

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

Annegret Mündermann^{1,2}, Christopher Klenk³, Christian Billich⁴, Corina Nüesch^{1,2}, Geert Pagenstert¹, Arno Schmidt-Trucksäss³, Uwe Schütz^{4,5}

¹Orthopaedics and Traumatology Hospital, University of Basel, Basel, Switzerland

²Department of Biomedical Engineering, University of Basel, Basel, Switzerland

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

⁵Orthopaedic 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

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 ± 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 event³⁴, 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 system¹⁸,
11 respiratory⁴³ and skeletal muscle³⁸, and the gastrointestinal system³⁸. Interestingly, to date
12 information on the effects of ultramarathon running on articular cartilage is scarce. A
13 previous study³⁷ 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 Dahlberg³¹ 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 joints³⁷, 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)³³, and load-induced changes in serum COMP predict
28 cartilage thickness changes in patients with knee OA.⁶ COMP levels are sensitive to exercise
29 bouts of walking (30 minutes²⁰; 4000 steps⁵) and running (30 minutes^{25, 26}; marathon (42
30 km)²²) but not to deep knee bends (120 in 30 minutes²⁵). Previous studies have shown that
31 COMP levels continue to increase throughout ultramarathon running races^{14, 38} 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.^{19, 44} 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^{2, 40}, 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.^{7, 39} Stromelysin-1 (MMP-3) is in part responsible for the degradation of
41 non-collagen matrix proteins in cartilage in rheumatoid arthritis and OA², and increased
42 levels of MMP-3 and stromelysin-2 (MMP-10) are found in articular cartilage and synovium
43 of these patients.^{10, 27, 42} 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).⁴

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).²⁴ Cleavage of type II collagen by collagenases yields
51 fragments, such as the C2C epitope (COL2-3/4Clong mono)²⁹, 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 21³⁶, 36 runners (4 female; mean \pm 1
60 standard deviation; age, 49.0 ± 10.7 years; height, 174 ± 8 cm; body mass start, 70.2 ± 10.2
61 kg, body mass finish, 65.2 ± 8.5 kg; body mass index, start: 23.1 ± 2.3 kg/m², finish: $21.4 \pm$
62 1.9 kg/m²) 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.³⁶ All runners were official race participants meeting the ultramarathon
69 registration requirements: ≥ 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.³⁵⁻³⁷ 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°C), and transferred to -80°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 ± 1.4 km/h (mean \pm 1 standard
 108 deviation) and lost an average of 5.3 ± 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>t₀</i> <i>Pre-race</i> <i>(N=36)</i>	<i>t₁</i> <i>After 1002 km</i> <i>(N=36)</i>	<i>t₂</i> <i>After 2132 km</i> <i>(N=30)</i>	<i>t₃</i> <i>After 3234 km</i> <i>(N=29)</i>	<i>t₄</i> <i>After 4038 km</i> <i>(N=26)</i>	<i>P-value</i> <i>finisher^a</i>
<i>Body mass (kg)</i>	70.6 (9.9)	67.6 (9.2)	66.4 (8.8)	65.6 (8.8)	65.2 (8.5)	<.001
<i>Mean running speed (km/h)</i>		8.40 (1.25)	8.46 (1.39)	8.43 (1.47)	8.41 (1.44)	.955
<i>Mean daily running time (h)</i>		8.1 (1.1)	7.7 (1.3)	7.9 (1.2)	7.8 (1.6)	.226

^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.

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

<i>Cartilage biomarker</i>	<i>t₀</i> <i>Pre-race</i> <i>(N=36)</i>	<i>t₁</i> <i>After 1002 km</i> <i>(N=36)</i>	<i>t₂</i> <i>After 2132 km</i> <i>(N=30)</i>	<i>t₃</i> <i>After 3234 km</i> <i>(N=29)</i>	<i>t₄</i> <i>After 4038 km</i> <i>(N=23)</i>	<i>P-value finisher^a</i>
<i>COMP (ng/mL)</i>	2.19 (0.42)	2.67 (0.48)	2.61 (0.60)	2.57 (0.40)	2.69 (0.53)	<.001
<i>MMP-1 (ng/mL)</i>	20.07 (25.06)	20.88 (30.28)	26.32 (23.21)	33.93 (30.08)	32.04 (23.42)	0.328
<i>MMP-3 (ng/mL)</i>	25.36 (16.16)	31.15 (20.16)	33.26 (16.03)	36.48 (18.65)	37.77 (16.43)	0.046
<i>MMP-9 (ng/mL)</i>	232.71 (207.83)	444.11 (453.74)	484.47 (295.11)	568.10 (311.10)	541.52 (262.67)	<.001
<i>C2C (ng/mL)</i>	166.45 (48.77)	185.92 (60.76)	175.63 (47.24)	154.24 (44.83)	162.17 (29.31)	.190
<i>CPII (μg/mL)</i>	2.98 (1.45)	3.64 (2.02)	3.00 (1.34)	2.63 (1.37)	2.64 (6.43)	.067
<i>C2C:CPII</i>	0.061 (0.014)	0.058 (0.018)	0.063 (0.017)	0.065 (0.018)	0.064 (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

