Comparative MRI analysis of T2 changes associated with single and repeated bouts of downhill running leading to eccentric-induced muscle damage

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Marqueste T, Giannesini B, Le Fur Y, Cozzone PJ, Bendahan D. Comparative MRI analysis of T2 changes associated with single and repeated bouts of downhill running leading to eccentric-induced muscle damage. J Appl Physiol 105: 299–307, 2008. First published May 1, 2008; doi:10.1152/japplphysiol.00738.2007.—Although the exact mechanisms are still unclear, it is commonly acknowledged that acute eccentric exercise alters muscle performance, whereas the repetition of successive bouts leads to the disappearance of the deleterious signs. To clarify this issue, we measured blood creatine kinase and lactate dehydrogenase activities and proton transverse relaxation time (T2) in various leg muscles 72 h after single and repeated bouts of exhausting downhill running sessions (~15°, 1.5 km/h) with either 4 or 7 days elapsed between bouts. After a single exercise bout, T2 and enzyme activities initially increased and recovered rapidly. When exercise bouts were repeated over a short time period (4 days), initial changes did not recover and endurance time throughout additional exercise sessions was significantly reduced. On the contrary, with a longer resting time between exercises (7 days), the endurance time of additional running sessions was significantly longer and muscle changes (T2 increase, muscle edema, and enzyme activity changes) slowly and completely reversed. Significant correlations were found between T2 changes and enzyme activities. T2 changes in the soleus and gastrocnemius muscle heads were differently affected by lengthening contractions, suggesting a muscle specificity and indicating that muscle alterations might be linked to different anatomical properties, such as fiber pennation angles, typology, and/or the exhausting nature of the downhill running sessions. We documented a “less muscle injury” effect due to the repetition of exercise bouts at a low frequency (i.e., 1 session per week) in accordance with the delayed muscle inflammation. This effect was not observed when the between-exercise resting time was shorter.

downhill running; delayed-onset muscle soreness; rat; lengthening; functional magnetic resonance imaging; transverse relaxation time

The lengthening of a contracting muscle, for example, during downhill running, is well known to be linked to eccentric-induced muscle damage. The terms “eccentric” or “lengthening” actually illustrate the imbalance between the external strength imposed to the muscle and the strength produced by the muscle itself (15). This situation is quite common during downhill running, activation of an antagonist muscle group, and when muscles are exposed to unaccustomed or high levels of repeated activity. It is invariably linked to structural and functional muscle damage (16, 19).

As previously mentioned by Faulkner et al. (16), whole body exercise performed by mice, rats, and humans has been systematically associated with postexercise muscle alterations (2, 18, 30, 45). During eccentric exercise, cytoskeletal, sarcolemmal, and microtubular lesions together with sarcoplasmic reticulum fragmentation and mitochondrial swelling have been reported (1, 2). A local production of inflammatory mediators can be detected 3 h after the end of exercise, whereas muscle edema and a significant increase in blood enzyme activities have been described 2–5 days after exercise as a sign of a systemic inflammatory response. Muscle regeneration then starts 4–6 days after the eccentric session and can last several weeks (24, 59).

Paradoxically, when eccentric contractions are repeated after a long-enough resting period, i.e., 1 wk, muscle alterations are milder and the corresponding functional recovery is faster. This “less injury” phenomenon is known as the protective effect or the “repeated bout effect” (8, 29, 35, 37) and could be mediated by neural, muscular, mechanical, and cellular adaptations. However, the exact mechanisms still remain to be elucidated (7, 37). Most of the aforementioned studies have been conducted in healthy human subjects, and very few have included multiple exercise repetitions. Such protocols can be achieved in animals, and standardized exercise bouts can be reliably repeated to induce reproducible muscle alterations and to study the corresponding muscle changes.

