Localization of muscle damage within the quadriceps femoris induced by different types of eccentric exercises

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This study examined localization of muscle damage within the quadriceps femoris induced by different types of eccentric exercises by using transverse relaxation time ($T_2$)-weighted magnetic resonance imaging (MRI). Thirty-three young males performed either of the following three exercises: single-joint eccentric contraction of the knee extensors (KE), eccentric squat (S), or downhill walking (DW) (n=11/exercise). KE and S consisted of 5-set×10-lowering of 90% one-repetition maximum load. DW was performed for 60 minutes with −10% slope, 6 km/h velocity, and 20% body mass load carried. At pre- and 24-, 48-, and 72-hours post-exercise, $T_2$-MRI values for the rectus femoris (RF), vastus intermedius (VI), vastus lateralis (VL), and vastus medialis (VM) at proximal, middle, and distal sites were calculated. Additionally, soreness felt when static pressure was applied to these sites and maximal isometric knee extension torque were measured. Maximal torque significantly ($P<.05$) decreased (7%-15%) at 24-48 hours after all exercises. $T_2$ significantly increased (3%-9%) at 24-72 hours after all exercises, with heterogeneities within the muscles found in each exercise. Effect size and peak change of $T_2$, as well as soreness, overall indicated that the proximal RF after KE and middle VM after S and DW were most affected by these exercises. The VL did not show any significant $T_2$ increase after all exercises. These results suggest that muscle damage specifically localizes at the proximal RF by KE and at the middle VM by S and DW, while the VL is least damaged regardless of the exercises.

KEYWORDS
downhill walking, knee extension, maximal strength, muscle soreness, squat, $T_2$-MRI

1 | INTRODUCTION

It is well documented that exercise-induced muscle damage (EIMD) occurs after a bout of unaccustomed exercise that involves eccentric (lengthening) contractions.1 Suggested primary theories regarding the mechanisms for the eccentric EIMD are disruption of weaker sarcomeres and/or excitation-contraction coupling machinery caused by active muscle lengthening.2 Typical symptoms of EIMD include reduced muscle function, delayed onset muscle soreness, and increased plasma creatine kinase (CK) activity lasting for several days post-exercise.3 In EIMD-related studies, the quadriceps femoris (QF) has often been selected for examination due to its fundamental roles in daily and sports activities,4,6 while studies on more vulnerable upper limb muscles (eg, elbow flexors) have also provided valuable information regarding the etiology of such damage.1,2 The QF performs an eccentric contraction when lowering a load in a controlled manner during squat or knee extension exercise.7 Downhill walking is also a representative form of the QF eccentric exercise, in which the muscle functions as a brake (performs a rapid eccentric contraction) to absorb shocks at...
each step. Indeed, previous studies have demonstrated that EIMD markers such as maximal strength, muscle soreness, and/or plasma CK activities were significantly altered after above-mentioned exercises.

The QF is composed of four muscles: the rectus femoris (RF), vastus intermedius (VI), vastus lateralis (VL), and vastus medialis (VM). They all function as knee extensors, but the RF also acts as a hip flexor because of its bi-articular nature. It is reported that the activation level/pattern of the RF differs along the muscle during a hip flexion or differs from the mono-articular vasti muscles during a knee extension depending on whether the exercise involves hip joint torque exertion. Given these facts, it is reasonably assumed that the degree of EIMD would vary between the RF and others and possibly along the RF, and its site would also be influenced by exercises performed. Some studies have explored this issue in other muscle groups and indeed found heterogeneous EIMD in the plantar flexors but not in the elbow flexors after eccentric exercises. On the other hand, little attention has been paid on such heterogeneity of the EIMD within the QF, for example, by examining only the VL as a representative of the whole QF or by using an average value across all the vasti muscles (explained later).

EIMD can be directly assessed by histological analyses of muscle biopsies, but its invasive nature prohibits repetitive and multiple measurements. Thus, EIMD is often evaluated based on changes in such indirect parameters as muscle function (eg, strength) or intramuscular proteins in blood (eg, CK), which cannot detect potential localizations of damage. Muscle soreness is another often-used parameter in related studies, which can at least in part differentiate the site and degree of soreness by pressing or palpating several sites of the muscle. However, soreness is usually measured as a whole muscle. On the other hand, magnetic resonance imaging (MRI) is a powerful non-invasive alternative tool allowing for spatially resolved analysis of muscle tissue. More specifically, transverse relaxation time ($T_2$)-weighted MRI can provide information on water content of muscle tissue given as a $T_2$ value, which can be then used as an index of the inflammatory edema induced by eccentric exercise. Indeed, high correlation ($r > 0.9$) has been reported between changes in $T_2$ and plasma CK activity after eccentric exercise. It should be noted that a $T_2$ increase is a delayed consequence of cytoskeletal disruptions, thus not directly reflecting muscle damage severity, and its manifestation timing could vary among muscles. Nevertheless, the literature well agrees with the usefulness of $T_2$-MRI to quantitatively localize the damaged site within a muscle group.

A few studies reported a difference among the QF in a degree of $T_2$ changes after voluntary eccentric exercise. For example, Prior et al. reported that a $T_2$ increase after single-joint eccentric contractions of the knee extensors was greater in the RF than in the vasti (averaged across VI, VL, VM). Black and McCully found a similar result that a $T_2$ increase after the same exercise as above was greater in the RF than in the VL. On the other hand, Fulford et al. reported that $T_2$ significantly increased after squat exercise in all of the vasti muscles but not in the RF. These results imply that localization of damage differs depending on exercises. However, in these studies, in-depth analysis such as a comparison between sites along a muscle and/or differentiation of each of the vasti muscles were not made because it was not the purpose of their studies. To our knowledge, no studies have examined eccentric exercise-induced $T_2$ changes for a wide range of the QF while also focusing on whether its localization differs among exercises. Elucidating this subject will contribute to our better understanding of load sharing strategies among synergists during exercise and help decide which site should be chosen in future studies evaluating damage severity with relation to the eccentric exercises adopted. Moreover, considering that inflammatory responses, if tightly regulated, are integral to muscle repair and regeneration, it can also be valuable information to exercise practitioners for prescribing exercises based on which muscle or site is specifically targeted in the program.

