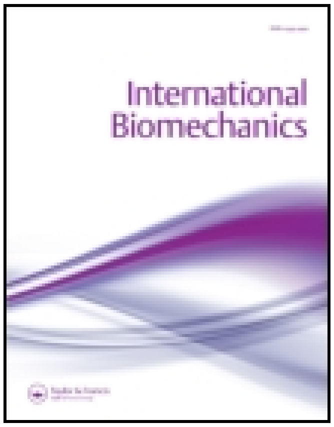




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# A longitudinal study of muscle rehabilitation in the lower leg after cast removal using magnetic resonance imaging and strength assessment

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Magnetic resonance imaging (MRI) was used to investigate muscle rehabilitation following cast immobilization. The aim was to explore MRI as an imaging biomarker of muscle function. Sixteen patients completed an eight-week rehabilitation programme following six weeks of cast immobilization for an ankle fracture. MRI of the lower leg was performed at two-week intervals for 14 weeks. Total volume and anatomical cross-sectional areas at 70% of the distance from lateral malleolus to tibial tuberosity (ACSA) were measured for tibialis anterior (TA), medial and lateral gastrocnemius (GM and GL) and soleus (SOL). Pennation angle of muscle fascicules was measured at the same position in GM. Fractional fat/water contents and $T_2$ relaxation times before and after exercise were calculated. Strength was measured as maximum isometric torque developed in plantar- and dorsi-flexion. Torque increased by (mean [SD]) 1.10 (0.32) N m day$^{-1}$ in males, 0.74 (0.43) N m day$^{-1}$ in females in plantar-flexion (0.9% of final strength per day), and 0.36 (0.15) N m day$^{-1}$ in males, 0.28 (0.19) N m day$^{-1}$ in females in dorsi-flexion (1.1% per day). Neither difference between males and females was significant. Volume and ACSA of muscles recovered by week 14 apart from SOL which was still 6.8% smaller ($p = 0.006$) than the contralateral leg. $T_2$ peaked at the end of the cast period for TA and SOL, and at week 8 for GM before returning to baseline. Pennation angle recovered rapidly following cast removal. Quantitative MRI can generate markers of muscle biomechanics and indicates that many of these return to baseline within eight weeks of remobilization.

**Keywords:** muscles; magnetic resonance imaging; injury biomechanics; rehabilitation; biomarkers; exercise

## Introduction

Lower leg immobilization with a cast is a standard of care after fracture of the ankle. Inactivity or disuse as a result of immobilization causes numerous adaptive changes in the skeletal muscle, such as a decrease in muscle protein turnover (Gibson et al. 1987), alterations in muscle architecture (Blazevich & Sharp 2005), changes in expression of genes associated with the regulation of muscle mass (Jones et al. 2004), with the ubiquitin–proteasome and Akt pathways (Urso et al. 2006), and reductions in mitochondrial capacity (Gram et al. 2014), with reduced muscular function as a final outcome. This was recently reviewed by Bostock et al. (2013). Loss of muscle mass (muscular atrophy) is a recognized consequence of muscle disuse, with loss of muscle strength being pronounced (Berg et al. 1991, 1997; Hather et al. 1992; Greenhaff 2006).

Few longitudinal studies have been conducted to study the reversibility of the muscular changes induced by immobilization in humans (Vandenborne et al. 1998; Stevens et al. 2004; Grosset & Onambele-Pearson 2008). Some of the more detailed studies are limited to case reports on individuals (Vandenborne et al. 1998; Grosset & Onambele-Pearson 2008). Others used larger cohorts of patients but with limited time points (Stevens et al. 2004) or shorter periods of immobilization (Wall et al. 2014). Muscle activation was shown to increase rapidly, while muscle hypertrophy followed more slowly in nine young subjects using magnetic resonance imaging (MRI) to measure muscle cross-sectional area immediately following cast removal for ankle fracture and again five and 10 weeks later (Stevens et al. 2006). If MR imaging is to serve as a biomarker, it is important to know more about possible differences in response to immobilization

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following injury between various lower leg muscles and the effectiveness of rehabilitation at more closely spaced time points. In addition, the effects on recovery of patient age and sex remain unclear.

