Type II Cleavage Assay and Procollagen Type II C-Propeptide  
88 Assay (IBEX Technologies Inc. Montreal, Quebec, Canada)). All biomarkers were  
89 determined simultaneously for each sample upon thawing the sample to avoid refreezing  
90 samples. All samples of each participant were tested on the same plate to avoid any errors  
91 due to plate-to-plate differences. Intra-assay variability was assessed as relative coefficients  
92 of variation (CV%) between duplicates and was 4.8% for COMP, 3.7% for MMP-1, 7.0% for  
93 MMP-3, 2.7% for MMP-9, 6.9% for C2C, and 7.3% for CPII.

94

#### 95 *Statistical analysis*

96 All statistical analyses were performed using SPSS Version 21 (IBM Corporation,  
97 Armonk, NY). All parameters were tested for normal distribution using Kolmogorow  
98 Smirnow tests. Linear mixed models were used to detect significant changes in serum

99 biomarker levels over time with time-varying covariates body mass, running speed, and daily  
 100 running time, and posthoc least square tests. Because not all runners completed the entire race,  
 101 missing data were handled by imputing values using the last observation carried forward  
 102 method, and all models were rerun. Race finishing was used as between subject factor in the  
 103 models (finisher versus non-finisher). The significance level for all statistical tests was set a  
 104 priori to .05.

105

## 106 **Results**

107 Participants ran with an average running speed of  $8.2 \pm 1.4$  km/h (mean  $\pm$  1 standard  
 108 deviation) and lost an average of  $5.3 \pm 2.7$  kg of body mass (Table 1). Six runners dropped  
 109 out in MI2, one in MI3 and four in MI4. Age, height, body mass, running speed and  
 110 biomarker levels after MI1 and MI2 did not differ between groups by time of dropout  
 111 ( $P > .029$ ). The following reasons for drop-out were reported: shin splint (N=4), thigh splint  
 112 (N=2), foot pain with purulence (N=1), phlegmon finger treated by surgery (N=1), proximal  
 113 tibia fracture (N=1), anterior pelvic ring fracture (N=1; participant with focal cartilage defect  
 114 in patellofemoral joint), and respiratory infection (N=1). All other participants with focal  
 115 cartilage defects completed the race. None of the biomarker results differed between

Table 1. Mean (1 standard deviation) time varying covariates body mass, running speed and daily running time before and throughout the multistage ultramarathon.

| <i>Parameter</i>                   | <i>t<sub>0</sub></i><br><i>Pre-race</i><br><i>(N=36)</i> | <i>t<sub>1</sub></i><br><i>After 1002 km</i><br><i>(N=36)</i> | <i>t<sub>2</sub></i><br><i>After 2132 km</i><br><i>(N=30)</i> | <i>t<sub>3</sub></i><br><i>After 3234 km</i><br><i>(N=29)</i> | <i>t<sub>4</sub></i><br><i>After 4038 km</i><br><i>(N=26)</i> | <i>P-value</i><br><i>finisher<sup>a</sup></i> |
|------------------------------------|--|---|---|---|---|---|
| <i>Body mass (kg)</i>              | 70.6<br>(9.9)  | 67.6<br>(9.2)   | 66.4<br>(8.8)   | 65.6<br>(8.8)   | 65.2<br>(8.5)   | <b>&lt;.001</b>                               |
| <i>Mean running speed (km/h)</i>   |  | 8.40<br>(1.25)  | 8.46<br>(1.39)  | 8.43<br>(1.47)  | 8.41<br>(1.44)  | .955  |
| <i>Mean daily running time (h)</i> |  | 8.1<br>(1.1)  | 7.7<br>(1.3)  | 7.9<br>(1.2)  | 7.8<br>(1.6)  | .226  |

<sup>a</sup>—results of the linear mixed models on the runners who completed the race (N=23). Note: The results of the linear mixed models did not change when data of non-finishers were considered using the last observation carried forward approach.