In this study, we aimed to characterize localizations of EIMD induced by different types of eccentric exercises of the QF by using $T_2$-weighted MRI. As the exercise tasks, we chose single-joint eccentric contraction of the knee extensors (KE), eccentric squat (S), and downhill walking (DW) based on previous studies. In addition to the $T_2$-weighted MRI, we measured muscle soreness at several sites in the anterior thigh as a supplementary index, acknowledging that it is a poor reflector of muscle damage. Maximal isometric knee extension strength was also measured because it is suggested to be the most reliable index for quantifying muscle damage of a whole muscle group. We hypothesized that changes in $T_2$ and soreness would be heterogeneous within the QF, especially between the RF and vasti, and along the RF, if any. We also hypothesized that such localization would differ among the exercises.

## 2 MATERIALS AND METHODS

### 2.1 Subjects

A total of 33 healthy young males were recruited and placed to either one of the three groups that performed KE, S, or DW (n=11/exercise) by intentionally matching the baseline physical characteristics across the groups to avoid any confounding factors. No significant ($P > 0.05$) differences in their mean (±SD) age (25.8±3.8 years), height (1.71±0.06 m), body mass (66.2±9.9 kg), and knee extension strength (224.8±46.7 Nm) were found among the groups. None of the subjects had been involved in any type of systematic ($≥30$ min/d, $≥2$ d/wk) resistance, aerobic, or flexibility training program, and experienced strenuous mountain trekking and/or DW (other than
those encountered in daily activities) in the past 6 months, and none had been resistance-trained (eg, a weight lifter) prior to 6 months. Subjects were instructed not to perform any unfamiliar activities and any interventions that could affect the recovery such as massage, icing, and nutritional supplementations during the experimental period. This study was approved by the Ethics Committee of Waseda University and was consistent with institutional ethical requirements for human experimentation in accordance with the Declaration of Helsinki. Prior to any measurements, the subjects visited the laboratory and were fully informed about the procedures and possible risks involved as well as the purpose of the study, and written informed consent was obtained. Before and 24, 48, and 72 hours after the eccentric exercises, measurements of T$_2$-MRI, muscle soreness, and isometric knee extension torque in this order were performed. The eccentric exercises and measurements were conducted as follows.

2.2 | Eccentric exercises

A traditional knee extension machine (Nitro Leg Extension; Nautilus, Vancouver, Washington, USA) for KE and smith machine (Smith Machine; Nautilus) for S were used for these exercises. The KE was performed on the right side unilaterally with the hip joint angle fixed at ~80° (anatomical position=0°) and the knee joint angle ranging from ~10° to ~100° (anatomical position=0°). The S was performed with the legs shoulder-width apart, both the hip and knee joint angles ranging from ~0° (when standing) to ~90° (when squatting at the bottom). The KE and S consisted of 5-set×10-lowering of 90% one-repetition maximum (1RM) load, based on a previous study that showed symptoms of EIMD (including a T$_2$ increase) after performing KE using a similar protocol. To determine a 1RM load for each subject, the subjects started lifting a light load first, performing KE using a similar protocol. To determine a 1RM load, based on a previous study that showed symptoms of EIMD (including a T$_2$ increase) after performing KE using a similar protocol. To determine a 1RM load for each subject, the subjects started lifting a light load first, and gradually increased the load until they could not lift it in the prescribed range of motion. During the 1RM measurement, sufficient rest intervals between trials were set, and the examiner(s) helped lower the load to avoid any influence of subjects’ fatigue on the 1RM measurement as well as on the subsequent eccentric exercises. After at least a 5-minutes rest from the determination of 1RM, the subjects performed 2-3 reps of the single-joint eccentric contraction of the knee extensors or eccentric squat to familiarize themselves with the exercises. During these exercises, the examiner(s) lifted the load to the starting position (knee joint angle at ~10° for KE and the standing position for S), and the subject lowered the load to the finish position in a controlled manner over a 3-second count, with a help of a metronome. After ~3-minute rest from the familiarization, the prescribed exercise was performed. 3-second between-repetition and 2-minute between-set intervals were set.

Due to the difference in the exercise type of DW from KE and S, we could not match the protocol for the three exercises. Therefore, we used a typical protocol for the DW to induce EIMD based on previous studies that found symptoms of EIMD after performing DW. DW was performed on a treadmill for 60 minutes under the condition of ~10% slope, 6 km/h velocity, and 20% body mass load carried in a backpack at each subject’s own preferred stride and pitch. The subjects first performed DW at a slow velocity to familiarize themselves for ~30 seconds and gradually increased the velocity to 6 km/h and continued the exercise for 60 minutes under the supervision of the examiner.

2.3 | Maximal isometric knee extension torque

Subjects performed an isometric knee extension of the right side with maximal effort on a specially designed dynamometer (VTK-002; Vine, Tokyo, Japan). They sat on the device with the hip and knee joints fixed at 80° and 70°, respectively. Prior to the measurement, subjects performed an adequate warm-up, consisting of submaximal contractions of 30%, 50%, and 80% of maximal effort to familiarize themselves with the measurement. After at least a 1-minute rest, two maximal knee extensions with a 1-minute rest in-between trials were performed. Subjects were asked to develop torque gradually over 5 seconds to reach maximum and then to sustain maximum effort for 2 seconds, with verbal encouragement provided by the examiner. Additional trials were performed if the difference in the peak torque of the two trials was more than 10%. The torque signals were amplified by a strain amplifier (DPM-711B; Kyowa, Tokyo, Japan) and analogue-digital converted (PowerLab, ADInstruments, Sydney, New South Wales, Australia) into a computer at 1000 Hz with a 10-Hz low-pass filter. The highest knee extension torque was adopted. Day-to-day reproducibility (separated by 2-5 days) of the maximal torque for this method was examined on eight subjects. The coefficient of variation (CV) and intraclass correlation coefficient (ICC) were 6.2% and .93, respectively.

