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# Quantitative Magnetic Resonance Imaging Assessment of the Quadriceps Changes during an Extreme Mountain Ultramarathon

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1 Quantitative Magnetic Resonance Imaging  
2 assessment of the Quadriceps changes during an  
3 extreme mountain ultramarathon

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25 **ABSTRACT**

26 **Introduction/Purpose**

27 Extreme ultra-endurance races are growing in popularity but their effects on skeletal  
28 muscles remain mostly unexplored. This longitudinal study explores physiological  
29 changes in mountain ultramarathon (MUM) athletes' quadriceps using quantitative  
30 magnetic resonance imaging (qMRI) coupled with serological biomarkers.

31 The study aimed to monitor the longitudinal effect of the race and recovery, and to  
32 identify local inflammatory and metabolic muscle responses by codetection of biological  
33 markers.

34 **Methods**

35 An automatic image processing framework was designed to extract imaging-based  
36 biomarkers from qMRI acquisitions of the upper legs of 20 finishers at three time points.  
37 The longitudinal impact of the race was demonstrated by analyzing the image markers  
38 with dedicated biostatistical analysis.

39 **Results**

40 Our framework allows a reliable calculation of statistical data not only inside the whole  
41 quadriceps volume but also within each individual muscle heads. Local changes in MRI  
42 parameters extracted from quantitative maps were described and found significantly  
43 correlated with principal serological biomarkers of interest. A decrease in the PDFF after  
44 the race and a stable paramagnetic susceptibility value were found. Pairwise post hoc  
45 tests suggested that the recovery process differs among the muscle heads.

46 **Conclusion**

47 This longitudinal study conducted during a prolonged and extreme mechanical stress,  
48 showed that quantitative MRI-based markers of inflammation and metabolic response  
49 can detect local changes related to the prolonged exercise, with differentiated  
50 involvement of each head of the quadriceps muscle as expected in such eccentric load.  
51 Consistent and efficient extraction of the local biomarkers enables to highlight  
52 interplay/interactions between blood and MRI biomarkers. This work indeed proposes  
53 an automatized analytic framework to tackle the time consuming and mentally  
54 exhausting segmentation task of muscle heads in large multi-time-points cohorts.

55 **KEYWORDS**

56 Musculoskeletal; statistical analysis; image processing; functional variation

## 57 INTRODUCTION

58 Mountain ultra-endurance running consists of running/walking outdoors over a distance longer  
59 than the traditional marathon on mountain trails with significant upward and downward slopes.  
60 This sport has become increasingly popular over the last decade.

61 Because of the inherent challenge for the body, the mountain ultramarathon (MUM) is  
62 considered an outstanding model to investigate the adaptive responses to extreme load and  
63 mechanical stress(1) in skeletal muscles. Studies have been carried out to explore the impact  
64 of these prolonged cardiovascular and muscle efforts, demonstrating, for instance, multiple  
65 visceral changes with inflammatory effects(2–4) or an impairment of neuromuscular  
66 function(5). Among all muscles, the quadriceps are particularly targeted because extensive  
67 downhill running causes repeated eccentric contractions and high mechanical stress, ultimately  
68 leading to muscle damage(6,7).

69 Magnetic Resonance Imaging (MRI) is a reliable, non-irradiating and non-invasive imaging  
70 method for tissue characterization and quantitative assessment of tissue integrity through its  
71 magnetic properties. Indeed T2 MR relaxation times, and its corresponding rates R2 ( $=1/T2$ )  
72 denote the characteristic time constants of the recovery back toward equilibrium of the xy  
73 (transverse) components of the magnetization. After being disturbed, the MR signal source, i.e.  
74 nuclear magnetization, does not spontaneously recover very fast, but relies almost entirely on  
75 interactions of hydrogen nuclei with the surrounding material to re-equilibrate(8). Because T2  
76 is sensitive to low-frequency components, in tenth of ms, water molecules diffuse and sample  
77 many different environments on the cellular level within the timescale of relaxation. In many  
78 (although by no means all) situations, very rapid exchange may occur between bulk water and  
79 bound and interfacial water in biological systems. This is believed to be the origin for many T2  
80 changes in various pathologies, such as edematous changes following insults to tissue. Changes  
81 in tissue water and protein content have been shown to affect relaxation. The measure of

82 average water proton relaxation rate will depend on how effectively, and at what rate, these  
83 effects are spread through the rest of the water population. Moreover, the existence of water in  
84 separate compartments that are only slowly exchanging gives rise to more complex  
85 behavior(9). Many studies witness that edematous areas in the muscle appear bright in T2-  
86 weighted images because the T2 relaxation time becomes longer, and T2 maps show elevated  
87 quantitative values in the matching area(10,11). T2 maps have, therefore, been used to monitor  
88 inflammation in muscle diseases, before and after medical interventions or treatment(12–14),  
89 or to predict return to play(15). T2\* is the "effective" T2 resulting from inhomogeneities in the  
90 main magnetic field or susceptibility-induced field distortions produced in the tissue, related to  
91 the presence of chemical or paramagnetic substances such as fat or hemorrhage(16). However,  
92 the first step in decoding the various effects of prolonged exercise stress on the body using  
93 qMRI markers is to develop systematic and comprehensive noninvasive exploration strategies  
94 to consistently extract the biomarkers reflecting the physiological consequences of such  
95 challenges.

96 From that perspective, magnetic resonance imaging (MRI) appears to be a unique imaging  
97 modality for extracting anatomical and structural features of muscle tissue(17,18). Recently,  
98 quantitative imaging methods such as chemical shift-encoded MRI(19,20) and MR  
99 relaxometry mapping(21,22) have allowed users to understand chemical alterations  
100 noninvasively at the imaged pixel size.

101 Several postprocessing methods need to be sequentially applied to extract a quantitative index  
102 from MRI data. These methods include the segmentation of muscle heads on multiple large 3D  
103 images, the extraction of image features in each area of interest, and statistical analysis. Each  
104 of these steps is an area of research in its own right. Although various alternatives have been  
105 proposed to target each of these challenges(17,18), they are rarely applied to cohorts or  
106 longitudinal studies.

107 The aim of the study was to quantify the response of the quadriceps muscle to a MUM's  
108 extreme loading conditions with quantitative MRI (qMRI) and to monitor the longitudinal  
109 effect of race and recovery. To do so, we propose an automatized and integrated analysis  
110 pipeline that enables the extraction of local quantitative imaging markers. Hence, the relation  
111 of these new markers with standard blood biomarkers was investigated to provide further  
112 insight into the local inflammatory and metabolic muscle responses.

113

## 114 **MATERIALS AND METHODS**

### 115 **Subjects and experimental study design**

116 We performed a longitudinal study on the athletes who participated in the "Tor des Géants"  
117 MUM in 2014, including three MRI sessions carried out just before the start and, for those who  
118 finished the race, immediately upon arrival and after 48 hours of recovery. Biological samples  
119 were also drawn at four time points with an additional sample obtained at half-race. The  
120 flowchart of the study is shown in Figure 1.

121 This study was approved by the local ethical committee (Aosta Valley, Azienda USL 101/946),  
122 and the experimental plan was conducted in accordance with the Helsinki Declaration (2001).  
123 Subjects were recruited through mailing and public announcements to registered runners by  
124 race organizers. Exclusion criteria were smoking, substance abuse, regular intake of  
125 medications, medical or psychiatric illness, and any contraindication to MRI (e.g.,  
126 claustrophobia, nonremovable metal devices) or abnormalities detected upon laboratory  
127 screening. There were 50 experienced runners who volunteered and provided informed written  
128 consent to participate in this study.