Table 2. Mean (1 standard deviation) serum biomarker concentrations before and throughout the multistage ultramarathon.

| <i>Cartilage biomarker</i> | <i>t<sub>0</sub></i><br><i>Pre-race</i><br><i>(N=36)</i> | <i>t<sub>1</sub></i><br><i>After 1002 km</i><br><i>(N=36)</i> | <i>t<sub>2</sub></i><br><i>After 2132 km</i><br><i>(N=30)</i> | <i>t<sub>3</sub></i><br><i>After 3234 km</i><br><i>(N=29)</i> | <i>t<sub>4</sub></i><br><i>After 4038 km</i><br><i>(N=23)</i> | <i>P-value finisher<sup>a</sup></i> |
|----------------------------|--|---|---|---|---|-------------------------------------|
| <i>COMP (ng/mL)</i>        | 2.19<br>(0.42)   | 2.67<br>(0.48)  | 2.61<br>(0.60)  | 2.57<br>(0.40)  | 2.69<br>(0.53)  | <b>&lt;.001</b>                     |
| <i>MMP-1 (ng/mL)</i>       | 20.07<br>(25.06)   | 20.88<br>(30.28)  | 26.32<br>(23.21)  | 33.93<br>(30.08)  | 32.04<br>(23.42)  | 0.328                               |
| <i>MMP-3 (ng/mL)</i>       | 25.36<br>(16.16)   | 31.15<br>(20.16)  | 33.26<br>(16.03)  | 36.48<br>(18.65)  | 37.77<br>(16.43)  | <b>0.046</b>                        |
| <i>MMP-9 (ng/mL)</i>       | 232.71<br>(207.83)                                       | 444.11<br>(453.74)  | 484.47<br>(295.11)  | 568.10<br>(311.10)  | 541.52<br>(262.67)  | <b>&lt;.001</b>                     |
| <i>C2C (ng/mL)</i>         | 166.45<br>(48.77)  | 185.92<br>(60.76)   | 175.63<br>(47.24)   | 154.24<br>(44.83)   | 162.17<br>(29.31)   | .190                                |
| <i>CPII (μg/mL)</i>        | 2.98<br>(1.45)   | 3.64<br>(2.02)  | 3.00<br>(1.34)  | 2.63<br>(1.37)  | 2.64<br>(6.43)  | .067                                |
| <i>C2C:CPII</i>            | 0.061<br>(0.014)   | 0.058<br>(0.018)  | 0.063<br>(0.017)  | 0.065<br>(0.018)  | 0.064<br>(0.015)  | .407                                |

COMP—cartilage oligomeric matrix protein; MMP—matrix proteinases; C2C— C-terminal neopeptide generated by the collagenase-mediated cleavage of collagen type II triple helix; CPII— procollagen type II C-terminal propeptide; CPII—ratio of C2C and CPII reflecting collagen turnover. A—results of the linear mixed models on the runners who completed the race (N=23). Note: The results of the linear mixed models did not change when data of non-finishers were considered using the last observation carried forward approach.

116 participants with or without focal cartilage defect.

117 Serum concentrations of COMP, MMP-9 and MMP-3 changed significantly  
 118 throughout the multistage ultramarathon (Table 2). On average, concentrations increased  
 119 during MI1 by 22.5% (change MI1, COMP: [0.29;0.71] ng/mL), 22.3% (MMP-3:  
 120 [0.24;15.37] ng/mL), and 95.6% (MMP-9: [81.7;414.5] ng/mL), and remained stable  
 121 throughout MI2, MI3 and MI4 (Figure 1). Changes in serum COMP, MMP-3, and MMP-9  
 122 concentrations during MI1 did not differ between finishers and non-finishers (time×finishing  
 123 group interaction: P=.387, P=.620, and P=.945, respectively). Serum concentrations of MMP-  
 124 1, C2C, CPII, and C2C:CPII did not change significantly throughout the multistage  
 125 ultramarathon (Table 2). The results of the linear mixed models did not change when data of  
 126 non-finishers were considered using the last observation carried forward approach.

127 The time varying covariate body mass was significantly associated with changes in  
 128 COMP, MMP-3, and MMP-9 throughout the multistage ultramarathon (COMP: Wald