2.4 | T$_2$-MRI

Before the scanning, ink lines were drawn transversely at the proximal (30%), middle (50%), and distal (70% and 80%, explained below) sites of the thigh length (the distance from the great trochanter of the femur to the articular cleft between the femoral and tibial condyles), and oil capsules were put as markers on the skin surface at the lateral side. Subjects lay supine with their legs fully extended and muscles relaxed in a magnet bore (Signa EXCITE 1.5T; GE Medical Systems, Waukesha, Wisconsin, USA). T$_2$-weighted MRIs (echo times: 25, 50, 75, and 100 ms, repetition time: 2000 ms, matrix: 256×256, field of view: 24 cm, slice thickness: 1.5 cm, gap: 1 cm) of the whole right thigh in the transverse plane were recorded using an 8-channel body array coil (GE Medical Systems). To obtain images reproducibly between sessions, a specific slice was always set using the same anatomical...
markers. Namely, the most proximal slice was always set at the proximal edge of the femoral head, and nearest slices to the markers were used for analysis. Images were analyzed with ImageJ software (National Institute of Health, Bethesda, Maryland, USA). Regions of interest were drawn in each slice by manually tracing the border of the anatomical cross-sectional area of each of the QF muscles at the proximal (30%), middle (50%), and distal (70% for the RF, VI, and VL and 80% for the VM) sites (Figure 1). The reason for choosing 80% for the VM was that this muscle’s insertion (distal end) is located at a more distal site than those of the other three muscles. Care was taken to exclude visible adipose and connective tissue incursions.

$T_2$ relaxation time was calculated by least-squares analysis, fitting the signal intensity at each of the four echo times ($n\times25$ ms: 25, 50, 75, 100 ms) to a monoexponential decay using the following equation:

$$S_n = S_0 \exp(-\frac{TE_n}{T_2}) \quad (n = 25, 50, 75, \text{and } 100 \text{ ms}),$$

where $TE$ is echo time, $S_0$ is signal intensity at 0 ms, and $S_n$ is signal intensity at $TE_n$.

Day-to-day reproducibility of the $T_2$ values was assessed in a pilot study on eight subjects for the above-mentioned 12 sites (3 sites×4 muscles). The CV and ICC of the two measurements separated by 3-7 days were 1.9% and .82, respectively. Absolute $T_2$ values (ms) were used in between-time comparisons for each site, and absolute change values from pre ($\Delta T_2$: ms) were used in between-site comparisons for each exercise (detailed in the Statistical analysis section).

### 2.5 Muscle soreness

Muscle soreness was assessed using a 0-10 scale (0=no pain, 10=maximal pain) when static pressure was applied to several sites in the right anterior thigh (modified from in which a similar measurement was performed on the biceps brachii). Subjects lay supine with their legs fully extended and muscles relaxed on a bed. In addition to the transverse lines at the proximal, middle, and distal sites used for the MRI measurement, longitudinal lines at the lateral, central, and medial sides were drawn so that these lines ran along approximately the center of the VL, RF, and VM, respectively. The location of the VI was not taken into consideration at this point because the VI is located in the deep area of the QF and thus invisible. By using an algesiometer (Matsumiya Ika Seiki, Tokyo, Japan), 20 N pressure was applied to the cross-points of these lines (proximal-middle-distal×lateral-central-medial=9 sites) (Figure 1). This pressure was hard enough to induce an uncomfortable feeling in the sites but did not necessarily induce pain sensation at pre. One measurement was performed for each site at each time point. An order of the measurement sites was randomly chosen by the examiner. Absolute values (a.u.) were used in between-time

FIGURE 1 Images for the measurement sites of the MRIs and muscle soreness. Examples of $T_2$ maps superimposed on $T_2$-MRIs at the proximal, middle, and distal sites scanned before (pre) and 72 hours after the single-joint eccentric contractions of the knee extensors for one subject and the algesiometer are shown. MRI, magnetic resonance imaging; RF, rectus femoris; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis
comparisons for each site and absolute change values from pre (Δsoreness: a.u.) were used in between-site comparisons for each exercise (detailed in the Statistical analysis section).

2.6 Statistical analysis

Descriptive data are presented as means±SDs. All data were analyzed using the SPSS software (version 23.0; IBM Corp, Armonk, New York, USA). Changes in maximal torque were compared by a one-way (four time points) repeated measures analysis of variance (ANOVA) followed by a Dunnett’s post hoc test for each exercise. Changes in $T_2$ and soreness were compared by a two-way (4 time points×12 sites for $T_2$ and 9 sites for soreness) ANOVA for each exercise. When a significant time point-site interaction effect was found, a Dunnett’s post hoc test was performed to compare changes from pre for each site for each exercise. In addition, as timing of $T_2$ and soreness manifestations could vary among sites and individuals, peak values for $T_2$ (peak Δ$T_2$) and soreness (peak Δsoreness) were calculated by subtracting the pre-exercise value from the peak value post-exercise for each site, regardless of at which time point the peak value occurred, and compared among sites by a one-way ANOVA (12 sites for $T_2$ and 9 sites for soreness) followed by a Tukey post hoc test for each exercise. Dunnett’s and Tukey’s tests both control the type 1 error rate.30 Dunnett’s test was used for comparisons between time points, because this test is the only multiple comparison that allows for testing means against a control mean (ie, each time point post-exercise vs pre).30 Tukey’s test was used for comparisons between sites (12 sites for $T_2$ and 9 sites for soreness) based on that this test has more statistical power than Bonferroni when the number of comparisons is large (eg, >5).30 As indices of effect size, Cohen’s $d$ (for post hoc comparisons) and partial $\eta^2$ (for ANOVA) were also calculated. Sphericity was checked by Mauchly’s test in ANOVA and $P$ values were modified with Greenhouse-Geisser correction when necessary. Statistical significance was set at $P<.05$.