129 The Tor des Géants is a 330 km long ultradistance trail running, with considerable  
130 positive/negative elevation changes (+24,000 m) in the Valley of Aosta (Italy). It is considered



131 one of the most difficult mountain marathon races in the world since the ultra-endurance  
132 activity is associated with high altitude exposure and sleep deprivation. The altitude along the  
133 course ranges between 322 and 3,300 m, with 25 mountain passes over 2,000 m. The maximum  
134 time allowed to complete the race is 150 h, and in 2014, the best performance was 71 h 49 min  
135 among 740 starters and 446 (60%) finishers (<http://www.tordesgeants.it/>).

136 The present experimental design was thus a longitudinal study with repeated assessments at  
137 four checkpoints:

- 138 - The first point (prerace: Pre) was at the start location. The data collection was  
139 performed within 4 days before the race and consisted of MRI acquisition and  
140 biological sampling.
- 141 - The second point was located halfway through the race (middle: Mid). Only biological  
142 sampling was performed.
- 143 - The third one was at the arrival of the race (arrival: Post): athletes who finished the race  
144 were transported by car to the laboratory and were evaluated (MRI and biological  
145 sampling) within 1 h after finishing the race.
- 146 - The last point (recovery: Post+3) was 48-72 h after arrival time. Both MRI and  
147 biological sampling were acquired.

#### 148 **MRI acquisitions**

149 At Pre, Post and Post+3 time points, MRI acquisitions were performed on site using a mobile  
150 1.5 T MR scanner system (MAGNETOM Avanto, Siemens Healthcare, Erlangen, Germany,  
151 located within a truck from Alliance Medical, England). A standard coil configuration was  
152 used: a flexible 4-channel array surface coil, combined with 4 elements of the spine coil  
153 embedded in the bed, resulting in an 8-channel coil in total.

154 Three MRI acquisitions of the legs were sequentially performed:

- 155 - A three-dimensional (3D) isotropic gradient dual-echo sequence was used. The coronal  
156 acquisition included the entire upper leg (from the tibial tuberosity to the anterior  
157 superior iliac spine). The reconstruction of the water and fat images from the acquired  
158 dual-echo data sets was performed inline (Syngo software, Siemens healthcare,  
159 Erlangen, Germany) using a Dixon approach enabling four 3D isotropic in-phase, out-  
160 phase, fat and water coronal images to be calculated inline on the MR scanner, hereafter  
161 denoted water (W), fat (F), in-phase (IN) and out-phase (OUT) images, respectively.
- 162 - A 3D spoiled gradient echo sequence (3D GRE) was used for simultaneous estimation  
163 of proton density fat fraction (PDFF), T2\*, and internal magnetic susceptibility ( $\chi$ )  
164 quantification. Eight echoes were acquired in the transverse plane with a flyback  
165 readout gradient (first echo: 1.58 ms and echo spacing: 2.52 ms). TR and flip angle  
166 were adjusted to minimize the T1-related bias. Phase and magnitude images were  
167 systematically reconstructed. Prescription of localization was performed using  
168 anatomic images from an isotropic 3D gradient echo acquisition. For standardization  
169 purposes, the central partition in the z-direction was planned at a 15 cm distance from  
170 the upper part of the patella using the sagittal multiplanar reconstruction of the first  
171 acquired 3D gradient isotropic sequence.
- 172 - A 2D multiecho T2 weighted spin-echo sequence (meSE) with 16 echo times (TEs)  
173 ranging from 10 to 178 ms, with its central slice was also planned at the same location  
174 as previously described.

175 Each individual sequence was individually optimized. Anatomical regions explored by each  
176 technique are summarized in Figure 2a, and the main MR parameters are listed in Supplemental  
177 Digital Content 1, MR imaging acquisition parameters.

178

## 179 **MR Image postprocessing**

180 PDFF,  $\chi$ , and  $T_2^*$  were calculated from 3D-GRE acquisition using the reconstruction method  
181 described in (19–22) which encompassed two main steps: fat-water separation (provided  
182 parametric  $T_2^*$  and PDFF maps) and magnetic susceptibility quantification. Meanwhile,  
183  $T_2$  parametric maps were calculated from the meSE sequences.

184 An automatic segmentation based on external-force-driven deformable registration was  
185 performed on the T1-W images (23) to obtain the quadriceps heads of interest.

186 Briefly, dedicated in-house postprocessing pipelines were required 1) to simultaneously  
187 compute the main parametric maps (fat fraction – PDFF, internal magnetic susceptibility ( $\chi$ )  
188 and  $T_2^*$  from the 3D GRE sequence,  $T_2$  parametric map from the meSE sequence) and 2) to  
189 correct the results of the automatic muscle segmentation and to adjust their resolutions to  
190 different image sequences (Figure 2). These steps allowed us to extract metrics from the four  
191 parametric maps: PDFF,  $\chi$ ,  $T_2^*$  and  $T_2$ . All details about parametric map reconstruction and  
192 muscle segmentation are provided in Supplemental Digital Contents 2, quantitative maps  
193 computation, and 3, muscle segmentation procedure.

194

### 195 **Biological sampling and analysis**

196 Blood and urinary samples were collected at each of the four sessions (Pre, Mid, Post, Post+3)  
197 within 10 min after arrival at each key point. Blood samples were drawn from an antecubital  
198 vein into a dry, heparinized, or EDTA tube according to the analysis to be performed and  
199 immediately centrifuged. Since it was not possible to carry out all the investigations on the  
200 same day by point-of-care technologies, plasma and serum were frozen at  $-80^\circ\text{C}$  within 20 min  
201 after blood collection for later analysis of muscle injury markers and biochemical variables.  
202 The hematology parameters (hemoglobin, red blood cells, white blood cells) were directly  
203 analyzed by a pocH-100i™ automated hematology analyzer (Sysmex, Villepinte, France).  
204 Cobas 8000 (RocheDiagnostics, Mannheim, Germany) was used to perform serial

205 determinations for C-reactive protein (CRP), urinary creatinine, creatinine, calcium, chlorine,  
206 potassium, sodium, and cholesterol. The osmolality and urinary osmolality were measured on  
207 an Arkray Osmo Station OM-6050 (Menarini, Florence, Italy).

208 All blood biomarkers analyzed in this study are listed in Supplemental Digital Content 4,  
209 complete list of 58 biological markers analyzed in the study.

210

## 211 **Statistical analysis**

212 Missing values in biological datasets were estimated (see Supplemental Digital Contents 5,  
213 biological data preprocessing for missing data) to employ paired statistical tests and for  
214 subsequent correlation analysis between MRI and biological markers. The significance level  
215 was set at  $P < .05$  for all statistical tests.

### 216 *Longitudinal statistical analysis*

217 For the qMRI longitudinal data, we used a statistical analysis of repeated measures with  
218 adaptation to the data normality, as the normal distribution could not be assumed. For each  
219 qMRI calculated index of each of the 9 muscle volumes (4 quadriceps muscle heads per leg  
220 and the total quadriceps volume), we tested the normality of the data at 3 time points with the  
221 Shapiro-Wilk test. For the global effect test, one-way ANOVA designed for repeated measures  
222 was conducted for all data normally distributed at all the time points; otherwise, the Friedman  
223 test was employed.

224 While performing ANOVA, the sphericity of the data was verified by using Mauchly's test. If  
225 the sphericity assumption was violated, the Greenhouse-Geisser correction method was used  
226 on the P-value of ANOVA. After the global effect test, a post hoc test was performed to  
227 compare each pair of time points. The type of post hoc test depended on the normality of  
228 differences between two time points: dependent t-test for normally distributed differences and

229 Wilcoxon signed rank test otherwise. The obtained P-values were adjusted with the Bonferroni  
230 adjustment method for multiple comparisons.

231 A similar strategy was applied to the biological marker data with 58 variables at 4 time points.

232 Relative variations of a biomarker  $X$  at each time point ( $RV(X_n), n \in \{Mid, Post, Post + 3\}$ )  
233 compared to time point  $Pre$  were also computed with:

$$234 \quad RV(X_n) = \frac{\overline{X}_n - \overline{X}_{Pre}}{\overline{X}_{Pre}} \times 100\%$$

235 where  $\overline{X}_n$  is the average value of biomarker  $X$  at time point  $n$ .