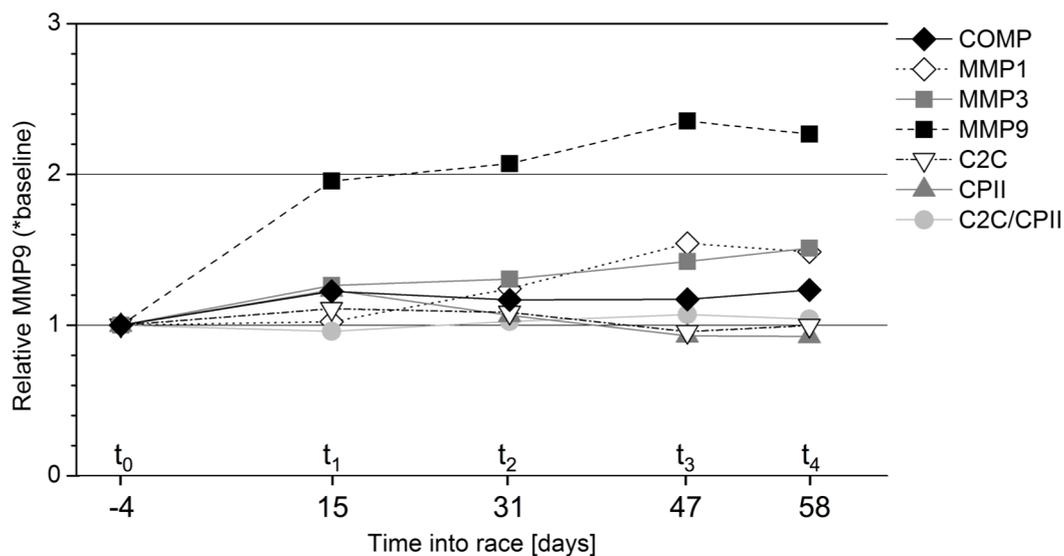


Figure 1. Mean relative changes in cartilage biomarkers normalized to baseline level. COMP—cartilage oligomeric matrix protein; MMP—matrix proteinases; C2C— C-terminal neopeptide generated by the collagenase-mediated cleavage of collagen type II triple helix; CII—procollagen type II C-terminal propeptide; C2C:CII—ratio of C2C and CII reflecting collagen turnover.

129 Z=3.411, P=.002; MMP-3: Wald Z = 2.472, P=.013; MMP-9: Wald Z = 2.226, P=.026). The  
 130 time varying covariates running speed and daily running time were not associated with  
 131 changes in any cartilage biomarker. Changes in MMP-3 were associated with changes in  
 132 COMP throughout the ultramarathon race (MMP-3: Wald Z=3.476, P=.001) where in 68% of  
 133 runners ultramarathon-induced changes in MMP-3 levels explained more than 30% of  
 134 ultramarathon-induced changes in COMP levels. Figure 2 shows an example of the  
 135 relationship between MMP-3 and COMP levels for one participant. Changes in MMP-1,  
 136 MMP-9, C2C, CII or C2C:CII were not associated with changes in COMP.

137

### 138 Discussion

139 The purpose of this study was to determine serum changes in cartilage biomarkers  
 140 during a multistage ultramarathon race. COMP, MMP-3, and MMP-9 levels increased within  
 141 the first 11 days of the ultra-marathon race and remained elevated throughout the remainder

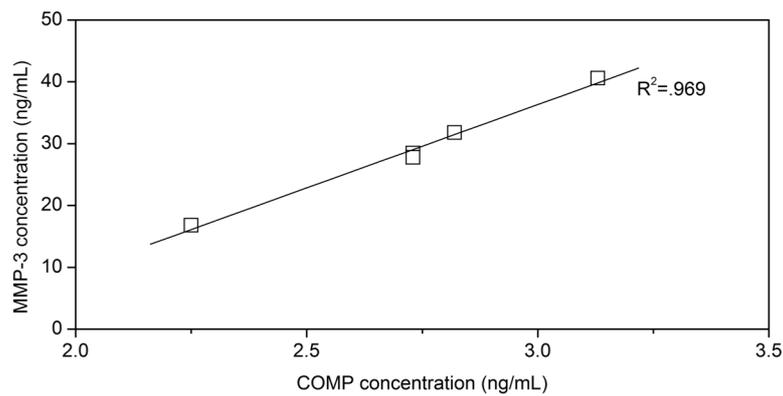


Figure 2. Relationship between MMP-3 and COMP levels for one participant.

142 of the race. MMP-1, C2C and CPII levels and C2C:CPII did not change throughout the race.  
 143 The time varying covariate body mass was associated with changes in COMP, MMP-3, and  
 144 MMP-9 throughout the multistage ultramarathon. Changes in MMP-3 were associated with  
 145 changes in COMP throughout the ultramarathon race. The results provide first evidence that  
 146 only some cartilage biomarkers are sensitive to extreme running exercise and that changes in  
 147 these biomarkers are correlated.