Exercise-induced muscle damage can be investigated directly using histological and biochemical analyses of muscle biopsies. Indirect evidence can be obtained from blood measurements of the activity of muscle enzymes such as creatine kinase (CK). Isometric force deficit measurements also provide valuable information (2, 7, 60). However, the invasive nature of muscle biopsies prohibits repetitive measurements, whereas muscular enzyme activity measured in the blood provides neither qualitative nor quantitative information regarding specific muscle damages. Magnetic resonance imaging (MRI) is actually a noninvasive alternative to both methods and allows us to distinguish changes in each muscle specifically. More particularly, transverse relaxation time (T2) measurements can provide direct information regarding eccentric exercise-induced muscle alterations. It has been shown that T2 increases 12–24 h (up to 3–5 days) after an eccentric exercise and can remain elevated for a period of 3 mo (34, 53), whereas it increases immediately after a concentric exercise (17). A direct relationship between T2 increase and CK blood level has been reported in humans during the days following an eccentric exercise, hence indicating that T2 changes could quantitatively illustrate...
the damage severity (29, 49). In addition, previous studies (13, 17, 44) have reported that “edema-like changes” could account for the increased signal intensity observed in T2-weighted images following eccentric exercise. This would be due to an increased muscle “free water” concentration.

The aim of the present study was to investigate and compare T2 changes occurring in the gastrocnemius-soleus muscle complex during single and repeated bouts of downhill running sessions performed until exhaustion. This procedure is well known to induce “eccentric-type” muscle damage throughout the repetition of stretch-shortening cycles (26). Muscle damage was mechanically evaluated from the measurements of electrically elicited force under isometric conditions.

The purpose of the present study was to compare T2 changes, serum muscle enzyme activities, and the isometric force deficit throughout running sessions repeated with a resting period of either 1 wk or 4 days. We hypothesized that the latter protocol would lead to a protective effect illustrated by milder muscle changes. We also aimed at determining whether the gastrocnemius-soleus complex was homogeneously affected throughout lengthening contractions or whether a muscle specificity could be observed.

MATERIALS AND METHODS

Animal Care and Feeding

Female Sprague-Dawley rats (Janvier Laboratory, Le-Genest-St-Isle, France) weighing 275–300 g were used for these experiments, following the guidelines of the National Research Council Guide for the care and use of laboratory animals and the French Law on the Protection of Animals, with a university committee-approved protocol. Rats were housed in an environmentally controlled facility (12:12-h light-dark cycle, 22°C) and received water and standard food ad libitum until the time of experiments.

Experimental Protocol

Animals were randomly assigned to four groups according to the number of running sessions performed on a dedicated treadmill (Fig. 1). In the first group (group A, n = 7), animals did not perform any running session during the whole protocol, whereas animals in groups B (n = 9), C (n = 7), and D (n = 8) completed one, three, and three downhill running sessions, respectively. In groups C and D, the three repeated bouts of exercise were interspersed by a week and by 4 days, respectively. As illustrated in Fig. 1, MRI and blood enzyme measurements were performed for each animal before any session of exercise (t0) and 72 h after each exercise session. T2 and CK values have been previously described to peak at 3 days after an eccentric exercise (41, 49, 50). Animals in group A were investigated at day 7 (t1), day 14 (t2), and day 21 (t3) after the initial investigation (t0). Measurements in group B were performed three times after the control investigation, i.e., 72 h (t1), 10 days (t2), and 17 days (t3) after the single bout of exercise.

Eccentric Exercise Protocol

As we previously reported (33), running sessions were performed at a constant speed (1.5 km/h) on a dedicated treadmill (Medical Développement, Saint Etienne, France) until exhaustion. The treadmill slope was negative, i.e., −15°, so that rats ran downhill during the trials. Time to exhaustion was defined as the time when animals lost their righting reflex and could no longer run on the treadmill (33).