### 236 *Associations between image markers and biological markers*

237 The potential associations between image markers and biological markers were evaluated by  
238 calculating the repeated measure correlation coefficient(24) between each image marker and  
239 each biological marker.

240

## 241 RESULTS

### 242 MR markers: parametric mapping analysis

243 Of the 50 athletes that were included in the study, 31 finishers completed the race (62%) in an  
244 average time of  $124 \pm 13$  h. Among them, only those with quadriceps muscle MRI datasets  
245 available at all the time points ( $n=24$ ) and with quantitative maps free of artifacts at all three  
246 time points were retained for final analysis ( $n=20$ ). Artifacts leading to data exclusions were  
247 mostly motion-related. They were specifically due to uncontrolled motion of the legs typically  
248 observed immediately after long races potentially combined with those related to sudden sleep  
249 attacks during MR acquisitions. Demographic data and global qMRI metrics collected at each  
250 time point are reported in Supplemental Digital Content 6, demographic data and qMRI metrics  
251 of the subject population.

252 The first step was to explore differences among all quadriceps muscle heads. Figure 3 shows a  
253 matrix of the t-test results when comparing qMRI metrics ( $\chi$ , PDFF, T2, T2\*) between all  
254 muscle heads at the three MR acquisition time points (Pre, Post, Post+3). When focusing only  
255 on right/left differences of the corresponding muscle heads,  $\chi$ , T2\* and PDFF showed similar  
256 tendency. At the same time, there was no significant difference between right and left muscle  
257 heads for  $\chi$  and T2\* at any time point, and PDFF exhibited a significant difference between  
258 vastus lateralis (VL) heads only at a single time point (Post+3). On the other hand, the T2  
259 metric showed a different pattern with a significant difference between the rectus femoris (RF)  
260 heads of the two legs at the three time points (Pre, Post, Post+3) and a significant difference  
261 between the left and right vastus medialis (VM) at time point Pre. Different muscle heads also  
262 had different qMRI values, especially the RF, which showed significant differences compared  
263 to all the other muscle heads most of the time. These results highlight the need to consider

264 individual muscle behavior separately and that pooling quadriceps muscle heads may result in  
265 a loss of information.

266 When analyzing temporal changes on the repeated measures of qMRI metrics, we observed a  
267 significant time effect (i.e., race effect) on T2\* and T2 mean values. This is shown in Figures  
268 4 and 5 with typical source images (see also Supplemental Digital Content 6 - Demographic  
269 data and qMRI metrics of the subject population, for summary values and Supplemental Digital  
270 Content 7 - Specific longitudinal T2\* analysis, for a local analysis of right VI in T2\* for an  
271 example subject). Both T2\* and T2 were significantly longer at arrival for most of the muscle  
272 heads. They significantly decreased after recovery for the VM and the vastus intermedius (VI)  
273 while not returning to baseline values at that time of measurement.

274 PDFF and  $\chi$  showed only a small time effect (Figure 6-7). Pooling all muscles, PDFF had a  
275 tendency to decrease after the race ( $2.88 \pm 0.53$ ) compared to baseline ( $3.16 \pm 0.45$ ) while  
276 increased to higher values than baseline ( $3.17 \pm 0.46$ ) after 48 h of recovery. When considering  
277 each muscle, the time effect reached significance for both VI, right VM and left VL muscle  
278 heads (Figure 6). Despite a similar trend as the PDFF, time-effect changes in  $\chi$  did not reach  
279 significance for most muscle heads, except for left VL and left VM (L).

280 When focusing on T2\* and T2 findings, the vastus group exhibited stronger variations than the  
281 RF muscle group. The VM and VI had larger changes than the VL or the average of all muscles.  
282 T2\* values appeared less sensitive to muscle changes than T2, as illustrated in Figure 3, but  
283 most of the statistical tests were significant except for some muscle heads on both legs between  
284 time points Post and Post+3.

### 285 **Biological marker analysis**

286 The longitudinal variations in biological markers throughout the race are shown in  
287 Supplemental Digital Content 8, Values of urinary and blood biomarkers at 4 time points. As

288 main blood biomarkers of muscle damage, serum creatine kinase (CK) and myoglobin levels  
289 peaked at +6598% and +4159% at Mid, while serum lactate dehydrogenase (LDH) levels  
290 peaked at +240% at Post and were elevated at Mid at +197% (all P-values of post hoc test  
291 against Pre are less than 0.01, see Figure 8). For most of the biomarkers, the biomarker values  
292 at Pre were significantly different from those at the other time points (Mid, Post, Post+3), while  
293 the difference between Mid and Post was nonsignificant.

#### 294 **Correlation between qMRI features and main blood biomarkers**

295 We presented our results with a heatmap produced within R software (Supplemental Digital  
296 Content 9, Correlation between biological markers and image features extracted from  
297 quadriceps muscles in each qMRI quantitative map). An interactive version of this heatmap  
298 can be found online  
299 ([http://amp.pharm.mssm.edu/clustergrammer/viz\\_sim\\_mats/5bed953490fbf7585f568](http://amp.pharm.mssm.edu/clustergrammer/viz_sim_mats/5bed953490fbf7585f5680c2/Correlation_Mean.txt)  
300 [0c2/Correlation\\_Mean.txt](http://amp.pharm.mssm.edu/clustergrammer/viz_sim_mats/5bed953490fbf7585f5680c2/Correlation_Mean.txt)) thanks to Clustergrammer(25). Significant correlations between  
301 qMRI features and biological markers were found:

302 - The T2 mean extracted from the whole quadriceps showed a significant time effect at all time  
303 points of the race and a relatively strong correlation with blood/urine biomarkers such as  
304 myoglobin (0.69), CK (0.78) and LDH (0.72). At the level of individual muscles, the  
305 correlation coefficient is higher for VI and VM muscles.

306 - T2\* exhibited similar results but with lower correlation coefficients: 0.54 for myoglobin, 0.57  
307 for CK and 0.61 for LDH.

308 - Meanwhile, neither the mean global PDFFF nor  $\chi$  showed any significant correlation  
309 coefficients with biological markers in this study.

310



311 **DISCUSSION**

312 While ultraloading events such as MUMs are recognized to endanger the musculoskeletal  
313 system, little is known about the exact consequences of the acute biomechanical burden on  
314 musculoskeletal tissues. Although (26) recommends the use of MRI as the method of choice  
315 for studying endurance-related damage, to date, there has been only one longitudinal  
316 observational field cohort study, focusing primarily on changes in overall body composition  
317 (27) or morphological tissue damage(28).

318 The challenge and novelty of this work are two-fold: 1) first, to provide a technological  
319 approach specifically developed enabling the extraction of qMRI parameters for longitudinal  
320 skeletal muscle studies; 2) then, to demonstrate that qMRI brings unique insights into the  
321 response of quadriceps muscle exposed to prolonged mechanical stress of a 330 km ultra-  
322 endurance event.

323 To do so, we built a standardizing and automatic analysis pipeline that segments, calculates,  
324 and extracts all qMRI markers in each quadriceps muscle's heads that can be robustly  
325 propagated to the different follow-up time points, thus enabling longitudinal monitoring.

326 Beyond the scope of this work, our first contribution is to empower a comprehensive and  
327 multiparametric quantification of MRI biomarkers in thigh skeletal muscle, including T2 and  
328 T2\* relaxation times, together with fat content (PDFF) and internal magnetic susceptibility  $\chi$ .  
329 Secondly, the analysis framework enabled analysis of each quadriceps muscle head over  
330 different time points (see Supplemental Digital Content 6, Demographic data and qMRI  
331 metrics of the subject population, and Figures 4-7).