148 Of the known potential cartilage biomarkers, COMP has been used most often as  
 149 surrogate measure of cartilage degradation in studies on the effect of exercises of different  
 150 intensities on articular cartilage. Interestingly, the magnitude of increase in COMP in our  
 151 study (+22.5%) was not greater than that reported for marathon and single stage  
 152 ultramarathon races. For instance, COMP levels did not change more than after other  
 153 physical activities such as walking 14 km uphill<sup>30</sup>, walking for 30 minutes,<sup>21</sup> walking 4000  
 154 steps at slow, medium or fast walking speed,<sup>5</sup> or running for 30 minutes.<sup>25</sup> Moreover,  
 155 increases in COMP after a marathon range from 17 to 60%.<sup>14, 22, 23</sup> Kim et al. reported a 1.9-  
 156 and 3-fold increase in COMP levels after 100 km and 200 km, respectively, of a 200 km  
 157 single stage ultramarathon in two separate studies (mean race time, 32.5 hours).<sup>13, 14</sup> In a  
 158 single stage ultramarathon study by Shin et al.<sup>38</sup>, COMP levels increased by 130.7% at 100

159 km to 160.4% at 200 km and 194.1% at 308 km (mean race time, 61.5 hours). All of these  
160 studies have in common that COMP concentrations continued to increase throughout these  
161 single stage marathon<sup>14, 23</sup> or single stage ultramarathon races.<sup>13, 14, 38</sup> In contrast, serum  
162 COMP levels in our study remained stable throughout the multistage ultramarathon race after  
163 the initial 1002 km. Because the second blood draw was taken 11 days into the race (after  
164 1002 km), information regarding a potential initial continuous increase or a peak in COMP  
165 level between days 1 and 11 of the race is not available.

166 Previous studies have reported a recovery of COMP levels within 30 minutes to  
167 several days for light (30-minute walking<sup>21</sup> or running<sup>25</sup>) and intense exercise (marathons<sup>14, 22,</sup>  
168 <sup>23</sup> and ultramarathons<sup>14</sup>), respectively. Moreover, Mündermann et al.<sup>22</sup> have shown that  
169 COMP levels in runners with faster marathon finishing times return to pre-race levels within  
170 24 hours of the marathon but not in those with slower marathon finishing times. The authors  
171 attributed these differences to different relative load between runners because of greater  
172 number of steps taken during the race in slower runners or differences in fitness among  
173 runners. In addition, a predefined walking exercise (4000 steps) at varying walking speeds  
174 (slow, medium, fast) resulted in systematic changes in COMP levels and these changes were  
175 related to differences in joint mechanics<sup>5</sup>. Accordingly, one could expect that changes in  
176 COMP during the multistage ultramarathon are associated with running speed and/or daily  
177 running time. However, the linear mixed models with time varying covariates did not reveal  
178 such an association in this group of experienced ultramarathon runners. Interestingly, a 3-  
179 week multistage cycling race did not result in changes in COMP levels in pro-cyclists<sup>3</sup>. Like  
180 running, cycling is characterized by high cyclic joint loads (e.g. several times body weight at  
181 the knee<sup>15</sup>), but unlike in running, joint forces rise and fall without an impact peak caused by  
182 the collision of the body with the ground. The lack of changes in COMP levels in a  
183 multistage cycling race and increases in COMP levels in a multistage running race suggests

184 that COMP levels are sensitive to repetitive impact loads most likely of articular cartilage and  
185 not of other musculoskeletal tissues.

186 The main differences between single stage and multistage ultramarathons are the  
187 much longer distances covered and the daily (usually overnight) resting times in multistage  
188 races. Based on COMP data from marathons and single stage ultramarathons, one would  
189 expect the magnitude of changes in COMP levels to increase with increasing distance with a  
190 gradual increase in levels throughout a race. The fact that COMP levels did not increase more  
191 during the multistage ultramarathon than reported increases in shorter single stage races  
192 suggests that the daily resting time may have been sufficient for tissue recovery to some  
193 extent. Slower runners took more time each day to complete the daily stage and hence had  
194 shorter overnight resting times implying less recovery. However, daily running time was not  
195 associated with changes in COMP. Hence, even in slower runners, overnight resting times  
196 may have been sufficient for preventing further increase in COMP levels throughout the race.  
197 It appears that cartilage reached a steady state during the race, which is further supported by  
198 previous reports<sup>35, 37</sup> of an initial T2\* increase in articular cartilage of the ankle and the knee  
199 followed by a subsequent T2\* decrease (ankle)<sup>37</sup> and steady-state (knee)<sup>35</sup> in these runners.  
200 The changes in COMP levels reported here support the previous suggestion of the ability of  
201 the normal cartilage matrix at the ankle joints to partially regenerate with continuing  
202 multistage ultramarathon load.<sup>37</sup> Participants of multistage ultramarathon races represent a  
203 unique sample of athletes that are extremely well conditioned because of extreme training  
204 regimens possibly explaining the smaller increases in COMP levels compared to those  
205 reported in marathon and single stage ultramarathon runners. These results are relevant not  
206 only for ultramarathon runners but also for elite athletes training for marathons requiring high  
207 weekly running distances or for extreme expeditions of several days or week.