Noninvasive Investigation of Muscle Function

Animal preparation. Rats were initially anesthetized in an induction chamber with 4% isoflurane (Forene; Abbott France, Rungis, France) mixed in 33% O2 (0.5 l/min) and 66% N2O (1 l/min). The right hindlimb was shaved, and electrode cream for electrical stimulation was applied at knee and heel levels to optimize electrical stimulation. An anesthetized rat was placed supine in a home-built cradle that was especially designed for the strictly noninvasive functional investigation of the right gastrocnemius muscle (14, 22, 23). This cradle integrates a hydraulic ergometer and two rod-shaped transcutaneous electrodes connected to an electrical stimulator (type 215/T; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany) and located above the knee and at the heel level, respectively. The foot was positioned on the pedal of the ergometer, and the hindlimb was immobilized in the cradle and centered inside a 30-mm-diameter 1H-MR Helmholtz imaging coil. The gastrocnemius muscle was passively stretched at rest by adjusting the pedal position to modify the angle between the foot and the lower hindlimb to give maximum isometric twitch tension in response to supramaximal square-wave pulses (6–8 mA, 1-ms duration). Throughout the experiment, anesthesia was maintained by gas inhalation with a face mask continuously supplied with 2.5% isoflurane in 33% O2 (0.4 l/min) and 66% N2O (0.8 l/min). Corneas were protected from drying by application of ophthalmic cream (Lacrigel; Europhila, Monte Carlo, Monaco). The face mask was connected to an open-circuit gas anesthesia machine (Isotec 3; Ohmeda Medical, Hatfield, UK). Exhaled and excess gases were removed through a canister filled with activated charcoal and mounted on an electrical pump extractor (Equipement Vétérinaire Minerve, Esteray, France). During anesthesia, the animal’s body temperature was maintained using an electric heating blanket (Prang + Partner, Pfungen, Switzerland) included in a feedback loop with a temperature control unit and a rectal probe (Harvard Apparatus).

Stimulation protocol and force measurement. The stimulation protocol consisted of 6 min of repeated isometric contractions at a frequency of 3.3 Hz that were electrically induced with square-wave pulses (6–8 mA, 1-ms duration). Electrical signal coming out from the pressure transducer was amplified (Gould), converted to a digital signal, and processed on a personal computer using ATS software (Sysma, Aix-en-Provence, France).

Fig. 1. Schematic diagram of the experimental design showing the 4 exercise protocols and the recording times. Arrows refer to time points (t) at which MRI measurements, maximal isometric force, and intracardiac blood samplings were performed. Triangles indicate the downhill running sessions, and open boxes refer to resting periods. As indicated, animals in group A did not perform exercise, whereas a single bout of eccentric exercise was done by animals in group B. Three bouts of eccentric exercise were performed in both groups C and D, with a shorter repetition rate in group D.
MR acquisition. MR data were acquired at 4.7 T on a Biospec 47/30 Avance MR system (Bruker, Karlsruhe, Germany). Five non-contiguous axial imaging slices (2-mm thickness, 1-mm gap) were selected across the lower hindlimb based on a set of scout images. Multiecho T2-weighted images of these slices (8 echo times equally spaced from 16 to 128 ms, 1,000-ms repetition time, 4 × 4-cm field of view, 256 × 128 matrix size, total acquisition time 4.57 min) were recorded at rest.

MR data processing. MR data were processed using a custom-written software developed on the IDL platform (Interactive Data Language; Research Systems, Boulder, CO). T2-weighted images obtained at 16, 48, 80, and 112 ms were used to generate T2 maps on the basis of a pixel-by-pixel analysis using a single-exponential function to fit the 1H-MRI signal decay. Because of imperfect refocusing (hermite refocusing pulses), even echoes (32, 64, 96, and 128 ms) were excluded from the analysis. Mean T2 values for tibialis anterior, soleus, and red-mixed and white gastrocnemius regions were measured on T2 maps by manually outlining regions of interest using a custom-written image analysis program. Anatomical, histochemical (3), and MRI studies (47) were used as a reference for muscle identification. These measurements were done and averaged for the two slices with the largest sections. The surface of the whole set of muscles located in the anterior and posterior tibial compartments (including soleus and gastrocnemius) was measured separately, and the ratio between the posterior compartment to the whole muscle surface was calculated.

Enzyme Activities

After MR measurements, transcardiac blood samples (0.25 ml) were obtained with a thin needle carefully introduced in the heart (0.6 × 25 mm) during the anesthetic epoch. Plasma was immediately separated after blood centrifugation (15 min at 4,000 rpm) in EDTA-treated tubes. Plasmatic activities of lactate dehydrogenase (LDH; EC 1.1.1.27) and creatine kinase (CK) (EC 2.7.3.2) were spectrophotometrically determined.

Statistical Analysis

All data are means ± SD, with differences evaluated using either one-way (type of muscle: degree of freedom = 5) or two-way (time: degree of freedom = 3; groups: degree of freedom = 3) repeated-measures analysis of variance (ANOVA) with the α level of significance set at P < 0.05. When justified by ANOVA results, specific comparisons among experimental conditions were performed using post hoc Tukey’s tests. Pearson product moment tests were performed to investigate relationships between T2 changes and corresponding values of blood CK and LDH activities. Data processing was done using SigmaStat (Jandel, Chicago, IL).