MRI can delineate soft tissues and measure chemical and structural changes non-invasively and so has great potential for the development of imaging biomarkers of physiological processes or pathology. The ratio of fat to water, as measured by the Dixon MR technique, is an important indicator of muscle quality since a muscle with a high proportion of fat has less contractile tissue (and thus lower ability to generate force) than an equally sized muscle with a lower proportion of fat. $T_2$ values are also an indicator of muscle quality since they relate to various aspects of the muscle such as fibre type (Kuno et al. 1990), water content and fat content. Muscle atrophy, involving atrophy of type 2 fibres, in particular, is associated with lengthening $T_2$ relaxation times (Hatakenaka et al. 2001). Inactivity has been found to be associated with decreases in strength and increases in fat in young (Manini et al. 2007) and older people (Marcus et al. 2010), with exercise training reducing the quantity of intramuscular fat (Marcus et al. 2010). In another study, however, five-day limb immobilization resulted in no measurable changes in muscle fat (Wall et al. 2015).

We have previously reported the utility of MRI as an imaging biomarker to measure changes in muscle properties during a prolonged period of lower leg immobilization in patients after ankle injury (Psatha et al. 2012). The purpose of this study was to monitor the changes in those same muscles during a rehabilitation programme that immediately followed immobilization and to determine the effects of that programme on muscle function. Using MRI, we studied the plantar flexors and extensors of the same patients at two-weekly intervals during eight weeks of rehabilitation following cast removal. In addition, muscle strength gains were assessed during the rehabilitation period using maximal voluntary isometric contractions. Our aim was to investigate the utility of MRI as an imaging biomarker of changes in muscle structures and function, and to generate MRI reference data for the kinetics of the atrophy and recovery of major lower limb muscles during and after limb immobilization.

## Methods

### *Participants*

Eighteen patients (eight males, 10 females) (aged over 18) had one lower leg immobilized with a below knee cast following an ankle fracture not requiring manipulation or fixation (13 right, five left). Initially identified when they attended the Accident and Emergency Department, they were given an information sheet and invited to take part in the study. Further contact was made after 24 h and, if suitable on pre-screening and willing to take part, patients were invited to return to the clinic where the study was explained in more detail. Participants then gave written informed consent to the study, as approved by the North of Scotland Research Ethics Service (reference number 08/S0801/101) and NHS Research and Development. All then underwent a full screening process and had their first MR scan. Patients were excluded if, within the previous two years, they reported taking medication that might affect muscle function, had diabetes, uncontrolled thyroid disease, known contraindication to MR scanning, body mass index over 35, moderate to severe cardiac illness or thrombophlebitis. In addition, eligible patients were also excluded if they lived too far from the hospital and could not easily attend for the MRI examinations and rehabilitation sessions. All participants gave written informed consent, as approved by the North of Scotland Research Ethics Service (reference number 08/S0801/101).

### *Study design*

Cast application defined study day 0 and cast removal occurred after six weeks (study day 43). Subsequently, participants undertook an eight-week period of strength training with strength assessment every two weeks. Subjects were invited for MRI examinations (Philips Achieva 3.0 T) on study days 3, 5, 8, 15, week 4 (day 29), week 6 (day 43, cast removed), week 8 (day 57), week 10 (day 71), week 12 (day 85) and week 14 (day 99). Because of patient or MRI machine availability, the actual scan days did not always coincide with the study days and not every patient had every scan. Full details of the cast phase were reported previously (Psatha et al. 2012) and here we describe the recovery phase following cast removal. Some cast data are included for comparison and to enable generation of an estimate of pre-injury values for image-derived parameters.

### *MRI protocols*

Imaging was done using a Philips Achieva 3.0-T whole-body MRI scanner using a Philips 16 channel SENSE XL Torso coil enclosing the lower legs, which were imaged simultaneously. Patients lay supine with their feet going in first. The knees were extended and relaxed with the feet and legs strapped into a custom-made Perspex frame using Velcro straps. The angle of the ankle for both legs was defined originally by the cast leg. Scan positions were defined with respect to the tibial tuberosity and the inferior tip of lateral malleolus using cod liver oil softgel capsules as described previously (Psatha et al. 2012). The distance between these landmarks was calculated and a marker was placed at 70% of this distance craniad from the malleolus, corresponding to the bulkiest part of the calf muscles. This marker was used for reproducible slice positioning during image acquisition and subsequent analysis. A high-resolution 3D $T_1$-weighted gradient-echo image was used as the anatomical reference for all subsequent scan sequences. Details of the imaging protocols have been described fully elsewhere (Psatha et al. 2012) and are described here briefly under each measurement. Quality assurance for MRI was performed every month using two phantoms to ensure that the required properties of the scanned objects were properly measured.