208 Cyclic loading enhances COMP expression in a fully developed pericellular matrix.<sup>9</sup>  
209 While some data on the effects of running on COMP are available, little is known on the  
210 effects of running on other cartilage biomarkers. COMP levels are a measure of intact COMP  
211 or COMP fragments in blood. However, it is unclear if these fragments are present because of  
212 simple turnover or cartilage breakdown. Hence, markers reflecting tissue metabolism must  
213 also be considered. MMP-3 and MMP-9 levels but not MMP-1, C2C, or CPII levels changed  
214 during the multistage ultramarathon. Interestingly, COMP, MMP-3, and MMP-9 but not  
215 MMP-1 levels changed during immobilization during a 21-day bed-rest study.<sup>16</sup> Hence,  
216 COMP, MMP-3, and MMP-9 systematically respond to extreme load and to unloading  
217 emphasizing their importance in the mechanobiology of articular cartilage. MMP-3 is in part  
218 responsible for the degradation of non-collagen matrix proteins in cartilage in rheumatoid  
219 arthritis and OA<sup>2</sup> and MMP-9 and MMP-13 coordinate cartilage collagen and aggrecan  
220 breakdown. The association of changes in MMP-3 levels with changes in COMP levels  
221 indicate that MMP-3 may be involved in the degradation of COMP. This result supports  
222 findings of in situ experiments where digestion of human articular cartilage with MMP-3, -12,  
223 or -13 but not with MMP-2, -8, or -9 yielded fragments of COMP.<sup>45</sup> MMP-1 degrades  
224 collagen types I, II, and III in the extracellular matrix, and mediates cartilage destruction.<sup>2, 40</sup>  
225 The lack of changes in MMP-1, C2C and CPII levels, and in C2C:CPII suggest that the  
226 extreme running load did not affect collagen turnover. Similarly, COMP, MMP-3, and MMP-  
227 9 but not MMP-1 levels changed in a 21-day bed rest study<sup>16, 17</sup> suggesting that MMP-1 is not  
228 sensitive to loading.

229 Henrotin et al.<sup>12</sup> observed decreases in Coll2-1 levels (a denaturation epitope located  
230 in the triple helical domain of the type II collagen molecule that is made available by  
231 unwinding of the triple helix<sup>11</sup>) after a marathon, which they interpreted as a protective effect  
232 of long distance running on cartilage. In contrast, we did not observe changes in C2C or CPII

233 levels or in C2C:CPII during the multistage ultramarathon suggesting that the balance  
234 between collagen II synthesis and degradation was unaffected by the extreme running load.  
235 However, because the second sample was taken after about 1000 km, it is possible that we  
236 were unable to detect subtle changes early in the race. Moreover, it is possible that extreme  
237 load does not initiate collagen turnover but causes reorganization or loss of organization of  
238 the matrix and degradation of proteoglycans resulting in an increases in glucosaminoglycan  
239 content<sup>31, 32</sup>, which has also been indicated by previously observed changes in T2\* of  
240 articular cartilage at the ankle during a multistage ultramarathon.<sup>37</sup>

241         Some discrepancies between our results and the literature may have been caused by  
242 methodological differences. For instance, while many studies used a blood sample taken  
243 within 2 hours prior to the race as baseline value, in other studies baseline samples were  
244 taken 24 hours before the marathon<sup>41</sup>, 6 to 10 hours before the ultramarathon<sup>14</sup>, and up to 4  
245 days before the multistage marathon in our study. Moreover, none of the studies specified  
246 whether physical activity prior to the baseline sample was controlled or restricted which may  
247 influence baseline levels.<sup>21</sup> Interestingly, most studies<sup>13, 14, 22, 38</sup> on marathon and  
248 ultramarathon running involve participants with an average age around 50 years who were  
249 experienced ultramarathon runners when 25% of the population between 45 and 64 years  
250 suffer from arthritis or joint pain.<sup>1</sup> Some runners had focal lesion in the patellofemoral joint  
251 without any symptoms, and the MR signal did not change throughout the race. Hence, the  
252 patellofemoral joint may not have been adversely affected by the extreme running exercise on  
253 flat ground. Further, it is possible that only athletes without any joint degeneration affecting  
254 joint mechanics will participate in such a physically and mentally demanding sports. Based  
255 on the literature it is also feasible that a stringent training regimen over a long time may  
256 protect against cartilage degeneration in the tibiofemoral and ankle joints as previously  
257 shown in animal studies<sup>8, 28</sup> and suggested by Schütz et al.<sup>37</sup>