3 RESULTS

3.1 Eccentric exercises

The load used for each exercise was 55.4±9.5 kg, 101.0±17.4 kg, and 13.8±2.6 kg for the KE, S, and DW, respectively. All subjects completed the prescribed protocols and pre- and post-exercise measurements.

3.2 Maximal isometric knee extension torque

A one-way ANOVA found a significant main effect of time in the maximal knee extension torque for each of the KE ($P=.007$, partial $\eta^2=.327$), S ($P=.013$, partial $\eta^2=.297$), and DW ($P=.038$, partial $\eta^2=.241$) (Figure 2). Dunnett’s post hoc tests showed that the knee extension torque significantly decreased from baseline at 24 hours (−15.4%, $P=.003$, $d=1.09$) and 48 hours (−11.6%, $P=.027$, $d=0.72$) after the KE, at 24 hours (−12.7%, $P=.006$, $d=0.56$) after the S, and at 24 hours (−7.0%, $P=.013$, $d=0.29$) after the DW (Figure 2).
Partial $\eta^2 = 0.177$, $S$ ($P = 0.042$, partial $\eta^2 = 0.143$) and DW ($P = 0.039$, partial $\eta^2 = 0.155$) (Table 1). Dunnett’s post hoc tests showed that KE caused significant ($P < 0.05$) $T_2$ increases at the proximal ($d = 0.98 - 1.09$), middle ($d = 0.98 - 1.00$), and distal ($d = 0.85 - 1.06$) RF at 48 and 72 hours, at the middle VI at 48 hours ($d = 0.6$), and at the proximal ($d = 0.77 - 0.8$) and middle ($d = 0.72 - 0.75$) VM at 24, 48, and 72 hours. S increased $T_2$ at the middle VM at 24 and 48 hours ($d = 0.69 - 0.75$). After DW, significant $T_2$ increases were found at the proximal RF at 72 hours ($d = 0.61$) and at the middle VM at 48 hours ($d = 0.92$). Among the sites, Cohen’s $d$ was the highest at the proximal RF after KE and at the middle VM after S and DW (Table 1). Figure 3 shows the peak $\Delta T_2$ for each site for each exercise. A one-way ANOVA followed by a Tukey post hoc test showed that the peak $\Delta T_2$ at the proximal RF after KE was significantly higher than that of the proximal, middle, and distal VL ($P < 0.05$, Cohen’s $d = 0.93 - 1.04$). No significant differences were found among the sites in the peak $\Delta T_2$ after S or DW.

### 3.4 Muscle soreness

A two-way ANOVA found a significant time point-site interaction effect in soreness changes for each of the KE ($P < 0.001$, partial $\eta^2 = 0.236$), S ($P < 0.001$, partial $\eta^2 = 0.201$), and DW ($P = 0.001$, partial $\eta^2 = 0.182$) (Table 2). Dunnett’s post hoc tests showed that soreness developed at all sites at least at some point within 72 hours after all exercises ($P < 0.05$, $d = 0.71 - 2.65$). Among the sites, Cohen’s $d$ was the highest at the middle RF after KE and at the middle VM after S and DW (Table 2). Peak $\Delta$ soreness values for each site for each exercise are shown in Figure 4. A one-way ANOVA followed by a Tukey post hoc test showed that peak $\Delta$ soreness at the central proximal ($d = 0.79 - 1.26$) and central middle ($d = 0.84 - 1.23$) sites after KE were significantly ($P < 0.05$) higher than those at the lateral proximal, lateral distal, central distal, and medial distal sites. Peak $\Delta$ soreness after S was higher at the medial middle site than the lateral proximal, central proximal, central middle, and medial distal sites ($P < 0.05$, $d = 0.87 - 1.57$). After DW, peak $\Delta$ soreness was higher at the medial middle site than the lateral proximal, lateral distal, central proximal, central distal, and medial distal sites ($P < 0.05$, $d = 0.96 - 1.19$).

### 4 DISCUSSION

The main findings of the present study were that after the eccentric exercises, (a) $T_2$ increases were heterogeneous not only among but also along the QF muscles, even within the vasti, and (b) the most affected sites varied among the exercises. Furthermore, it was also revealed that all exercises did not induce a significant $T_2$ increase at any sites of the VL, suggesting that the VL is least susceptible among the QF.

Maximal knee extension torque significantly decreased after all exercises (Figure 2). $T_2$ significantly increased at least at some point after all exercises (Table 1), but its change was heterogeneous within the QF and most affected sites varied among exercises. These results indicate that some damage was induced in the QF by each exercise, but the most affected site was different among exercises. Changes in soreness (Table 2) at least in part supported the changes in $T_2$. It should be noted that soreness is a subjective scale by nature and influenced by interindividual and possibly by intermuscle/site differences in several factors such as inherent pain sensitivity, innervation density of nociceptors, and thickness of muscle and subcutaneous tissues. It is also worth noting that the VI, the underlying muscle beneath the other superficial ones, could have influenced the results. These factors to some extent would account for the difference in the results between $T_2$ and soreness. However, the present finding supports the usefulness of $T_2$-MRI to differentiate the damage severity within a muscle.