RESULTS

Table 1. Power, total work, and duration of exercise bouts and maximal isometric force measured at times indicated after each exercise bout

<table>
<thead>
<tr>
<th>Group</th>
<th>1st Exercise Bout</th>
<th>2nd Exercise Bout</th>
<th>3rd Exercise Bout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Power, W</td>
<td>Work, kJ</td>
<td>Duration, min</td>
</tr>
<tr>
<td>A</td>
<td>1.11±0.02</td>
<td>15.6±1.5</td>
<td>233±22</td>
</tr>
<tr>
<td>B</td>
<td>1.11±0.01</td>
<td>16.0±0.4</td>
<td>241±6</td>
</tr>
<tr>
<td>C</td>
<td>1.11±0.03</td>
<td>15.9±1.4</td>
<td>237±21</td>
</tr>
<tr>
<td>D</td>
<td>1.11±0.03</td>
<td>15.9±1.4</td>
<td>237±21</td>
</tr>
</tbody>
</table>

Results are means ± SD. Power and total work were computed in accordance with the speed and slope of the treadmill and based on the respective animal’s weight for each bout. Duration represents the time to exhaustion measured at the end of each bout of downhill running exercise. Maximal isometric force, measured at indicated times (t1, t2, and t3), respectively, 3 days after each exercise bout, is the remaining force compared with that preexercise (t0), which is 100%. *P < 0.05; **P < 0.01; ***P < 0.001 compared with maximal isometric force measured in each rat at t0. *P < 0.05; **P < 0.01 compared with the respective values measured during the first bout of downhill exercise. *P < 0.05 compared with group D values at the second bout.

Time to Exhaustion, Power, and Total Work

The time to exhaustion was not different among groups during the first downhill running bout, averaging 237 ± 17 min (Table 1). The second bout’s endurance time was significantly longer (t1, t2, t3). Results are means ± SD. *P < 0.05; **P < 0.01; ***P < 0.001 compared with the respective control level measured at t0 within each group.
shorter in both groups C and D, although it was comparatively longer in group C than group D. During the third exercise session, the time to exhaustion measured in group C was similar to the control value, illustrating a complete recovery. On the contrary, an additional reduction (181 ± 4 min) was measured in group D. As indicated in Table 1, changes in the total work were similar to changes measured for the time to exhaustion.

**Isometric Force**

Maximal isometric force was measured throughout a standardized muscle stimulation protocol at t0, t1, t2, and t3. At t0, i.e., before any eccentric exercise session, maximal isometric force did not differ among the four groups, averaging 8.5 ± 2.6 N, and did not change throughout the whole protocol in group A (Table 1). A transient and significant reduction was measured at t1, i.e., 72 h after the first bout of eccentric exercise, in both groups B and C, whereas a complete recovery was measured afterward. On the contrary, a continuous force reduction was measured at t1 (59% of the preexercise isometric force), t2 (46%), and t3 (44%) in group D.

**LDH and CK Activities**

Under control conditions (t0), LDH and CK activities ranged between 30.2 and 45.3 U/l (Fig. 2A) and 176.3 and 291.9 U/l (Fig. 2B), respectively, and did not vary among groups. Similarly, no activity change was measured in group A during the whole protocol. For groups B, C, and D, who experienced repeated downhill running exercises, both LDH (group B, 143 ± 14 U/l; group C, 148 ± 13 U/l; group D, 172 ± 13 U/l) and CK activities (group B, 775 ± 78 U/l; group C, 609 ± 72 U/l; group D, 884 ± 72 U/l) increased significantly at t1, i.e., 72 h after the first bout of exercise. In group B, CK activity returned to control value as soon as 1 wk after the single exercise bout, whereas LDH activity remained significantly high at t2 and reached the initial control value at t3. In group C, CK activity gradually decreased after the first session of exercise and reached the control value 72 h after the last exercise bout. On the contrary, LDH activity decreased but still remained significantly high at that time. For animals in group D, which performed three bouts of exercise at the highest repetition rate, blood enzymes activities always remained dramatically high compared with control conditions.