258

## 259 **Conclusions**

260 The results of this study provide evidence that physical load affects some cartilage  
261 biomarkers (COMP, MMP-9, and MMP-3 but not MMP-1, C2C, CPII, or C2C:CPII) and that  
262 the magnitude of these changes appear to be limited by providing regular short recovery  
263 periods throughout ultra-running exercises in highly adapted ultra-endurance athletes. While  
264 COMP levels may play an important role in the mechanotransduction of ambulatory load to  
265 chondrocytes, the role of COMP concentration on cartilage health in this population remains  
266 unclear. Nonetheless, elevated COMP levels indicate increased COMP turnover in response  
267 to extreme running, and the association between load-induced changes in MMP-3 and  
268 changes in COMP suggests the possibility that MMP-3 may be involved in the degradation of  
269 COMP. The lack of changes in MMP-1, C2C, CPII, and C2C:CPII indicate that these  
270 markers are not involved in load-induced changes in articular cartilage.

271

## 272 **Author disclosures**

273 We declare that we have no conflicts of interest in the authorship or publication of this  
274 contribution

275

## 276 **References**

- 277 1. Barbour KE, Helmick CG, Boring M, Zhang X, Lu H, Holt JB. Prevalence of Doctor-  
278 Diagnosed Arthritis at State and County Levels - United States, 2014. *MMWR Morb*  
279 *Mortal Wkly Rep.* 2016;65(19):489-494.
- 280 2. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front*  
281 *Biosci.* 2006;11:529-543.
- 282 3. Corsetti R, Perego S, Sansoni V, et al. Osteocartilaginous metabolic markers change over  
283 a 3-week stage race in pro-cyclists. *Scand J Clin Lab Invest.* 2015;75(6):523-530.

- 284 4. De Ceuninck F, Sabatini M, Pastoureau P. Recent progress toward biomarker  
285 identification in osteoarthritis. *Drug Discov Today*. 2011;16(9-10):443-449.
- 286 5. Denning WM, Becker Pardo M, Winward JG, et al. Ambulation speed and corresponding  
287 mechanics are associated with changes in serum cartilage oligomeric matrix protein.  
288 *Gait Posture*. 2016;44:131-136.
- 289 6. Erhart-Hledik JC, Favre J, Asay JL, et al. A relationship between mechanically-induced  
290 changes in serum cartilage oligomeric matrix protein (COMP) and changes in  
291 cartilage thickness after 5 years. *Osteoarthritis Cartilage*. 2012;20(11):1309-1315.
- 292 7. Fernandes JC, Martel-Pelletier J, Lascau-Coman V, et al. Collagenase-1 and collagenase-  
293 3 synthesis in normal and early experimental osteoarthritic canine cartilage: an  
294 immunohistochemical study. *J Rheumatol*. 1998;25(8):1585-1594.
- 295 8. Galois L, Etienne S, Grossin L, et al. Moderate-impact exercise is associated with  
296 decreased severity of experimental osteoarthritis in rats. *Rheumatology (Oxford)*.  
297 2003;42(5):692-693; author reply 693-694.
- 298 9. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage  
299 oligomeric matrix protein (COMP). *Biorheology*. 2003;40(1-3):101-109.
- 300 10. Hembry RM, Bagga MR, Reynolds JJ, Hamblen DL. Immunolocalisation studies on six  
301 matrix metalloproteinases and their inhibitors, TIMP-1 and TIMP-2, in synovia from  
302 patients with osteo- and rheumatoid arthritis. *Ann Rheum Dis*. 1995;54(1):25-32.
- 303 11. Henrotin Y, Addison S, Kraus V, Deberg M. Type II collagen markers in osteoarthritis:  
304 what do they indicate? *Curr Opin Rheumatol*. 2007;19(5):444-450.
- 305 12. Henrotin Y, Labasse A, Franck T, Bosseloir A, Bury T, Deberg M. Collagen catabolism  
306 through Coll2-1 and Coll2-1NO2 and myeloperoxidase activity in marathon runners.  
307 *Springerplus*. 2013;2(1):92.
- 308 13. Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and  
309 inflammation during a 200 km run. *Eur J Appl Physiol*. 2007;99(4):443-447.
- 310 14. Kim HJ, Lee YH, Kim CK. Changes in serum cartilage oligomeric matrix protein  
311 (COMP), plasma CPK and plasma hs-CRP in relation to running distance in a  
312 marathon (42.195 km) and an ultra-marathon (200 km) race. *Eur J Appl Physiol*.  
313 2009;105(5):765-770.
- 314 15. Kutzner I, Heinlein B, Graichen F, et al. Loading of the knee joint during ergometer  
315 cycling: telemetric in vivo data. *J Orthop Sports Phys Ther*. 2012;42(12):1032-1038.