332 Our main findings are that the cumulative biomechanical stress of a 330 km MUM onto  
333 quadriceps muscles translated in our subjects to a clear inflammatory burden with an increase  
334 in T2 and T2\* relaxation times. On the other hand, muscular metabolic and energy storage

335 status probed fat content (PDFFF) remained relatively stable with a small decrease in PDFFF,  
336 contrasting with the high energy expenditure that mainly relies on lipids. Below is a detailed  
337 discussion of each qMRI finding.

338 *T2 and T2\* changes: MR markers of the muscular inflammatory burden*

339 T2 and T2\* changes (Supplemental Digital Content 6, Demographic data and qMRI metrics of  
340 the subject population, and Figures 4, 5) are an expression of the well-known sensitivity of  
341 qMRI T2 and T2\* relaxation time parameters to overall water content variations and water  
342 redistribution between intracellular and extracellular space(18,22,29–32). Our findings  
343 confirm previous studies that reported with other approaches a significant inflammatory  
344 response after MUM(5,33), mainly related to repetitive eccentric muscle contractions resulting  
345 in microscopic muscle damage and subsequent edema (5,6,34). In our study, T2 and T2\*  
346 remained elevated even after a few days of recovery, suggesting that 48-72 h is not sufficient  
347 for a full recovery in most subjects and confirming previous findings by Robach *et al*(35).

348 Relaxometry changes demonstrated at the muscular level were corroborated by a large increase  
349 in skeletal myocyte injury-related biomarkers (CK, myoglobin and LDH) that was also  
350 measured in our subjects. These blood biomarkers are known to witness microlesions and early  
351 signs of cellular inflammatory processes preceding water redistribution from intracellular to  
352 extracellular compartments and have also been shown to reflect the extent of cellular  
353 damage(33,36). Among the other findings in the biological profiles, WBC activation,  
354 particularly neutrophil activation, is known to trigger cellular and humoral inflammatory  
355 responses(37) characterized by elevated CRP levels and the presence of leucocytes in the  
356 extracellular and extravascular spaces in response to long-term exercise(33,38). The intrinsic  
357 strength of MR imaging relies on its capacity to precisely reveal the local changes, while the  
358 evident correlation of quantified T2 and T2\* parameters with biological markers most likely

359 could reflect a difference in the temporal response of the compared biomarkers. If the tendency  
360 are similar, the amplitude of the correlation could only be due to a difference in the temporal  
361 occurrence of the inflammation signature at the blood and tissue level, hence also during the  
362 tissue recovery process.

363 The development of peripheral and muscular edema together with a total body water increase  
364 have been reported in the context of ultramarathon running(27), with a 6% increase in total  
365 body water after a 1,200 km run over 17 consecutive days and an increase in total body water  
366 associated with tissue edema(39). Our measures of the total quadriceps muscle volume  
367 (excluding superficial tissues) illustrated the same trend but with a small increase in quadriceps  
368 muscle volume (+3.3% at the end of the race and +3.4% after 48-72 h recovery relative to  
369 baseline; see Supplemental Digital Content 6, Demographic data and qMRI metrics of the  
370 subject population): number of voxels from the 3D GRE sequence, expressing muscle volume).  
371 This observation suggests that among the potential mechanisms involved, the T2 and T2\*  
372 changes are more likely related to water redistribution than to a net muscular tissue water  
373 content increase.

374 An interesting finding is the observable difference in T2 and T2\* response among the different  
375 quadriceps muscle heads. Indeed, while the VI and VL exhibited both higher T2 and T2\*  
376 increases, the RF showed almost no variations. These findings confirm previous studies  
377 exploring MUM and long-distance running adaptation strategies. Sloniger *et al.*(40) For  
378 instance, uphill running requires considerably greater activation of the vastus group and less  
379 activation of the RF than horizontal running(40). Ultrarunners are also known to modify their  
380 running pattern by increasing their stride frequency and by reducing the vertical oscillations to  
381 minimize the load and the associated pain occurring in lower limbs mainly during the eccentric  
382 phases(41). Impact forces when running downhill can be moderated by increasing knee flexion  
383 at initial contact and reducing stride length(42). This shock attenuation strategy is also

384 associated with a less pronounced heel strike and a forward leaning trunk. Altogether, these  
385 protective mechanical adaptations are likely to be specific to MUM and to differentially impact  
386 the quadriceps muscles, inducing less stimulation of the RF compared to the vastus muscles,  
387 which is clearly illustrated here.

388 From a mechanistic point of view, T2 and T2\* MR relaxation measures are well established  
389 and sensitive markers of exercise response. For instance, Le Rumeur *et al.*(32) have shown that  
390 T2 relaxation times can detect different effects of dynamic exercise in trained and untrained  
391 subjects and that there was a correlation between T2 increase and work intensity in healthy  
392 volunteers. Beyond the observed mean variations already discussed, individual variations  
393 observed in T2 and T2\* values (Figure 4 for T2\* and Figure 5 for T2) along such long-distance  
394 running are also probably determined by a complex system of regulatory mechanisms including  
395 individual response to inflammatory processes at each compartment level, and the intrinsic  
396 differences between T2 and T2\* mechanisms can probably provide further insights. Indeed,  
397 T2\* mapping differentiates from T2 mapping by its ability to characterize the relaxation of the  
398 transverse magnetization that is influenced by macroscopic (inhomogeneities of the magnetic  
399 field) and mesoscopic (structure of the tissue) magnetic field inhomogeneities(43–47). For  
400 example, reduced T2\* values have already shown the potential to describe structural alterations  
401 suggestive of ischemic alterations, collagen areas or hemorrhage, for instance, in myocardium  
402 and extracardiac tissues(48,49). According to this, histopathologic substrates of T2\* and a  
403 correlation of T2\* to flow analyses have been demonstrated(44–47,50). Recently, reduced T2\*  
404 values have been described in a group of hypertrophic cardiomyopathy patients potentially  
405 triggered through relative ischemia(51). In our case, explanation for the observed increased  
406 T2\* values could be related to increased perfusion and/or local variation of oxymyoglobin and  
407 oxyhemoglobin as oxygen suppliers are increased while deoxymyoglobin is decreased. Note

408 also that increased  $T2^*$  contrast will be associated with oxygenated proteins, while  
409 deoxygenated proteins that are paramagnetic would reduce local  $T2^*$  values.

410 Contrary to  $T2^*$ ,  $T2$  in muscle is known to have three components: ~18% of the signal had a  
411 very short  $T2$  (10 ms), 67% of the signal had an intermediate  $T2$  (~40 ms), and the remaining  
412 15% of the signal had a long  $T2$  (~170 ms)(52). The short  $T2$  component is the water bound to  
413 macromolecules, and the intermediate and long components represent intracellular and  
414 interstitial water, respectively. The literature also clearly describes a unidirectional flow of  
415 water from the “innermost” to “outermost” of three compartments connected in series  
416 (intracellularly bound water -> free intracellular water -> interstitial water -> lymphatic  
417 vessels). The latter three are subject to considerable variations as a result of normal  
418 physiological changes in the muscle. In our study and due to MR acquisition technical  
419 constraints, the relaxation in the tissue could be modeled only as a unique compartment. Using  
420 a single exponential to describe  $T2$  decay in muscle limits our understanding of the exact  
421 contributions of different physiological and biochemical responses to exercise.

422

### 423 *PDFF and muscular lipid storage*

424 Our study analyzed noninvasively the local variations in fat content in skeletal muscles during  
425 an ultra-endurance running event. With PDFF mapping, MRI is indeed the only noninvasive  
426 method that can enable simultaneous, reliable, and repeatable fat fraction estimates within each  
427 muscle's heads not achievable by alternative methods such as MR spectroscopy(53).