**MRI Analysis**

Figure 3A displays a typical axial T2-weighted image recorded from the rat lower hindlimb. T2 maps were generated from T2-weighted images from a pixel-by-pixel analysis and recorded before (Fig. 3B) and 72 h after (Fig. 3C) each eccentric session. The corresponding T2 values of soleus, tibialis anterior, and whole gastrocnemius muscles are represented in Fig. 4, A, B, and C, respectively. T2 values measured at rest ranged from 27.2 to 30.3 ms under control conditions and differed among neither muscles nor groups. Similarly to enzyme activities, T2 values did not change in animals of group A, which experienced no exercise. As a quality control, T2 values measured in the tibialis anterior muscle remained stable during the whole protocol within the four experimental groups (Fig. 4A). On the contrary, T2 changes were observed in soleus and gastrocnemius muscles. After the first exercise bout, a significant increase was measured in the soleus muscle and for the three groups (group B, +9.9%; group C, +9.5%; group D, +13.5%) (Fig. 4B). In group B, this transient increase was followed by a progressive decrease throughout the resting periods, i.e., t2 and t3. On the contrary, T2 values remained significantly elevated after the second exercise bout in both groups C (+7.1%) and D (+9.2%). It is noteworthy that the initial T2 value was reached back after the last exercise session in group C (+4.3%) but not in group D, in which the T2 value was still 12.2% higher. Similar changes were recorded in the gastrocnemius muscle. T2 value did not change in group A, whereas it increased and remained high in group D. On the
Contrary, a complete T2 recovery was measured at t3 in group C and earlier (at t2) in group B (Fig. 4C).

A detailed analysis of T2 changes in the mixed, white, and red portions of gastrocnemius muscle indicates a larger T2 increase in the mixed (Fig. 5A) and white (Fig. 5B) regions compared with the red one (Fig. 5C). In addition, 72 h after the second exercise bout, T2 remained significantly elevated in both the mixed and the white parts of the gastrocnemius muscle, whereas it was back to normal in the red portion.

Changes in the relative muscle surface of the posterior tibial compartment are displayed in Fig. 6 for the four groups. A significant swelling was recorded at t1 (ranging from +7.8 to +8.1%) and at t2 (from +5.2 to +9.1%) in the three exercising groups. On the contrary, this swelling only remained at t3 in group D (+8.7%), whereas the phenomenon reversed in both groups B and C.

Correlations Between MRI and Enzyme Measurements

T2 data from each muscle were gathered and plotted against CK and LDH activities for corresponding times regardless of the group (Fig. 7, A and B), and the results are summarized in Table 2. Positive correlations between T2 changes and CK or LDH activity were found for soleus, whole gastrocnemius, and the white, mixed, and red regions of the gastrocnemius muscle. For the tibialis anterior muscle, the absence of T2 changes (see Fig. 4. Proton T2 values measured in the tibialis anterior (A), soleus (B), and whole gastrocnemius muscles (C) before (t0) and at different times after an eccentric exercise (t1, t2, t3). Results are means ± SD. *P < 0.05; **P < 0.01; ***P < 0.001 compared with the respective control value measured at t0 for each group.

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Fig. 4A) was well correlated with limited changes in enzyme activities regardless of the group. The tibialis anterior muscle displayed the lowest T2 changes (Table 2). One-way ANOVA also indicated that T2 changes were significantly lower in the red region of the gastrocnemius muscle compared with the white (P < 0.001) and mixed portions (P < 0.01 for CK, P < 0.05 for LDH changes) and compared with the soleus muscle (P < 0.05 for CK, P < 0.001 for LDH changes). The highest T2 change in the gastrocnemius muscle was recorded in the white region, hence evidencing the large implication of fast-twitch fibers in this type of exercise.