### Image analysis

Image analysis tools were developed using MATLAB (The Mathworks Inc., Natick, MA, USA) and Java to measure cross-sectional areas, pennation angles, water/fat fraction and $T_2$ relaxation times (Psatha et al. 2012). Regions of interest (ROI) were defined in each muscle by segmenting the image around the muscle boundaries. All measurements were made in the same way on both the cast leg and the contralateral leg.

To measure muscle volume and anatomical cross-sectional area (ACSA), $T_1$-weighted spin-echo images (repetition time, TR = 568 ms, echo time, TE = 20 ms, acquisition matrix 508 × 362) were acquired in two blocks of 20 slices (slice thickness 2.5 mm, spacing 10 mm) to span the length of the lower leg. The ACSAs were measured at the 70% position for the main dorsi flexor of the foot, tibialis anterior (TA) and the plantar flexors gastrocnemius, considered separately as medialis (GM) and lateralis (GL), and soleus (SOL). Total muscle volume was calculated as the product of the sum of these ACSAs and the distance between the most distal part of the gastrocnemius and the most proximal part of the tibial plateau and expressed as a fractional change. This was found previously to reflect accurately the fractional change in the true muscle volume (Psatha et al. 2012).

Pennation angle in the medial gastrocnemius at the 70% position was measured from a high-resolution $T_1$-weighted spin-echo image sequence orientated perpendicular to the plane of the tibia and fibula. The acquisition matrix was 800 × 722, TR = 493 ms, TE = 12 ms, and the slice thickness was 2 mm with spacing 2.25 mm.

$T_2$ relaxation times were measured in each of TA, GM and SOL from −1/slope of the regression line of the natural log of the signal vs. echo time (TE). Eight spin echoes were recorded (TR = 3000 ms, TE 8 × 10 ms). The acquisition matrix was 200 × 94 (slice thickness 5.0 mm and spacing 5.0 mm). The final scans in an imaging session comprised measurements of $T_2$ in rested and exercised muscle. The exercise protocol consisted of repeated two-legged heel raises performed at a rate of 80 raises per minute. The duration of exercise was determined during the first visit when volunteers exercised until they reached rating 5 on the BORG scale of perceived exertion (Borg 1982) (which corresponds to heavy exercise; at this level the participant finds the exercise hard). This protocol was designed to exercise maximally each individual while taking account of initial differences in the fitness level. On average, the exercise lasted for about 2 min with the subject returning to the scanner within 3–4 min of the exercise ending.

Fractional water contents were calculated in the same ROIs as above using IDEAL (iterative decomposition of water and fat with echo asymmetry and least-squares estimation), which enables fractional fat and water contents to be measured on the assumption that the total signal arises only from fat and water (Glover 1991; Reeder et al. 2005). Results are shown as fractional water (fractional fat is equal to 1 − fractional water).

### Strength training and assessment

Patients undertook an eight-week schedule of supervised strength training of the injured lower leg, with two training sessions a week. A protocol of isokinetic exercise was performed using a KIN-COM dynamometer. Patients lay supine with their leg secured and an additional strap around the waist. The foot of the injured leg was secured to the footplate with the lateral malleolus aligned with the axis of rotation. The footplate was set with a constant lever arm of 21 cm and the resting position was 90°. Participants were asked to perform maximal plantar flexion between 100° and 120° of ankle flexion at an angular speed of 45° $s^{-1}$ and follow it by maximal dorsiflexion to return the ankle to the starting position at a speed of 60° $s^{-1}$. Our pilot experiments showed these slow velocities were better suited for exercising lower leg muscles predominated by slow-contracting type-I fibres compared with 180° $s^{-1}$ which was used in strength training of quadriceps containing equal proportions of type-I and type-II fibres (Johnson et al. 1973; Greenhaff 2006). The exercise protocol comprised six sets of 10 maximal movement repetitions with one-minute recovery between each. All participants were able to perform this exercise even during the initial stages of training after cast removal.