After the KE, significant $T_2$ increases were observed at least at one site of all muscles except for the VL (Table 1). Among the $T_2$-increased sites after the KE, an effect size analysis suggested that muscle damage was prominent at the proximal RF (Table 1). Moreover, peak $T_2$ change was greater at the proximal RF than at several other sites (Figure 3). This is further collaborated by the fact that peak soreness was greater at the central proximal and central middle sites than at all sites of the VL (Figure 4), although we cannot ignore the influence of the VI as mentioned. This supports the findings of Prior et al. and Black and McCully, in which a $T_2$ change was greater in the RF than in the vasti or VL after an KE exercise, while in their study, only the middle site (or average of several slices) along the thigh was examined. Taken together, these results indicate that the RF, especially at the proximal site, was most affected by the KE. The observed intermuscle differences would be mainly attributed to the difference between the bi- and mono-articular nature of the RF and the other vasti muscles, respectively. Namely, when the knee joint is flexed, the vasti muscles are always lengthened, while the length of the RF also depends on the hip joint angle. This would expose the RF to less eccentric stress in a daily activity, thus making it more responsive when isolated KE is performed thereby subjected to high eccentric stress. This theory could partly explain why QF muscle strain and tendon injuries in sports, which is often caused during eccentric muscle action (e.g., rapid change of direction), most frequently occur in the RF.

After the S, $T_2$ increased at the middle VM only, with significantly greater peak soreness at this site than several other sites. This indicates that EIMD was heterogeneous even within the vasti muscles (discussed later). First, the reason why $T_2$ did not increase in the RF could be explained again by its bi-articular nature. During the lowering phase
| TABLE 1 | $T_2$ values (ms) measured before and 24, 48, and 72 h after the tasks |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Pre  | 24 h  | 48 h  | 72 h  | Peak $d$ |
| KE              |      |      |      |      |          |
| RF              |      |      |      |      |          |
| Proximal        | 24.5±1.6 | 25.8±1.3 | 26.3±1.7 | 26.8±2.9 | 1.09b     |
| Middle          | 25.1±1.4 | 25.9±0.8 | 26.5±1.4 | 26.9±2.2 | 1.00      |
| Distal          | 25.0±1.5 | 25.7±0.9 | 26.5±2.0 | 26.7±1.7 | 1.06      |
| VI              |      |      |      |      |          |
| Proximal        | 30.3±2.6 | 31.3±2.2 | 31.0±1.8 | 30.8±1.9 | 0.42      |
| Middle          | 31.5±1.6 | 32.2±1.5 | 32.4±1.4 | 32.1±1.3 | 0.60      |
| Distal          | 30.5±1.4 | 31.3±1.1 | 31.2±1.0 | 31.2±1.5 | 0.64      |
| VL              |      |      |      |      |          |
| Proximal        | 29.6±2.4 | 30.1±1.8 | 29.3±2.5 | 30.0±1.7 | 0.24      |
| Middle          | 30.3±2.0 | 30.1±1.1 | 30.4±1.1 | 30.5±1.4 | 0.12      |
| Distal          | 29.4±2.0 | 29.0±1.2 | 29.5±1.4 | 29.7±1.5 | 0.17      |
| VM              |      |      |      |      |          |
| Proximal        | 29.5±1.4 | 30.7±1.6 | 30.7±1.7 | 30.7±1.6 | 0.80      |
| Middle          | 30.5±1.4 | 31.5±1.4 | 31.7±1.8 | 31.4±1.0 | 0.75      |
| Distal          | 28.3±1.4 | 29.4±1.3 | 29.2±2.1 | 29.5±1.8 | 0.82      |
| S               |      |      |      |      |          |
| RF              |      |      |      |      |          |
| Proximal        | 24.1±1.0 | 24.1±1.1 | 23.9±0.7 | 24.1±0.8 | 0.01      |
| Middle          | 24.5±0.9 | 24.2±1.0 | 24.9±1.1 | 24.8±1.2 | 0.28      |
| Distal          | 24.8±1.4 | 24.6±0.9 | 24.4±0.9 | 24.8±0.9 | 0.01      |
| VI              |      |      |      |      |          |
| Proximal        | 29.4±1.4 | 30.1±1.4 | 29.9±1.4 | 30.0±1.5 | 0.50      |
| Middle          | 31.0±1.6 | 31.9±1.4 | 31.3±1.3 | 31.4±1.2 | 0.28      |
| Distal          | 31.5±0.9 | 31.2±0.8 | 31.8±2.9 | 31.0±1.3 | 0.45      |
| VL              |      |      |      |      |          |
| Proximal        | 29.0±1.5 | 29.9±1.4 | 29.9±1.4 | 29.9±1.8 | 0.62      |
| Middle          | 30.2±1.2 | 30.5±0.8 | 30.6±1.0 | 30.5±1.0 | 0.36      |
| Distal          | 29.1±0.8 | 29.6±0.7 | 30.0±1.8 | 29.7±1.1 | 0.67      |
| VM              |      |      |      |      |          |
| Proximal        | 29.7±1.2 | 30.6±1.3 | 30.4±1.1 | 30.2±1.2 | 0.72      |
| Middle          | 30.2±1.3 | 31.0±1.0 | 31.1±1.1 | 30.5±1.0 | 0.75b     |
| Distal          | 29.5±1.6 | 30.0±1.3 | 31.0±2.4 | 30.6±1.6 | 0.74      |
| DW              |      |      |      |      |          |
| RF              |      |      |      |      |          |
| Proximal        | 23.7±1.4 | 23.8±1.2 | 23.9±1.2 | 24.5±1.2 | 0.61      |
| Middle          | 24.5±1.5 | 24.3±1.6 | 24.7±1.3 | 24.8±1.2 | 0.22      |
| Distal          | 24.6±1.7 | 24.5±1.3 | 24.8±1.7 | 25.0±1.0 | 0.29      |
| VI              |      |      |      |      |          |
| Proximal        | 29.6±1.4 | 29.8±0.9 | 29.5±1.0 | 29.5±1.2 | 0.17      |
| Middle          | 30.3±0.9 | 30.5±1.3 | 30.6±1.1 | 30.7±0.9 | 0.45      |
| Distal          | 30.0±1.1 | 29.9±0.8 | 30.1±1.2 | 30.0±0.9 | 0.09      |