428 Overall, muscular fat content showed a small decrease at the end of the race, but the changes  
429 were not statistically significant except in the most solicited muscle heads (vastus group) (see  
430 Figure 3).

431 Exploring muscular fat content variations during an MUM was of special interest since lipids  
432 are the main source of energy in low- to moderate-intensity exercises, such as long-distance  
433 events. Additionally, the highest rates of fat oxidation are observed in endurance athletes below  
434 65% (54,55) which is in accordance with what has been measured on the Tor des Géants MUM  
435 by Maufrais et al., who reported an average exercise intensity below 50% of  $VO_{2max}$  (56).

436 Muscle fat content is mainly represented by small quantities of triglycerides (TG) that are  
437 present as lipid droplets located between or inside muscle fibers (intramyocellular TG, or  
438 IMTG), while most fat storage is mainly located in subcutaneous and deep visceral adipose  
439 tissue (57). There is now clear evidence that the IMTG pool acts as a buffer regulating fatty  
440 acid (FA) flux in skeletal muscle both during exercise to maintain intracellular FA (metabolite)  
441 concentrations and in conditions of increased plasma FA provision (58,59). Our findings are in  
442 line with other studies that also reported no or minimal reduction of IMTG using biopsies  
443 before and after exercise (60–62). Using  $^1H$ -MR spectroscopy, other authors reported larger  
444 changes with an ~25% IMTG decrease after 2-3 h of exhaustive running at 50-65%  
445  $VO_{2max}$  (63,64). Both approaches have been challenged as suffering from intrinsic bias: the  
446 direct biopsy approach is subject to between-biopsy variability (reported up to 23%), while  $^1H$ -  
447 MR spectroscopy modeling accuracy may be biased by water content changes and assumptions  
448 of FA compositions in IMTG (65). All existing studies have explored fat content changes in  
449 short-duration (up to 3 h) and high-intensity (up to 80%  $VO_{2max}$ ) exercises that profoundly  
450 differ from MUM, which intrinsically limits comparison.

451 While the IMTG pool appeared to behave as a buffer to facilitate mitochondrial oxidation and  
452 skeletal muscle energy supply, our blood lipid profiles showed a marked reduction in  
453 circulating TG and low-density lipoprotein (LDL), since the lipoprotein lipase released by  
454 endothelial cells during exercise has been shown to increase FA availability (66). At the same  
455 time, we observed an increase in high-density lipoprotein (HDL) cholesterol that coincides

456 with significant increases in lipoprotein lipase activity(67) as already reported in long-distance  
457 exercise(68).

458

#### 459 *Paramagnetic susceptibility $\chi$*

460 Magnetic susceptibility  $\chi$  is a physical property that describes the response of a medium being  
461 placed within a magnetic field(69). A positive susceptibility value characterizes a paramagnetic  
462 medium (O<sub>2</sub>, salts) with a magnetic field increase, while most biological tissues, including  
463 water and fat, are diamagnetic with negative values inducing a local decrease of the initial  
464 magnetic field.

465 All magnetic susceptibility measured in our subjects and all time points were paramagnetic  
466 coherently with the metabolic consumption of oxygen in tissues: the weakly diamagnetic  
467 oxyhemoglobin releases strongly paramagnetic O<sub>2</sub> and deoxyhemoglobin molecules(70). The  
468 magnetic susceptibility  $\chi$  remained very stable with only a minimal increase at arrival of  
469  $1.75\pm 0.43$  ppm vs.  $1.49\pm 0.34$  ppm at baseline (see Supplemental Digital Content 8, Values of  
470 urinary and blood biomarkers at 4 time points), as expected in low-intensity aerobic exercise  
471 where the average intensity remains below 50% of VO<sub>2</sub>max(56), explaining the lack of  
472 significant variations over time. Indeed, measurable changes in magnetic susceptibility-related  
473 measures in skeletal muscles reported in the literature occurred only in critical conditions such  
474 as cuff compression tests or ischemia(71). Our differences among muscle heads and groups  
475 are, however, in line with findings by others(71). This finding is explained by the differences  
476 among muscles in slow-twitch oxidative muscle fibers, different capillary density, and  
477 myoglobin content.

478 From a broader perspective, the proposed approach could also serve in diseases for longitudinal  
479 monitoring of muscular disorder progression or improvement due to various treatments.

480 Indeed, the motivation for both athletes and physicians during this study was also to serve and  
481 encourage technological developments that could, beyond a deeper understanding of muscle  
482 physiology during extreme exercise, also help, in turn, be a crucial adjunct to improve patient  
483 diagnosis, follow-up, and care, evaluate the effects of personalized-designed therapies, a highly  
484 critical objective not in clinical routine. Indeed, there are several clinical scenarios in which  
485 pathologies lead to inflammation or muscle wasting. Moreover, such an approach could be  
486 coupled to the other strengths of MRI, including in vivo dynamic imaging of muscle function  
487 and kinematics(72–75), muscle microstructure using diffusion MRI or measures of chemical  
488 changes associated with metabolic states and glycogen consumption using MR  
489 spectroscopy(76).

490

#### 491 **Limitations**

492 While we have studied the average response for all the subjects, there is an interindividual  
493 response heterogeneity which is likely influenced by factors such as age(77), training  
494 status(78), performance level, experience, and many others that likely modulated the  
495 inflammatory response but could not be taken into account due to our limited sample size.

496 In this paper, we used simple statistical descriptors to analyze the quantitative indexes extracted  
497 at each time point and within each muscle head, while radiomic features could have been  
498 explored for each quantitative index.

499 While considering the results on PDFF, it should be kept in mind that the PDFF values in our  
500 study are especially low since our subjects are professional athletes and there are estimation  
501 errors in PDFF map computation.



502 Recently, more specific acquisition methods and modeling, combining ultrashort-TE  
503 acquisition(79) and muscle-specific models of diffusion, might enable short-term  
504 distinguishing the T2 of each muscle compartment (T2 intracellular, T2 in the membrane, T2  
505 extracellular) and thus elucidating the different causes of changes in the parameters.

## 506 **Conclusion**

507 Using a model of prolonged and extreme mechanical stress, we showed that a dedicated  
508 analysis pipeline enables the extraction of local quantitative imaging markers of inflammation  
509 and metabolic response and the detection of local changes related to exercise. Our study  
510 confirms that prolonged MUMs induce singular muscle damages compared to other sporting  
511 events, while the extreme eccentric load (due to the 24000 m of downhill running) is likely the  
512 main trigger. When applied with proposed segmentation and analysis pipeline, qMRI can  
513 monitor these changes while differentiating the involvement of the heads of the quadriceps  
514 muscle.

515 There are many potential applications; for example, in sports science, our approach could be  
516 used to explore the impact of other exercise challenges or to quantify the effects of training  
517 strategies or training programs on specific individuals. Considering that MRI can also provide  
518 high-spatial-resolution anatomical images of muscle injuries and trauma, as well as information  
519 on muscle microstructure and metabolic variables such as glycogen consumption, qMRI would  
520 be a highly relevant noninvasive modality for investigating the relationship among force loss,  
521 muscle microstructure, performance and recovery in athletes as well as muscle wasting and  
522 disability in patients.