Strength was assessed by measuring the maximum torque at optimal ankle positions for the maximum isometric voluntary contraction (MVC), i.e. 90° for plantar-flexion and 105° for dorsi-flexion in the ankle joint using a constant arm length on the dynamometer of 21 cm (Fukunaga et al. 1996). Participants were instructed to exert their maximum effort for 5 s, first in plantar-flexion followed immediately by dorsi-flexion. This was performed three times at one-minute intervals.

### Statistical analysis

Results are plotted as a function of mean scan day and lateral error bars indicate the standard deviation of the actual scan days, which, especially towards the end of the study, could be taken anything up to three days either side of the designated study day. Study day 5 was taken to be the baseline and total volume and cross-sectional area are plotted as fractional change from baseline as the absolute values clearly depend on the size of each individual. Absolute values are plotted for pennation angle, $T_2$ relaxation time and fractional water content. Data were tested for normality and normally distributed data are shown as mean (standard deviation, SD), otherwise median [25, 75%] is given. Statistically significant differences over time from baseline for cast and contralateral legs separately and the difference between cast and contralateral legs were explored using repeated measures ANOVA. Pairwise comparisons were made using a Holm-Sidak test. Before-and-after comparisons were done using a paired $t$-test. Statistical and plotting software used was Sigmaplot for Windows version 11.0 (Systat Software Inc.).

## Results

From the 205 patients with one lower leg immobilized in a cast invited for screening, 129 met the inclusion criteria, but, of these, 55 were subsequently excluded on the grounds of distance or commitment. Of those remaining, 20 patients agreed to participate and 18 of these completed the cast part study and entered the rehabilitation phase, one of whom was in a cast for shorter than the expected six weeks of immobilization phase. Sixteen patients completed the study (Table 1). Twelve patients had the right and four the left lower leg immobilized. No differences were found between males and females for any of the measures except for the daily increment of strength gain and so data are shown combined unless stated otherwise. Total muscle volume increased following cast removal but still showed a significant deficit of 6% ($p = 0.007$) by the end of the study compared with the day 5 starting value (Figure 1). Similar recovery was seen in each muscle as shown by the separate ACSA values. In the cast leg, TA, GM and GL had recovered to match those in the contralateral leg by study day 71. The exception was SOL, which not only incurred the greatest loss but still had a significant deficit at the end of the study (−5.5% compared with day 5 baseline ($p = 0.01$), −6.8% compared with contralateral ($p < 0.006$) (Figure 2)). The small reduction in ACSA in the contralateral leg appeared to recover rapidly after cast

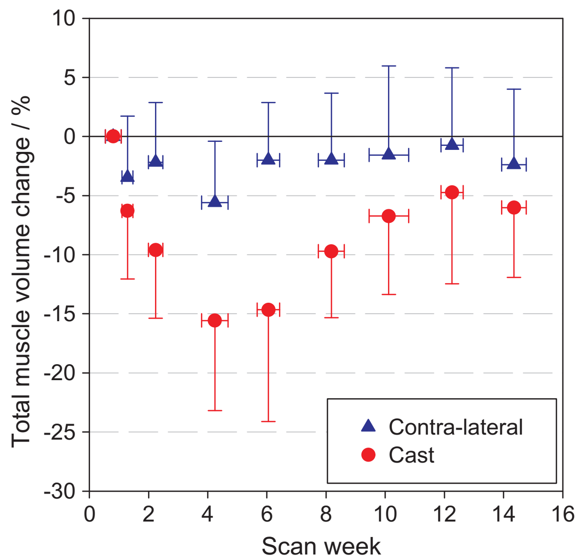


Figure 1. Total muscle volume increased after cast removal at week 6, but in the cast leg was still significantly different from baseline ($p = 0.007$) by the end of the study.

removal but did not return fully to baseline values in gastrocnemii, while SOL and TA both overshot the 5-day baseline; SOL by 2.3 (6.6)% and TA by 6.1 (6.1)%.