(Continues)
that showed significant $T_2$ increases in the RF, VI, and VM but not in the VL. Then, why was $T_2$ increased at the middle but not in the RF. It is possible that the middle VM is the most susceptible site within the vasti to eccentric stress, considering the fact that the middle VM was the only site that showed $T_2$ increase after all three exercises (Table 1). This may be related to the anatomical characteristics of the VM, being smaller and having to compensate for the strong lateral pull of the large VL to stabilize the patella during knee extension. Supporting this is the fact that the VL did not show any significant $T_2$ increase after all exercises, suggesting that the VL is the least susceptible to eccentric stress. This finding is consistent with that of Black and McCully who also showed significant $T_2$ increases in the RF, VI, and VM but not in the VL after single-joint eccentric contractions of the knee extensors. Using electromyogram (EMG), Hedayatpour and Falla showed that single-joint eccentric contractions of the knee extensors significantly altered EMG onset and amplitude of the VM and VL (no measurement on the RF and VI) in response to rapid destabilizing knee perturbation, with greater changes in the VM than the VL. This is in line with the current result in the sense that the VM was more affected than the VL by the eccentric exercise. Based on these, it is likely that the degree of damage would differ even within the vasti muscles, and the VM and VL would be the most and least, respectively, susceptible muscle among them. Further research is needed to clarify what factor(s) influences the heterogeneity within the vasti muscles.

The DW induced $T_2$ increases at the proximal RF and middle VM. This is a somewhat complicating result, but could be attributed to the above-mentioned theory. As mentioned earlier, because the RF is governed by the two motor nerves that reach the proximal and middle-distal sites, its activation level/pattern differs along the muscle. More specifically, the proximal site would activate more than the middle and/or the distal sites during a hip flexion. It is reported that during DW in a similar slope condition, the hip joint is kept at a relatively extended position (<20°) at the braking (eccentric) phase as well as throughout the execution of the exercise. Also, in the latter half of the braking phase of DW, the hip joint angle slightly decreases (from ~20° to ~0°) as the trunk and leg moves upward and downward, respectively, which can be considered a situation where the proximal RF undergoes an active lengthening (ie, eccentric contraction of the hip flexors). Although this theory is purely speculative in the absence of any data on the behavior of the RF during the exercise, its unique activation together with the potential vulnerability of the RF and VM (discussed above) could have resulted in the significant $T_2$ increase at the proximal RF and middle VM, after the DW. A similar explanation could account for the somewhat higher $T_2$ increase at the proximal than the middle and distal RF after the KE. In the KE, the hip joint was fixed at ~80°. However, even if a joint is fixed during a torque exertion, some biological compliance is inevitable due to compression of soft tissue, leading to small unavoidable changes in joint angle especially during a near-maximal contraction. In the KE, a relatively heavy load (90% 1RM) was applied to the leg. This may have caused a small change in the hip joint angle toward a more extended position by both the leg-lowering and compensating trunk-raising movements, subjecting the proximal RF to more eccentric stress than the other sites of the RF. Biomechanical evidence in conjunction with a $T_2$ change is needed to confirm these speculations.

As mentioned, a $T_2$ increase is a delayed response and could result in different timing in peak $T_2$ changes among the sites. Therefore, in addition to the comparisons of the time-course changes for each site (Table 1), we also compared

|       | Pre   | 24 h | 48 h   | 72 h   | Peak $d$ |
|-------|-------|------|--------|--------|---------|
| **RF** |       |      |        |        |         |
| Proximal | 28.7±1.2 | 29.2±1.2 | 28.8±1.2 | 28.6±1.4 | 0.42    |
| Middle  | 29.4±1.0 | 29.7±1.3 | 29.8±1.2 | 29.6±0.9 | 0.36    |
| Distal  | 28.5±0.9 | 28.5±1.0 | 28.7±0.9 | 28.5±0.5 | 0.22    |
| **VI**  |       |      |        |        |         |
| Proximal | 29.1±1.3 | 29.6±1.3 | 29.4±1.7 | 29.5±1.1 | 0.39    |
| Middle  | 30.2±1.5 | 30.3±1.3 | 31.3±0.8 | 30.6±1.1 | 0.92b   |
| Distal  | 28.8±1.5 | 28.8±1.2 | 29.3±1.3 | 29.5±1.3 | 0.36    |
| **VM**  |       |      |        |        |         |
| Proximal | 28.8±1.5 | 28.8±1.2 | 29.3±1.3 | 29.5±1.3 | 0.36    |

KE, single-joint eccentric contraction of the knee extensors; S, eccentric squat; DW, downhill walking; RF, rectus femoris; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

Significantly different from pre ($P<.05$).

The highest peak Cohen’s $d$ among the 12 sites after each exercise.
the peak change values among the sites (Figure 3). While significant differences among the sites were found after the KE, no such significant differences were found after the S and DW. This may be due to large interindividual differences in load sharing strategies\textsuperscript{25} and/or responses to eccentric exercises,\textsuperscript{26} as well as relatively lower $T_2$ increases (~3%) after the S and DW compared to the KE (3%-9%). In fact, these values are lower (for S and DW) or similar (for KE) compared to $T_2$ increases (5%-11%) observed in previous studies that performed eccentric exercises of the QF.\textsuperscript{8,24,37} In addition, maximal torque reductions in this study (eg, at 24 hours post-exercise: 15%, 13%, and 7% for the KE, S, and DW, respectively) are somewhat lower compared to those of other studies (>20%)\textsuperscript{8,9,38} that used the same or a similar exercise model. This may be attributed to the differences in a volume and/or degree of eccentric component of the exercise. For example, the previous studies used 10 repetitions×10-15 sets for single-joint eccentric contractions of the knee extensors\textsuperscript{38} or squat\textsuperscript{8} (vs 10×5 in this study), and a slope of −28% for downhill walking\textsuperscript{9} (vs −10%). In this sense, the current results may not necessarily be generalizable to such protocols. Indeed, it is possible that $T_2$ would increase in the VL as well after such more strenuous exercises. Nevertheless, in that case, it is logical to assume that more pronounced damage would also be found in other sites of the muscles (eg, proximal RF or middle VM) depending on the exercises. Therefore, we consider that this study could shed light on identifying the site most prone to damage by each exercise. Further research is warranted to clarify the effects of intensity and/or volume of various types of eccentric exercises on localization of muscle damage.