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532 **CONFLICT OF INTEREST**

533 The authors declare that there is no conflict of interest. The results of the present study do not  
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536 **REFERENCES**

- 537 1. Millet GP, Millet GY. Ultramarathon is an outstanding model for the study of adaptive  
538 responses to extreme load and stress. *BMC Med* [Internet]. 2012;10(1):1–3. Available  
539 from: <http://dx.doi.org/10.1186/1741-7015-10-77>
- 540 2. Andonian P, Viallon M, Le Goff C, et al. Shear-wave elastography assessments of  
541 quadriceps stiffness changes prior to, during and after prolonged exercise: A  
542 longitudinal study during an extreme mountain ultra-marathon. *PLoS One*.  
543 2016;11(11):1–21.
- 544 3. Zanchi D, Viallon M, Goff C Le, et al. Extreme mountain ultra-marathon leads to  
545 acute but transient increase in cerebral water diffusivity and plasma biomarkers levels  
546 changes. *Front Physiol*. 2017;7(JAN).
- 547 4. Belli T, Macedo DV, De Araújo GG, et al. Mountain ultramarathon induces early  
548 increases of muscle damage, inflammation, and risk for acute renal injury. *Front*  
549 *Physiol*. 2018;9:1368.
- 550 5. Saugy J, Place N, Millet GY, Degache F, Schena F, Millet GP. Alterations of  
551 Neuromuscular Function after the World’s Most Challenging Mountain Ultra-  
552 Marathon. *PLoS One* [Internet]. 2013;8(6):1–11. Available from:  
553 <http://dx.doi.org/10.1371%2Fjournal.pone.0065596>
- 554 6. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys*  
555 *Med Rehabil*. 2002;81(11 Supp):S52-69.
- 556 7. Guilhem G, Cornu C, Maffiuletti NA, Guével A. Neuromuscular adaptations to isoload  
557 versus isokinetic eccentric resistance training. *Med Sci Sports Exerc*. 2013;45(2):326–  
558 35.
- 559 8. Brown RW, Cheng YCN, Haacke EM, Thompson MR, Venkatesan R. Magnetic

- 560 Resonance Imaging: Physical Principles and Sequence Design: Second Edition. 2014.
- 561 9. Saab G, Thompson RT, Marsh GD. Multicomponent T2 relaxation of in vivo skeletal  
562 muscle. *Magn Reson Med*. 1999;42:150–7.
- 563 10. Ababneh ZQ, Ababneh R, Maier SE, et al. On the correlation between T2 and tissue  
564 diffusion coefficients in exercised muscle: Quantitative measurements at 3T within the  
565 tibialis anterior. *Magn Reson Mater Physics, Biol Med*. 2008;21:273–8.
- 566 11. Ploutz-Snyder LL, Nyren S, Cooper TG, Potchen EJ, Meyer RA. Different effects of  
567 exercise and edema on T2 relaxation in skeletal muscle. *Magn Reson Med*.  
568 1997;37:676–82.
- 569 12. Ran J, Ji S, Morelli JN, Wu G, Li X. T2 mapping in dermatomyositis/polymyositis and  
570 correlation with clinical parameters. *Clin Radiol*. 2018;73(12):1057.e13-1057.e8.
- 571 13. Maillard SM, Jones R, Owens C, et al. Quantitative assessment of MRI T2 relaxation  
572 time of thigh muscles in juvenile dermatomyositis. *Rheumatology*. 2004;43(5):603–8.
- 573 14. Thuny F, Lairez O, Roubille F, et al. Post-conditioning reduces infarct size and edema  
574 in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol*.  
575 2012;59(24):2175–81.
- 576 15. Biglands JD, Grainger AJ, Robinson P, et al. MRI in acute muscle tears in athletes: can  
577 quantitative T2 and DTI predict return to play better than visual assessment? *Eur*  
578 *Radiol*. 2020;
- 579 16. Welsch GH, Hennig FF, Krinner S, Trattnig S. T2 and T2\* Mapping. *Current*  
580 *Radiology Reports*. 2014. p. 60.
- 581 17. Froeling M, Oudeman J, Strijkers GJ, et al. Muscle Changes Detected with Diffusion-  
582 Tensor Imaging after Long-Distance Running. *Radiology [Internet]*. 2015;274(2):548–

- 583 62. Available from: <http://dx.doi.org/10.1148/radiol.14140702>
- 584 18. Maeo S, Ando Y, Kanehisa H, Kawakami Y. Localization of damage in the human leg  
585 muscles induced by downhill running. *Sci Rep.* 2017;7(1):5769.
- 586 19. Leporq B, Le Troter A, Le Fur Y, et al. Combined quantification of fatty infiltration, T  
587 1-relaxation times and T 2\*-relaxation times in normal-appearing skeletal muscle of  
588 controls and dystrophic patients. *Magn Reson Mater Physics, Biol Med [Internet].*  
589 2017 Aug;30(4):407–15. Available from: <https://doi.org/10.1007/s10334-017-0616-1>
- 590 20. Leporq B, Ratiney H, Pilleul F, Beuf O. Liver fat volume fraction quantification with  
591 fat and water T1 and T2\*estimation and accounting for NMR multiple components in  
592 patients with chronic liver disease at 1.5 and 3.0 T. *Eur Radiol.* 2013;23(8):2175–86.
- 593 21. Tawara N, Nitta O, Kuruma H, Niitsu M, Itoh A. T2 mapping of muscle activity using  
594 ultrafast imaging. *Magn Reson Med Sci.* 2011;10(2):85–91.
- 595 22. Patten C, Meyer RA, Fleckenstein JL. T2 mapping of muscle. *Semin Musculoskelet*  
596 *Radiol.* 2003;7(4):297–305.
- 597 23. Gilles B, De Bourguignon C, Croisille P, Millet G, Beuf O, Viallon M. Automatic  
598 segmentation for volume quantification of quadriceps muscle head: a longitudinal  
599 study in athletes enrolled in extreme mountain ultra-marathon. In: *ISMRM2016:*  
600 *International Society for Magnetic Resonance in Medicine.* Singapore;
- 601 24. Bakdash JZ, Marusich LR. Repeated measures correlation. *Front Psychol.*  
602 2017;42(2):261–7.
- 603 25. Fernandez NF, Gundersen GW, Rahman A, et al. Clustergrammer, a web-based  
604 heatmap visualization and analysis tool for high-dimensional biological data. *Sci Data.*  
605 2017;4:170151.

- 606 26. Lucas SJE, Helge JW, Schütz UHW, Goldman RF, Cotter JD. Moving in extreme  
607 environments: Extreme loading; carriage versus distance. *Extrem Physiol Med.*  
608 2016;5:6.
- 609 27. Schütz UHW, Billich C, König K, et al. Characteristics, changes and influence of body  
610 composition during a 4486 km transcontinental ultramarathon: Results from the  
611 Transeurope Footrace mobile whole body MRI-project. *BMC Med.* 2013;11(1):122.
- 612 28. Theysohn JM, Kraff O, Maderwald S, et al. MRI of the ankle joint in healthy non-  
613 athletes and in marathon runners: Image quality issues at 7.0 T compared to 1.5 T.  
614 *Skeletal Radiol.* 2013;42(2):261–7.
- 615 29. Fulford J, Eston RG, Rowlands A V, Davies RC. Assessment of magnetic resonance  
616 techniques to measure muscle damage 24 h after eccentric exercise. *Scand J Med Sci*  
617 *Sport.* 2014;25(1):e28-39.
- 618 30. Fleckenstein JL. Muscle water shifts, volume changes, and proton T2 relaxation times  
619 after exercise. *J Appl Physiol.* 1993;74(4):2047–8.
- 620 31. Fleckenstein JL, Watumull D, McIntire DD, Bertocci LA, Chason DP, Peshock RM.  
621 Muscle proton T2 relaxation times and work during repetitive maximal voluntary  
622 exercise. *J Appl Physiol.* 1993;74(6):2855–9.
- 623 32. Le Rumeur E, Carre F, Bernard AM, Bansard JY, Rochcongar P, De Certaines JD.  
624 Multiparametric classification of muscle T1 and T2 relaxation times determined by  
625 magnetic resonance imaging. The effects of dynamic exercise in trained and untrained  
626 subjects. *Br J Radiol.* 1994;67(794):150–6.
- 627 33. Skenderi KP, Kavouras SA, Anastasiou CA, Yiannakouris N, Matalas AL. Exertional  
628 rhabdomyolysis during a 246-km continuous running race. *Med Sci Sports Exerc.*  
629 2006;38(6):1054–7.