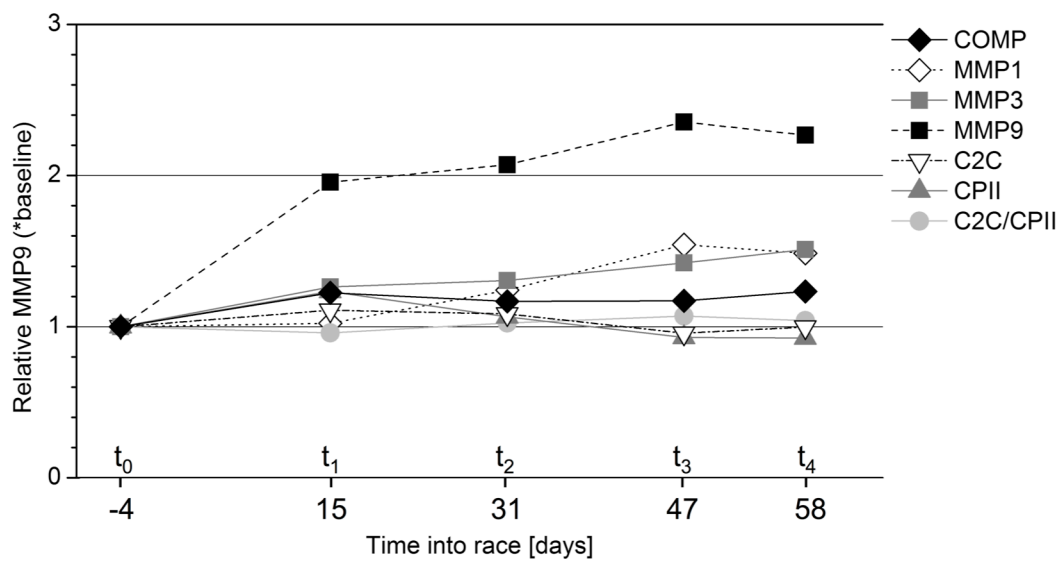


Figure 1. Mean relative changes in cartilage biomarkers normalized to baseline level. COMP—cartilage oligomeric matrix protein; MMP—matrix proteinases; C2C— C-terminal neopeptide generated by the collagenase-mediated cleavage of collagen type II triple helix; CII—procollagen type II C-terminal propeptide;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

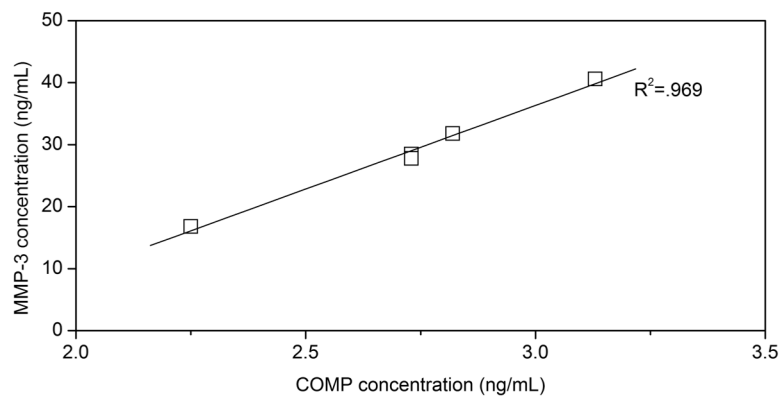


Figure 2. Relationship between MMP-3 and COMP levels for one participant.

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 uphill³⁰, walking for 30 minutes,²¹ walking 4000
 154 steps at slow, medium or fast walking speed,⁵ or running for 30 minutes.²⁵ Moreover,
 155 increases in COMP after a marathon range from 17 to 60%.^{14, 22, 23} 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).^{13, 14} In a
 158 single stage ultramarathon study by Shin et al.³⁸, 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^{14, 23} or single stage ultramarathon races.^{13, 14, 38} 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 walking²¹ or running²⁵) and intense exercise (marathons^{14, 22,}
168 ²³ and ultramarathons¹⁴), respectively. Moreover, Mündermann et al.²² 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 mechanics⁵. 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-cyclists³. Like
180 running, cycling is characterized by high cyclic joint loads (e.g. several times body weight at
181 the knee¹⁵), 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^{35, 37} of an initial T2* increase in articular cartilage of the ankle and the knee
199 followed by a subsequent T2* decrease (ankle)³⁷ and steady-state (knee)³⁵ 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.³⁷ 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.⁹
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.¹⁶ 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 OA² 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.⁴⁵ MMP-1 degrades
224 collagen types I, II, and III in the extracellular matrix, and mediates cartilage destruction.^{2, 40}
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^{16, 17} suggesting that MMP-1 is not
228 sensitive to loading.

229 Henrotin et al.¹² 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 helix¹¹) 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^{31, 32}, which has also been indicated by previously observed changes in T2* of
240 articular cartilage at the ankle during a multistage ultramarathon.³⁷

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 marathon⁴¹, 6 to 10 hours before the ultramarathon¹⁴, 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.²¹ Interestingly, most studies^{13, 14, 22, 38} 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.¹ 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^{8, 28} and suggested by Schütz et al.³⁷

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, Mündermann A, Koo S, et al. Vibration training intervention to maintain
320 cartilage thickness and serum concentrations of cartilage oligomeric 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, Müller 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