Because $T_2$ relaxation times in the contralateral leg remained constant throughout the cast and recovery periods, they were used as a comparison for the cast leg. In the cast leg, following a peak at the end of the cast period, $T_2$ values fell steadily and returned to baseline by week 14 for TA and SOL (Figure 3). In SOL, the difference between cast and contralateral legs was significant (weeks 8 and 10: $p < 0.001$, day 85 $p = 0.042$) until week 14 ($p = 0.12$). $T_2$ values in gastrocnemius, however, continued to rise for a further two weeks after cast removal before starting to return to baseline; the difference between cast and contralateral legs was significant ($p < 0.001$) independently of the study day.

Exercising muscle is expected briefly to increase the $T_2$ relaxation time, but no increase could be detected in TA in either leg (Figure 4). Both GM and SOL showed an increased $T_2$ after exercise. The difference between pre- and post-exercise for SOL did not change with time after cast removal ($p = 0.41$), but the mean increases were significantly different between legs: 1.53 (1.50) ms in the cast leg vs. 2.27 (1.54) ms ($p = 0.022$) in the contralateral leg, representing increases of 3.9 and 6.5%, respectively.

Table 1. Details of the patients who completed the study.

| | Age | Height (cm) | Weight (kg) | Left/right cast |
|---|---|---|---|---|
| Males (7) | 32 [22–39] | 177.7 (4.9) | 81.5 (16.3) | 2/5 |
| Females (9) | 53 [39–73] | 159.8 (9.1) | 72.5 (13.3) | 2/7 |

Note: Data are presented as mean (SD) or [range] as appropriate.