- 316 16. Liphardt A-M, Mündermann A, Koo S, et al. Relevance of immobility for serum levels  
317 of biomarkers for cartilage health. Paper presented at: 19th IAA Human in Space  
318 Conference 2014; June 2014, 2014; Waterloo, Canada.
- 319 17. Liphardt AM, Mundermann A, Koo S, et al. Vibration training intervention to maintain  
320 cartilage thickness and serum concentrations of cartilage oligometric matrix protein  
321 (COMP) during immobilization. *Osteoarthritis Cartilage*. 2009;17(12):1598-1603.
- 322 18. Lord R, George K, Somauroo J, et al. Alterations in Cardiac Mechanics Following Ultra-  
323 Endurance Exercise: Insights from Left and Right Ventricular Area-Deformation  
324 Loops. *J Am Soc Echocardiogr*. 2016;29(9):879-887 e871.
- 325 19. Morrison CJ, Butler GS, Rodriguez D, Overall CM. Matrix metalloproteinase  
326 proteomics: substrates, targets, and therapy. *Curr Opin Cell Biol*. 2009;21(5):645-653.
- 327 20. Mündermann A, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage  
328 oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in  
329 healthy adults. *Osteoarthritis Cartilage*. 2005;13(1):34-38.
- 330 21. Mündermann A, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage  
331 oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in  
332 healthy adults. *Osteoarthritis Cartilage*. 2005;13(1):34-38.
- 333 22. Mündermann A, Geurts J, Hugle T, et al. Marathon performance but not BMI affects  
334 post-marathon pro-inflammatory and cartilage biomarkers. *J Sports Sci*. 2016;May  
335 11:1-8.
- 336 23. Neidhart M, Müller-Ladner U, Frey W, et al. Increased serum levels of non-collagenous  
337 matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity)  
338 in marathon runners. *Osteoarthritis Cartilage*. 2000;8(3):222-229.
- 339 24. Nelson F, Dahlberg L, Laverty S, et al. Evidence for altered synthesis of type II collagen  
340 in patients with osteoarthritis. *The Journal of Clinical Investigation*.  
341 1998;102(12):2115-2125.
- 342 25. Niehoff A, Kersting UG, Helling S, et al. Different mechanical loading protocols  
343 influence serum cartilage oligomeric matrix protein levels in young healthy humans.  
344 *Eur J Appl Physiol*. 2010;110(3):651-657.
- 345 26. Niehoff A, Muller M, Bruggemann L, et al. Deformational behaviour of knee cartilage  
346 and changes in serum cartilage oligomeric matrix protein (COMP) after running and  
347 drop landing. *Osteoarthritis Cartilage*. 2011;19(8):1003-1010.