- 630 34. Fridén J, Lieber RL. Eccentric exercise-induced injuries to contractile and cytoskeletal  
631 muscle fibre components. *Acta Physiol Scand*. 2001;171(2):321–6.
- 632 35. Robach P, Boisson RC, Vincent L, et al. Hemolysis induced by an extreme mountain  
633 ultra-marathon is not associated with a decrease in total red blood cell volume. *Scand J*  
634 *Med Sci Sport*. 2014;24(1):18–27.
- 635 36. Overgaard K, Lindstrom T, Ingemann-Hansen T, Clausen T. Membrane leakage and  
636 increased content of Na<sup>+</sup>-K<sup>+</sup> pumps and Ca<sup>2+</sup> in human muscle after a 100-km run. *J*  
637 *Appl Physiol*. 2002;92(5):1891–8.
- 638 37. McCarthy DA, Dale MM. The Leucocytosis of Exercise: A Review and Model. *Sport*  
639 *Med*. 1988;6(6):333–63.
- 640 38. Hikida RS, Staron RS, Hagerman FC, Sherman WM, Costill DL. Muscle fiber necrosis  
641 associated with human marathon runners. *J Neurol Sci*. 1983;59(2):185–203.
- 642 39. Knechtle B, Duff B, Schulze I, Kohler G. A multi-stage ultra-endurance run over 1,200  
643 km leads to a continuous accumulation of total body water. *J Sport Sci Med*.  
644 2008;7(3):357–64.
- 645 40. Sloniger MA, Cureton KJ, Prior BM, Evans EM. Lower extremity muscle activation  
646 during horizontal and uphill running. *J Appl Physiol* [Internet]. 1997;83(6):2073–9.  
647 Available from: <http://jap.physiology.org/content/83/6/2073>
- 648 41. Degache F, Morin JB, Oehen L, et al. Running mechanics during the world’s most  
649 challenging mountain ultramarathon. *Int J Sports Physiol Perform*. 2016;11(5):608–14.
- 650 42. Gottschall JS, Kram R. Ground reaction forces during downhill and uphill running. *J*  
651 *Biomech*. 2005;38(3):445–52.
- 652 43. Van Oorschot JWM, Gho JMIH, Van Hout GPJ, et al. Endogenous contrast MRI of



- 653 cardiac fibrosis: Beyond late gadolinium enhancement. *J Magn Reson Imaging*.  
654 2015;41(5):1181–9.
- 655 44. Van Nierop BJ, Bax NAM, Nelissen JL, et al. Assessment of myocardial fibrosis in  
656 mice using a T2\*-weighted 3D radial magnetic resonance imaging sequence. *PLoS*  
657 *One*. 2015;10(6):e0129899.
- 658 45. Huang S yun, Li X hua, Huang L, et al. T2\* Mapping to characterize intestinal fibrosis  
659 in crohn’s disease. *J Magn Reson Imaging*. 2018;48(3).
- 660 46. Zia MI, Ghugre NR, Connelly KA, et al. Characterizing myocardial edema and  
661 hemorrhage using quantitative t2 and t2\* mapping at multiple time intervals post ST-  
662 segment elevation myocardial infarction. *Circ Cardiovasc Imaging*. 2012;5(5):566–72.
- 663 47. Messroghli DR, Moon JC, Ferreira VM, et al. Clinical recommendations for  
664 cardiovascular magnetic resonance mapping of T1, T2, T2 and extracellular volume: A  
665 consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR)  
666 endorsed by the European Association for Cardiovascular Imagin. *J Cardiovasc Magn*  
667 *Reson*. 2017;19(1):75.
- 668 48. Manka R, Paetsch I, Schnackenburg B, Gebker R, Fleck E, Jahnke C. BOLD  
669 cardiovascular magnetic resonance at 3.0 tesla in myocardial ischemia. *J Cardiovasc*  
670 *Magn Reson*. 2010;12(1):54.
- 671 49. Jahnke C, Gebker R, Manka R, Schnackenburg B, Fleck E, Paetsch I. Navigator-gated  
672 3D blood oxygen leveldependent CMR at 3.0-T for detection of stress-induced  
673 myocardial ischemic reactions. *JACC Cardiovasc Imaging*. 2010;3(4):375–84.
- 674 50. Aguor ENE, Arslan F, Van De Kolk CWA, et al. Quantitative T2 \* assessment of  
675 acute and chronic myocardial ischemia/reperfusion injury in mice. *Magn Reson Mater*  
676 *Physics, Biol Med*. 2012;25(5):369–79.

- 677 51. Lira FS, Yamashita AS, Uchida MC, et al. Low and moderate, rather than high  
678 intensity strength exercise induces benefit regarding plasma lipid profile. *Diabetol*  
679 *Metab Syndr.* 2010;3:31.
- 680 52. Belton PS, Jackson RR, Packer KJ. Pulsed NMR studies of water in striated muscle. I.  
681 Transverse nuclear spin relaxation times and freezing effects. *BBA - Gen Subj.*  
682 1972;286(1):16–25.
- 683 53. Nemeth A, Segrestin B, Leporq B, et al. Comparison of MRI-derived vs. traditional  
684 estimations of fatty acid composition from MR spectroscopy signals. *NMR Biomed.*  
685 2018;31(9):e3991.
- 686 54. Achten J, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that  
687 elicits maximal fat oxidation. *Med Sci Sports Exerc.* 2002;42(4):405–12.
- 688 55. Jeukendrup AE, Saris WHM, Wagenmakers AJM. Fat metabolism during exercise: A  
689 review - Part II: Regulation of metabolism and the effects of training. *Int J Sports Med.*  
690 1998;19(5):203–302.
- 691 56. Maufrais C, Millet GP, Schuster I, Rupp T, Nottin S. Progressive and biphasic cardiac  
692 responses during extreme mountain ultramarathon. *Am J Physiol - Hear Circ Physiol*  
693 [Internet]. 2016;310(10):H1340–8. Available from:  
694 <http://ajpheart.physiology.org/content/310/10/H1340>
- 695 57. Spriet LL. Regulation of skeletal muscle fat oxidation during exercise in humans. *Med*  
696 *Sci Sports Exerc.* 2002;34(9):1477–84.
- 697 58. Watt MJ, Cheng Y. Triglyceride metabolism in exercising muscle. *Biochim Biophys*  
698 *Acta - Mol Cell Biol Lipids.* 2017;1862(10 Pt B):1250–9.
- 699 59. Van Loon LJC. Use of intramuscular triacylglycerol as a substrate source during  
700 exercise in humans. *J Appl Physiol.* 2004;97(4):1170–87.

- 701 60. Stankiewicz-Choroszuca B, Górski J. Effect of decreased availability of substrates on  
702 intramuscular triglyceride utilization during exercise. *Eur J Appl Physiol Occup*  
703 *Physiol.* 1978;40(1):27–35.
- 704 61. Kiens B, Essen-Gustavsson B, Christensen NJ, Saltin B. Skeletal muscle substrate  
705 utilization during submaximal exercise in man: effect of endurance training. *J Physiol.*  
706 1993;469(459–78).
- 707 62. Guo Z, Burguera B, Jensen MD. Kinetics of intramuscular triglyceride fatty acids in  
708 exercising humans. *J Appl Physiol.* 2000;89(5):2057–64.
- 709 63. Krssak M, Petersen KF, Bergeron R, et al. Intramuscular glycogen and  
710 intramyocellular lipid utilization during prolonged exercise and recovery in man: A  
711 <sup>13</sup>C and <sup>1</sup>H nuclear magnetic resonance spectroscopy study. *J Clin Endocrinol Metab.*  
712 2000;85(2):748–54.
- 713 64. Décombaz J, Schmitt B, Ith M, et al. Postexercise fat intake repletes intramyocellular  
714 lipids but no faster in trained than in sedentary subjects. *Am J Physiol - Regul Integr*  
715 *Comp Physiol.* 2001;281(3):R760-9.
- 716 65. Watt MJ, Heigenhauser GJF, Spriet LL. Intramuscular triacylglycerol utilization in  
717 human skeletal muscle during exercise: Is there a controversy? *J Appl Physiol.*  
718 2002;93(4):1185–95.
- 719 66. Kiens B, Roepstorff C, Glatz JFC, et al. Lipid-binding proteins and lipoprotein lipase  
720 activity in human skeletal muscle: Influence of physical activity and gender. *J Appl*  
721 *Physiol.* 2004;97(4):1209–18.
- 722 67. Ferguson M a, Alderson NL, Trost SG, Essig D a, Burke JR, Durstine JL. Effects of  
723 four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. *J*  
724 *Appl Physiol.* 1998;85(3):1169–74.