DISCUSSION

The major result of the present study is that the eccentric exercise-induced muscle damages were not uniform within the soleus-gastrocnemius complex, but rather affected the mixed and white regions. In addition, we showed that repeated bouts of lengthening exercise could exert a protective effect on muscle performance as long as the repetition rate is smaller than once a week. This protective effect is actually illustrated by the whole set of measurements reported in the present study, including the electrically elicited force under isometric conditions, currently considered a reliable index of muscle injury (60). The beneficial aspects related to the less injuring effect of repeated exercise bouts are more particularly evidenced by the results obtained in group C. A transient alteration of enzymatic, MRI, and force indexes was measured after the first session of exercise and was followed by a progressive reversal throughout the repeated exercise bouts. This variable normalization related to such muscle damage as enzymatic activities, isometric force, and muscle edema/inflammation (T2 values) measured in group C was slower than the reversal process measured in animals of group B, which experienced a single exercise session. When the exercise bout repetition rate was larger than once a week (i.e., results from group D), no indication of a protective effect was observed. On the contrary, MRI and enzymatic measurements reached a peak after the first exercise bout and remained unchanged until the end of the protocol while two additional exercise bouts were performed. Moreover, no force and endurance recovery was observed. It is noteworthy that no alteration was found in the tibialis anterior muscle, a foot dorsiflexor muscle chosen on purpose as an internal control (2, 12).

Our MRI and enzymatic data are consistent with previous studies showing milder alterations in T2 and enzymatic activities when eccentric exercise bouts were repeated after a 2-mo resting period (20) or a week (35, 37). This less injuring effect would likely be due to the inflammatory phase occurring for a week period at the muscle level after a single eccentric exercise bout (16, 52). However, other studies have shown that muscle soreness, reduced range of motion, decreased maximal isometric force, and higher enzyme activities were not always exacerbated when exercise bouts were repeated within a shorter period of time i.e., 2–4 days (32, 40). Alternatively, one could suggest that changes in indicators of muscle damage were significantly blunted (10, 32). Studies conducted in humans have speculated that the repeated bout effect may be attributed to neural adaptation (35, 39, 43) even after a short-term repetition (5). Muscle damage and regeneration would represent the upstream trigger of this adaptive mechanism. In agreement with that, we (33) had already reported that during the first days following a single bout of downhill running, muscle sensory motor control was altered through the release...
of inflammatory mediators of the arachidonic acid pathway, thereby activating group III and IV muscle afferent fibers. This activation, due to inflammatory mediators, could alter both the central motor drive and muscle reflexes during exercise (4, 38), thereby leading to the observed decrease in muscle performance (Table 1) (8). At this early stage, according to the "muscle wisdom" concept, the feedback from damaged muscles afferents should reduce the central muscle activation as a protective effect, yet muscle edema and inflammation are still important (21). Moreover, if the resting period is too short, muscle regeneration cannot be initiated and further injuries can occur as a result of additional exercise bouts, thereby maintaining the muscle inflammatory status. In that respect, the whole set of indexes, including T2 values, edema, enzyme activities, and reduced muscle performance, further confirms these alterations in group D. On the contrary, such changes were not observed when exercise bouts were repeated over a period of 1 wk (i.e., in group C), illustrating the repeated bout effect. In keeping with that, the model proposed by Armstrong et al. (2) indicates that unaccustomed eccentric exercise could mechanically damage sarcomeric and connective tissue structure and result in a complete necrosis of the most severely injured fibers. The accompanying inflammatory process would be followed by the regeneration of more adapted muscle fibers, thereby explaining the protective effect of repeated exercise sessions of eccentric exercises (2).