- 348 27. Okada Y, Shinmei M, Tanaka O, et al. Localization of matrix metalloproteinase 3  
349 (stromelysin) in osteoarthritic cartilage and synovium. *Lab Invest.* 1992;66(6):680-  
350 690.
- 351 28. Otterness IG, Eskra JD, Bliven ML, Shay AK, Pelletier JP, Milici AJ. Exercise protects  
352 against articular cartilage degeneration in the hamster. *Arthritis Rheum.*  
353 1998;41(11):2068-2076.
- 354 29. Poole AR, Ionescu M, Fitzcharles MA, Billingham RC. The assessment of cartilage  
355 degradation in vivo: development of an immunoassay for the measurement in body  
356 fluids of type II collagen cleaved by collagenases. *J Immunol Methods.* 2004;294(1-  
357 2):145-153.
- 358 30. Pruksakorn D, Tirankgura P, Luevitoonvechkij S, et al. Changes in the serum cartilage  
359 biomarker levels of healthy adults in response to an uphill walk. *Singapore Med J.*  
360 2013;54(12):702-708.
- 361 31. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan  
362 content in knee cartilage: a four-month, randomized, controlled trial in patients at risk  
363 of osteoarthritis. *Arthritis Rheum.* 2005;52(11):3507-3514.
- 364 32. Saamamen AM, Kiviranta I, Jurvelin J, Helminen HJ, Tammi M. Proteoglycan and  
365 collagen alterations in canine knee articular cartilage following 20 km daily running  
366 exercise for 15 weeks. *Connect Tissue Res.* 1994;30(3):191-201.
- 367 33. Saberi Hosnijeh F, Runhaar J, van Meurs JB, Bierma-Zeinstra SM. Biomarkers for  
368 osteoarthritis: Can they be used for risk assessment? A systematic review. *Maturitas.*  
369 2015.
- 370 34. Schiffer T, Montiel G, Hildebrandt U, Predel HG, Knackstedt C. Der Marathonlauf als  
371 gesundheitliches Risiko? *Klinikerzt.* 2010;39:288-291.
- 372 35. Schütz UH, Billich C, Ellemann J, et al. The TEFr Field Study: Results of Continuous  
373 Biochemical and Morphological Cartilage Analysis of Hindfoot, Ankle, and Knee  
374 Joints in Course of a 4,500 km Ultramarathon Race throughout Whole Europe Using  
375 T2\*-mapping on a Mobile MRI Truck Trailer. Paper presented at: 101st Scientific  
376 Assembly and Annual Meeting of the Radiological Society of North America; Nov 29  
377 to Dec 4, 2015; Chicago, IL.
- 378 36. Schütz UH, Billich C, König K, et al. Characteristics, changes and influence of body  
379 composition during a 4486 km transcontinental ultramarathon: results from the  
380 TransEurope FootRace mobile whole body MRI-project. *BMC Med.* 2013;11:122.

- 381 37. Schütz UH, Ellermann J, Schoss D, Wiedelbach H, Beer M, Billich C. Biochemical  
382 cartilage alteration and unexpected signal recovery in T2\* mapping observed in ankle  
383 joints with mobile MRI during a transcontinental multistage footrace over 4486 km.  
384 *Osteoarthritis Cartilage*. 2014;22(11):1840-1850.
- 385 38. Shin KA, Kim AC, Kim YJ, et al. Effect of Ultra-marathon (308 km) Race on Bone  
386 Metabolism and Cartilage Damage Biomarkers. *Ann Rehabil Med*. 2012;36(1):80-87.
- 387 39. Tetlow LC, Adlam DJ, Woolley DE. Matrix metalloproteinase and proinflammatory  
388 cytokine production by chondrocytes of human osteoarthritic cartilage: associations  
389 with degenerative changes. *Arthritis Rheum*. 2001;44(3):585-594.
- 390 40. Valdes AM, Spector TD. Genetic epidemiology of hip and knee osteoarthritis.  
391 *Nat.Rev.Rheumatol*. 2011;7(1):23-32.
- 392 41. Vuolteenaho K, Leppanen T, Kekkonen R, Korpela R, Moilanen E. Running a marathon  
393 induces changes in adipokine levels and in markers of cartilage degradation--novel  
394 role for resistin. *PLoS One*. 2014;9(10):e110481.
- 395 42. Wolfe GC, MacNaul KL, Buechel FF, et al. Differential in vivo expression of  
396 collagenase messenger RNA in synovium and cartilage. Quantitative comparison with  
397 stromelysin messenger RNA levels in human rheumatoid arthritis and osteoarthritis  
398 patients and in two animal models of acute inflammatory arthritis. *Arthritis Rheum*.  
399 1993;36(11):1540-1547.
- 400 43. Wuthrich TU, Marty J, Kerherve H, Millet GY, Verges S, Spengler CM. Aspects of  
401 respiratory muscle fatigue in a mountain ultramarathon race. *Med Sci Sports Exerc*.  
402 2015;47(3):519-527.
- 403 44. Yong VW, Agrawal SM, Stirling DP. Targeting MMPs in acute and chronic neurological  
404 conditions. *Neurotherapeutics*. 2007;4(4):580-589.
- 405 45. Zhen EY, Brittain IJ, Laska DA, et al. Characterization of metalloprotease cleavage  
406 products of human articular cartilage. *Arthritis Rheum*. 2008;58(8):2420-2431.

407