- 725 68. Frias MA, Virzi J, Golaz O, Gencer B, Mach F, Vuilleumier N. Impact of long  
726 distance rowing on biological health: A pilot study. *Clin Biochem.* 2018;52(142–147).
- 727 69. Schenck JF. The role of magnetic susceptibility in magnetic resonance imaging: MRI  
728 magnetic compatibility of the first and second kinds. *Med Phys.* 1996;23(6):815–50.
- 729 70. Wang Y, Liu T. Quantitative susceptibility mapping (QSM): Decoding MRI data for a  
730 tissue magnetic biomarker. *Magn Reson Med.* 2015;73(1):82–101.
- 731 71. Wang C, Zhang R, Zhang X, et al. Noninvasive measurement of lower extremity  
732 muscle oxygen extraction fraction under cuff compression paradigm. *J Magn Reson*  
733 *Imaging.* 2016;43(5):1148–58.
- 734 72. Drace JE, Pelc NJ. Measurement of skeletal muscle motion in vivo with phase-contrast  
735 MR imaging. *J Magn Reson Imaging.* 1994;4(2):157–63.
- 736 73. Pappas GP, Asakawa DS, Delp SL, Zajac FE, Drace JE. Nonuniform shortening in the  
737 biceps brachii during elbow flexion. *J Appl Physiol.* 2002;92(6):2381–9.
- 738 74. Asakawa DS, Blemker SS, Gold GE, Delp SL. In vivo motion of the rectus femoris  
739 muscle after tendon transfer surgery. *J Biomech.* 2002;35(8):1029–37.
- 740 75. Zhong X, Epstein FH, Spottiswoode BS, Helm PA, Blemker SS. Imaging two-  
741 dimensional displacements and strains in skeletal muscle during joint motion by cine  
742 DENSE MR. *J Biomech.* 2008;41(3):532–40.
- 743 76. Kent-Braun J a, McCully KK, Chance B. Metabolic effects of training in humans: a  
744 <sup>31</sup>P-MRS study. *J Appl Physiol.* 1990;69(3):1165–70.
- 745 77. Manfredi TG, Fielding RA, O'Reilly KP, Meredith CN, Lee HY, Evans WJ. Plasma  
746 creatine kinase activity and exercise-induced muscle damage in older men. *Med Sci*  
747 *Sports Exerc.* 1991;23(9):1028–34.

- 748 78. Newton MJ, Morgan GT, Sacco P, Chapman DW, Nosaka K. Comparison of  
749 Responses to Strenuous Eccentric Exercise of the Elbow Flexors Between Resistance-  
750 Trained and Untrained Men. *J Strength Cond Res.* 2008;22(2):597–607.
- 751 79. Saab G, Thompson RT, Marsh GD. Multicomponent T2 relaxation of in vivo skeletal  
752 muscle. *Magn Reson Med.* 1999;42(1):150–7.
- 753 80. Kikinis R, Pieper SD, Vosburgh KG. 3D Slicer: A Platform for Subject-Specific Image  
754 Analysis, Visualization, and Clinical Support. In: Jolesz FA, editor. *Intraoperative*  
755 *Imaging and Image-Guided Therapy* [Internet]. New York, NY: Springer New York;  
756 2014. p. 277–89. Available from: [https://doi.org/10.1007/978-1-4614-7657-3\\_19](https://doi.org/10.1007/978-1-4614-7657-3_19)
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759 **FIGURE LEGENDS**

760 **Figure 1:** Flowchart summarizing the main steps of our study. MRI was performed at three  
761 time points: before (Pre), and participants who finished the race, immediately after (Post) and  
762 ~48-72 h after the race (Post+3). Meanwhile, biological sampling was performed at four time  
763 points (an additional sample was performed at half-race (Mid)).

764 **Figure 2:** MRI input sequences superposed on the isotropic water image (a), the coronal view  
765 of the automatic segmentation displayed with the isotropic water image as a background (b)  
766 and its 3D volume (c) rendered with 3DSlicer(80).

767 **Figure 3:** t-test matrix with color-coded P-values for multiple comparisons of qMRI metrics  
768 ( $\chi$ , PDFF, T2, T2\*) between muscle heads at all three acquisition time points (Pre, Post,  
769 Post+3). Abbreviations: VL – Vastus Lateralis, RF – Rectus Femoris, VM – Vastus Medialis,  
770 VI – Vastus Intermedius, r – right, l – left. For easy viewing, dotted diagonals highlight inter-  
771 leg (right/left) comparisons of the same muscle heads at each time point, while all other boxes  
772 are intra-leg and/or inter-head comparisons.

773 **Figure 4:** Variation of T2\* mean in the individual muscle heads of all finishers with an example  
774 of T2\* maps at the three MR acquisition time points relative to the race of the same subject. A  
775 P-value less than .05 indicates a significant change between two time points. Abbreviations:  
776 VL – Vastus Lateralis, RF – Rectus Femoris, VM – Vastus Medialis, VI – Vastus Intermedius.

777 **Figure 5:** Variation of T2 mean in the individual muscle heads of all finishers with an example  
778 of T2 maps at the three MR acquisition time points relative to the race of the same subject. A  
779 P-value less than .05 indicates a significant change between two time points. Abbreviations:  
780 VL – Vastus Lateralis, RF – Rectus Femoris, VM – Vastus Medialis, VI – Vastus Intermedius.

781 **Figure 6:** Variation of PDFF mean in the individual muscle heads of all finishers with an  
782 example of PDFF maps at the three acquisition time points relative to the race of the same

783 subject. A P-value less than .05 indicates significant changes between two time points.  
784 Abbreviations: VL – Vastus Lateralis, RF – Rectus Femoris, VM – Vastus Medialis, VI –  
785 Vastus Intermedius.

786 **Figure 7:** Variation of  $\chi$  mean in the individual muscle heads of all finishers with an example  
787 of  $\chi$  maps at the three acquisition time points relative to the race of the same subject. A P-value  
788 less than .05 indicates a significant change between two time points. Abbreviations: VL –  
789 Vastus Lateralis, RF – Rectus Femoris, VM – Vastus Medialis, VI – Vastus Intermedius.

790 **Figure 8:** P-values of statistical tests in our longitudinal analysis on the blood and urinary  
791 biomarker datasets. A P-value less than .05 indicates a significant change between two time  
792 points.

793 **Supplemental Digital Contents**

794 SDC1.docx MR imaging acquisition parameters

795 SDC2.docx Quantitative maps computation

796 SDC3.docx Muscle segmentation

797 SDC4.docx List of 58 biological markers

798 SDC5.docx Biological data preprocessing for missing data

799 SDC6.docx Demographic data and qMRI metrics of the subject population

800 SDC7.docx Specific longitudinal T2\* analysis

801 SDC8.docx Values of urinary and blood biomarkers at 4 time points

802 SDC9.docx Correlation between biological markers and image features extracted from  
803 quadriceps muscles in each qMRI quantitative map