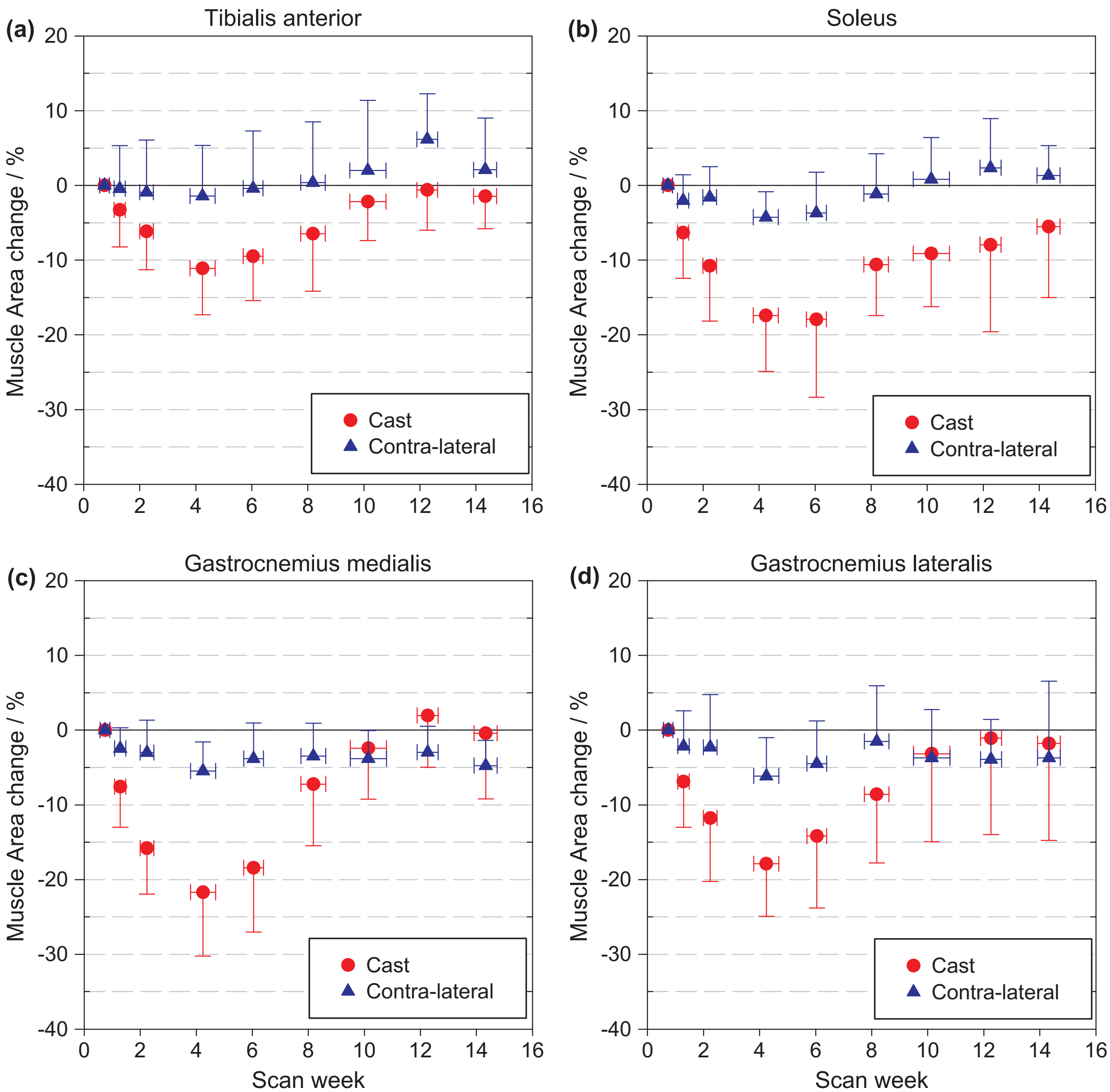


Figure 2. Changes in anatomical cross-sectional area of each muscle during casting and after cast removal normalized to a baseline value on study day 5.
Notes: SOL had the greatest amount to recovery but was still smaller than in the contralateral leg at the end of the study ($p = 0.006$). TA, GM and GL recovered to match those in the contralateral leg by week 10.

GM showed the largest difference between pre- and post-exercise, exercise leading to an increase of 9.1% for the cast leg and 12.1% for the contralateral leg. The increases found between cast (3.6 (1.8) ms) and contralateral (4.3 (2.2) ms) legs were not significantly different ($p = 0.12$).

The angle between the muscle fascicules of GM and the aponeurosis decreased significantly to 20.6° (3.5°) by week 4 in the cast leg ($p < 0.001$), but recovery was rapid following removal of the cast (Figure 5), returning to baseline values within the two weeks following cast removal. The mean value in the contralateral leg was 24.3° (3.6°) and no change was seen over the whole time course.

There was no clear evidence for any change in fractional water content in any muscle over the period of this study (weeks 6–14) (Figure 6). Although the fractional water content of the cast leg was almost always lower than the contralateral leg, this was only significant for SOL ($p < 0.001$) and showed no difference before and after removal of the cast.

