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

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► To cite this version:

Hoai-Thu Nguyen, Thomas Grenier, Benjamin Leporq, Caroline Le Goff, Benjamin Gilles, et al.. Quantitative Magnetic Resonance Imaging Assessment of the Quadriceps Changes during an Extreme Mountain Ultramarathon. *Medicine and Science in Sports and Exercise*, 2021, 53 (4), pp.869-881. 10.1249/mss.0000000000002535 . hal-02998029

HAL Id: hal-02998029

<https://hal.science/hal-02998029v1>

Submitted on 3 Jan 2021

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

4

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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 (χ)
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, χ , 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 (χ)
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, χ , 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°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 ± 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 (χ , 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, χ , T2* and PDFF showed similar
256 tendency. At the same time, there was no significant difference between right and left muscle
257 heads for χ 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 χ showed only a small time effect (Figure 6-7). Pooling all muscles, PDFF had a
275 tendency to decrease after the race (2.88 ± 0.53) compared to baseline (3.16 ± 0.45) while
276 increased to higher values than baseline (3.17 ± 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 χ 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 χ 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 χ .
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 χ*

460 Magnetic susceptibility χ 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 (O₂, 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 O₂ and deoxyhemoglobin molecules(70). The
468 magnetic susceptibility χ remained very stable with only a minimal increase at arrival of
469 1.75 ± 0.43 ppm vs. 1.49 ± 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 VO₂max(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.

523 **ACKNOWLEDGEMENTS**

524 This work has been performed within the framework of LABEX PRIMES (ANR-11-LABX-
525 0063) of Université de Lyon, within the program "Investissements d'Avenir" (ANR-11-IDEX-
526 0007) and France Life Imaging (ANR-11-INBS-0006) operated by the French National
527 Research Agency (ANR). H-T.Nguyen is working under doctoral contract with 488 SIS
528 doctoral school of Université de Lyon. The authors also thank warmly all ultra-trailers that
529 volunteered for the MUST project, Dr Guido Girardini from the Valle d'Aosta Regional for his
530 unfailing support and help, Hospital the Aosta Valley Regional Government, the VDA trailers
531 and Courmayeur city representatives for their technical assistance.

532 **CONFLICT OF INTEREST**

533 The authors declare that there is no conflict of interest. The results of the present study do not
534 constitute endorsement by ACSM and are presented clearly, honestly, and without fabrication,
535 falsification, or inappropriate data manipulation.

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- 757
- 758

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 (χ , 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 χ mean in the individual muscle heads of all finishers with an example
787 of χ 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