In summary, this study revealed that the single-joint eccentric contractions of the knee extensors caused $T_2$ increases in the overall QF except for the VL and especially at the proximal RF. The eccentric squat and downhill walking most affected the middle VM, based on $T_2$ and soreness changes. These results suggest that which site within the QF is most damaged by eccentric exercise varies among exercises. Although it is common to take biopsies\textsuperscript{39} or measure EMGs\textsuperscript{38,40} solely from the VL as the representative of the QF, our results suggest that the VL may not necessarily represent the whole QF or reflect its synergists, at least regarding damage severity. In this sense, it may be better to put particular focus on the proximal RF when implementing knee extension, and on the middle VM when conducting squat and downhill walking, to evaluate damage severity more precisely.

**PERSPECTIVES**

The present findings, together with previous reports, strongly indicate that the RF is more damaged by single-joint knee extension but less damaged by squat compared to the vasti. Considering that eccentric muscle action-induced strain injuries during sports often occur in the RF, single-joint knee extension exercise training (preferably with eccentric overload) should be effective in reducing risks of such injuries. Localization of muscle damage induced by downhill walking seems more complex than that of the other two exercises. Examination of fascicle behavior during exercise may provide information underpinning the heterogeneity of muscle damage along the RF and/or within the vasti, which requires further research. Recent studies\textsuperscript{15,16} have shown that shearwave elastography is also a relevant tool for the localization of muscle damage including non-superficial areas of muscles. Regardless of the devices used, evidence well supports
the existence of heterogeneity of muscle damage. However, it may also depend on a muscle group tested, because homogeneous muscle damage was reported in the elbow flexors. More research is needed to clarify what exercise most affects which site of the muscles in various muscle groups to better understand the muscle damage manifestations.

|      | Pre  | 24 h | 48 h | 72 h | Peak d |
|------|------|------|------|------|--------|
| KE   |      |      |      |      |        |
| Central |      |      |      |      |        |
| Proximal | 0.5±0.5 | 4.0±1.9<sup>a</sup> | 4.2±2.6<sup>a</sup> | 3.7±2.0<sup>a</sup> | 2.52    |
| Middle | 0.5±0.5 | 4.5±2.2<sup>a</sup> | 5.1±2.4<sup>a</sup> | 4.1±2.7<sup>a</sup> | 2.65<sup>b</sup> |
| Distal | 0.8±1.1 | 2.7±2.5<sup>a</sup> | 2.9±3.2<sup>a</sup> | 2.2±2.4 | 0.98    |
| Lateral |      |      |      |      |        |
| Proximal | 0.4±0.7 | 2.2±1.7<sup>a</sup> | 2.4±1.4<sup>a</sup> | 1.6±1.4<sup>a</sup> | 1.81    |
| Middle | 0.5±0.5 | 3.5±1.9<sup>a</sup> | 3.3±1.8<sup>a</sup> | 2.2±1.8<sup>a</sup> | 2.16    |
| Distal | 0.8±1.1 | 2.9±1.2<sup>a</sup> | 2.3±1.8<sup>a</sup> | 2.0±1.5 | 1.83    |
| Medial |      |      |      |      |        |
| Proximal | 0.9±0.8 | 3.1±1.1<sup>a</sup> | 2.9±1.8<sup>a</sup> | 2.7±0.6<sup>a</sup> | 2.55    |
| Middle | 1.2±0.9 | 3.5±1.9<sup>a</sup> | 4.1±2.0<sup>a</sup> | 3.2±2.2<sup>a</sup> | 1.87    |
| Distal | 0.5±0.7 | 2.3±1.9<sup>a</sup> | 2.1±1.1<sup>a</sup> | 1.1±1.0 | 2.23    |