Blood LDH and/or CK activity measurements have been widely used to illustrate eccentric exercise-induced skeletal muscle damages in both animals and humans (2, 16, 57). Although these measurements do correlate with histopathological signs of inflammation, they do not provide information related to the possible localization of muscle alterations (27). Our positive correlations between T2 measured in various muscle regions and CK or LDH activity do not imply a direct relationship between a given muscle damage and the overall muscle enzyme amount measured in the blood, but rather a higher contribution of a given muscle to the final amount of enzymes released from the muscles. Such a localization has been already reported in humans in a study showing that among the four heads of the quadriceps, rectus femoris experienced the greatest muscle injury during eccentric exercise (46). It has been proposed that this localized injury would result from the biarticular function of the muscle rather than its muscle fibers typology (46). This hypothesis is not consistent with other studies suggesting that muscle injury localization would be linked to fiber types composition (2, 19, 30, 31, 54), including preferentially type II fibers (18, 30) or both fast- and slow-twitch muscle fibers (2, 31, 54, 56). Considering that our running protocol was conducted until exhaustion, one could expect an initial fatigue of type I fibers from the soleus (45) and the red portion of the medial gastrocnemius with a compensatory type II fibers recruitment, thereby leading to an injury of the corresponding muscle region of the gastrocnemius. In that situation, these myofibers would be susceptible to injury due to their size and their type. In other models of injury, large but not small type II fibers have been reported to be preferentially injured. Accordingly, type II muscle fibers in the white region of the medial gastrocnemius are a bit larger than those in the red region (3). On the basis of previous ultrasonographic studies (6, 48) related to the behavior of muscle fascicles and pennation angle during shortening contractions, we should also consider that differences in fascicles architectures and the existence of mechanical links between muscles could also explain the different susceptibility of muscles to damage. One should keep in mind that muscle fibers are recruited differently during downhill running compared with other forms of muscle exercises such as pure lengthening contractions (35, 39). Indeed, during downhill running exercise, leg extensor muscles are alternatively involved in stretch-shortening cycles repetitions (26), whereas the muscle-lengthening contribution during the stretching step increases widely when fatigue occurs (25), i.e., until exhaustion.

In addition to T2 changes, MRI measurements illustrated an increased posterior tibial compartment volume, which includes damaged muscles such as soleus and gastrocnemius. It is noteworthy that both indexes have been commonly considered as signs of muscle edema (41, 43, 53) and that, regardless of exercise conditions, they have been linked to muscle damage (31, 41, 50, 51, 53 55, 58). Data presented in Fig. 5 might illustrate a milder effect of the exercise protocol on the slow-twitch fibers, within the red portion of the gastrocnemius muscle.

Muscle swelling and edema are actually considered as precursors of the exercise-induced fibers necrosis as reported in rodents (28). However, as illustrated by the results obtained in group D, muscle swelling and edema were maintained by the
short-term repetition protocol, and this inflammatory status could delay the regeneration of more adapted muscle fibers. In agreement with this hypothesis, a dramatic decrease in isometric force and time to exhaustion was measured in group D.

The exact meaning of T2 changes remains to be elucidated. Indeed, although T2 normally increases immediately after an exercise bout (23) and as a result of osmotic changes (47), a longer-term T2 increase has been reported after eccentric exercise sessions only (17, 53). Although this long-term T2 increase has been considered as a sign of inflammation/edema, the time scale actually seems inappropriate. Increased T2 values have been reported 2–3 mo after the exercise bout, whereas swelling is known to be resolved (17, 19, 52) at that time. This would suggest that the longer-term T2 increase measured after an eccentric exercise could reflect muscle long-lasting adaptations such as muscle fiber regeneration rather than only muscle swelling. Darr and Schultz (9) reported major satellite cell activation in fast- and slow-twitch damaged muscles within the days following a prolonged treadmill running session. This cellular activation was greater by far than that required to repair necrotic fibers, further suggesting that muscle fiber regeneration could account for prolonged T2 changes after lengthening exercise. However, the long-lasting elevated T2 value observed in group D, associated with a decrease in muscle properties, should be considered as a "chronic" muscle edema and inflammation that could be induced by the short-term repetition of muscular micro lesions. An inappropriate (too short) resting period would inhibit muscle fibers regeneration between exercise sessions.

T2 changes occurring in groups C and D illustrate that the delayed T2 increase reported in the present study is not simply related to muscle performance. Indeed, muscle performance was reduced in both groups after the first session, whereas T2 values remained high in group D and were significantly lower in group C, in which the last two exercise sessions were longer. Our results indicate that MRI measurements of various leg muscles can provide qualitative and quantitative information regarding specific muscle damages related to single or repeated bouts of downhill running conducted until exhaustion. In addition, we report that the gastrocnemius–soleus muscle complex was not homogeneously affected by repeated bouts of eccentric exercise. This heterogeneity could be related to different muscle fiber pennation angles and/or relative content of fiber types. The altered muscle performance due to the fast repetition of exercise bouts was associated with a dramatic T2 increase and muscle edema, whereas a less injuring effect during repeated bouts was related to T2 and size recovery. These results clearly illustrate that the beneficial repeated-bout effect could be obtained when exercise bouts are repeated over a long-term basis, thereby allowing the occurrence of a muscle inflammatory status.

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REFERENCES