|      |      |      |      |      |        |
|------|------|------|------|------|--------|
| S    |      |      |      |      |        |
| Central |      |      |      |      |        |
| Proximal | 0.6±0.9 | 2.0±2.1<sup>a</sup> | 2.5±1.8<sup>a</sup> | 1.4±1.5 | 1.34    |
| Middle | 0.5±1.2 | 2.7±2.1<sup>a</sup> | 2.6±1.7<sup>a</sup> | 1.5±1.6 | 1.43    |
| Distal | 0.4±0.9 | 3.5±2.4<sup>a</sup> | 2.9±1.7<sup>a</sup> | 1.7±1.5<sup>a</sup> | 1.84    |
| Lateral |      |      |      |      |        |
| Proximal | 0.4±0.7 | 2.8±2.1<sup>a</sup> | 3.2±2.1<sup>a</sup> | 2.3±1.6<sup>a</sup> | 1.79    |
| Middle | 0.6±0.8 | 4.0±2.0<sup>a</sup> | 3.5±1.6<sup>a</sup> | 2.8±1.3<sup>a</sup> | 2.29    |
| Distal | 0.6±0.9 | 3.4±2.1<sup>a</sup> | 3.9±2.5<sup>a</sup> | 2.6±1.7<sup>a</sup> | 1.76    |
| Medial |      |      |      |      |        |
| Proximal | 0.8±1.1 | 3.8±2.6<sup>a</sup> | 3.5±1.8<sup>a</sup> | 2.1±1.4<sup>a</sup> | 1.81    |
| Middle | 0.8±1.1 | 5.5±2.6<sup>a</sup> | 4.7±2.1<sup>a</sup> | 3.8±2.0<sup>a</sup> | 2.36<sup>b</sup> |
| Distal | 0.4±0.7 | 3.5±2.4<sup>a</sup> | 2.5±2.3<sup>a</sup> | 1.8±1.3<sup>a</sup> | 1.75    |
| DW   |      |      |      |      |        |
| Central |      |      |      |      |        |
| Proximal | 0.5±0.9 | 1.7±2.1<sup>a</sup> | 1.4±1.2<sup>a</sup> | 0.6±0.8 | 0.85    |
| Middle | 0.5±0.7 | 2.1±1.3<sup>a</sup> | 1.9±1.4<sup>a</sup> | 1.1±0.8 | 1.53    |
| Distal | 0.3±0.5 | 1.3±1.3<sup>a</sup> | 1.1±1.2<sup>a</sup> | 1.1±1.1<sup>a</sup> | 1.02    |
| Lateral |      |      |      |      |        |
| Proximal | 0.4±0.5 | 1.3±1.2<sup>a</sup> | 1.1±1.4 | 0.9±1.0 | 0.98    |
| Middle | 0.4±0.5 | 1.5±1.3<sup>a</sup> | 1.5±1.4<sup>a</sup> | 0.9±1.1 | 1.12    |
| Distal | 0.7±0.8 | 1.6±1.6<sup>a</sup> | 1.3±1.0 | 1.1±1.2 | 0.71    |
| Medial |      |      |      |      |        |
| Proximal | 0.8±1.3 | 2.2±2.1<sup>a</sup> | 2.3±1.7<sup>a</sup> | 1.7±1.3<sup>a</sup> | 0.91    |
| Middle | 0.5±0.7 | 3.1±1.3<sup>a</sup> | 2.5±1.6<sup>a</sup> | 1.8±1.1<sup>a</sup> | 2.49<sup>b</sup> |
| Distal | 0.5±0.7 | 1.6±1.4<sup>a</sup> | 0.8±1.2 | 0.9±1.0 | 0.99    |

KE, single-joint eccentric contraction of the knee extensors; S, eccentric squat; DW, downhill walking; RF, rectus femoris; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

<sup>a</sup>Significantly different from pre (<i>P</i> < 0.05).

<sup>b</sup>The highest peak Cohen’s <i>d</i> among the 9 sites after each exercise.
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22. LeBlanc AD, Jaweed M, Evans H. Evaluation of muscle injury using magnetic resonance imaging. *Clin J Sport Med*. 1993;3:26-30.

23. Foure A, Le Troter A, Guye M, Mattei JP, Bendahan D, Gondin J. Localization and quantification of intramuscular damage using statistical parametric mapping and skeletal muscle parcellation. *Sci Rep*. 2015;5:18580.

24. Black CD, McCully KK. Muscle injury after repeated bouts of voluntary and electrically stimulated exercise. *Med Sci Sports Exerc*. 2008;40:1605-1615.

25. Bouillard K, Hug F, Guevel A, Nordez A. Shear elastic modulus can be used to estimate an index of individual muscle force during a submaximal isometric fatiguing contraction. *J Appl Physiol*. 2012;113:1353-1361.

26. Nosaka K, Newton M, Sacco P. Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scand J Med Sci Sports*. 2002;12:337-346.

27. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med*. 1999;27:43-59.

28. Farr T, Nottle C, Nosaka K, Sacco P. The effects of therapeutic massage on delayed onset muscle soreness and muscle function following downhill walking. *J Sci Med Sport*. 2002;5:297-306.

29. Smith LL, McKune AJ, Semple SJ, Sibanda E, Steel H, Anderson R. Changes in serum cytokines after repeated bouts of downhill running. *Appl Physiol Nutr Metab*. 2007;32:233-240.

30. Field A. *Discovering Statistics Using IBM SPSS Statistics*. Thousand Oaks, California: Sage Publications Ltd; 2013:952.

31. Cross TM, Gibbs N, Houang MT, Cameron M. Acute quadriceps muscle strains: magnetic resonance imaging features and prognosis. *Am J Sports Med*. 2004;32:710-719.

32. Mendiguchia J, Alentorn-Geli E, Idoate F, Myer GD. Rectus femoris muscle injuries in football: a clinically relevant review of mechanisms of injury, risk factors and preventive strategies. *Br J Sports Med*. 2013;47:359-366.

33. Guilhem G, Doguet V, Hauraux H, et al. Muscle force loss and soreness subsequent to maximal eccentric contractions depend on the amount of fascicle strain in vivo. *Acta Physiol (Oxf)*. 2016;217:152-163.

34. Hedayatpour N, Falla D. Delayed onset of vastus muscle activity in response to rapid postural perturbations following eccentric exercise: a mechanism that underpins knee pain after eccentric exercise? *Br J Sports Med*. 2014;48:429-434.

35. Lay AN, Hass CJ, Gregor RJ. The effects of sloped surfaces on locomotion: a kinematic and kinetic analysis. *J Biomech*. 2006;39:1621-1628.

36. Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force development: physiological and methodological considerations. *Eur J Appl Physiol*. 2016;116:1091-1116.

37. Takahashi H, Kuno S, Miyamoto T, et al. Changes in magnetic resonance images in human skeletal muscle after eccentric exercise. *Eur J Appl Physiol Occup Physiol*. 1994;69:408-413.

38. Farup J, Rahbek SK, Bjerre J, de Paoli F, Vising K. Associated decrements in rate of force development and neural drive after maximal eccentric exercise. *Scand J Med Sci Sports*. 2016;26:498-506.

39. Dubowitz V, Sewry CA, Oldfors A. *Muscle Biopsy: A Practical Approach*, 4th edn. Amsterdam, Netherlands: Elsevier Health Sciences; 2013:592.

40. Giandolini M, Horvais N, Rossi J, Millet GY, Morin JB, Samozino P. Acute and delayed peripheral and central neuromuscular alterations induced by a short and intense downhill trail run. *Scand J Med Sci Sports*. 2016;26:1321-1333.

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