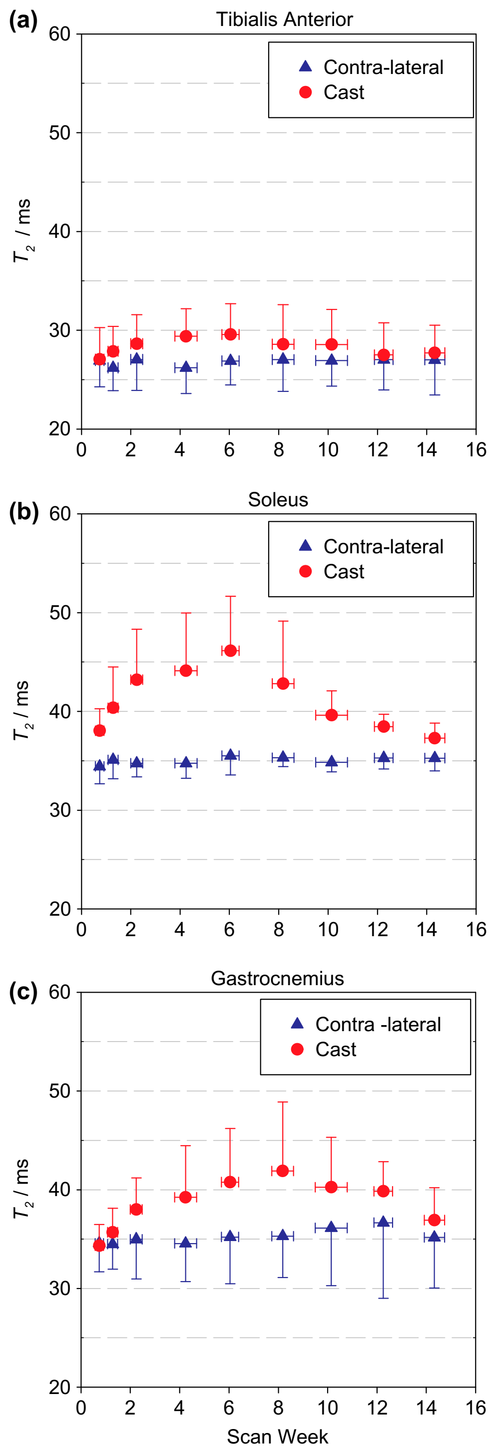


Figure 3. Measured $T_2$ values in three muscle groups.
Notes: Measurements in patients after cast removal (week 6) were taken before exercise. During rehabilitation, differences between cast and contralateral leg in TA were not significant. In SOL, the difference was significant on weeks 8, 10 ($p < 0.001$) and 12 ($p = 0.042$). For gastrocnemius, the difference between cast and contralateral legs was significant ($p < 0.001$) independently of rehabilitation period.

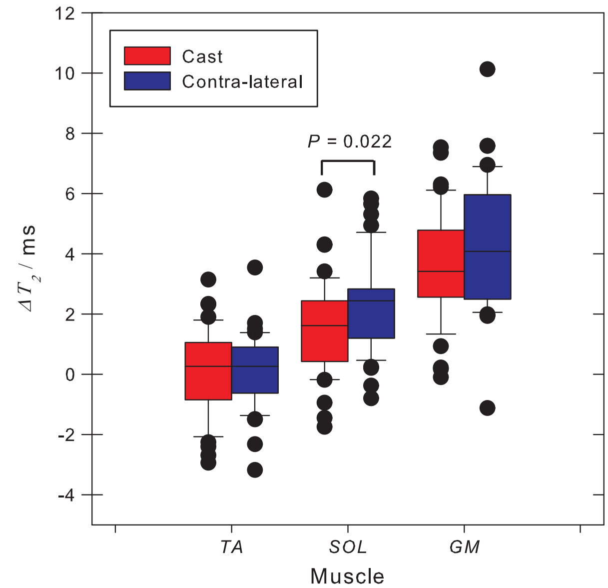


Figure 4. $T_2$ increased after exercise in SOL and GM, but this increase did not alter with time after cast removal.
Note: Only in SOL did the difference between the cast and contra lateral legs reach statistical significance.

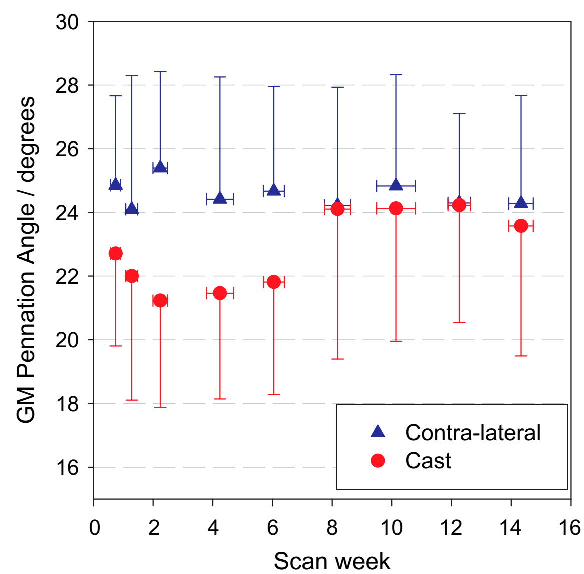


Figure 5. The muscle fibre pennation angle recovered rapidly after cast removal and returned to baseline values within two weeks.

The strength of the patients' lower leg muscles showed a significant deficit after removal of the cast, $p < 0.001$ for both plantar and dorsi flexors, compared with final values measured at week 14; being on average only 61% (13%) of the final value in dorsiflexion and 49% (16%) in plantar-flexion. Both increased significantly

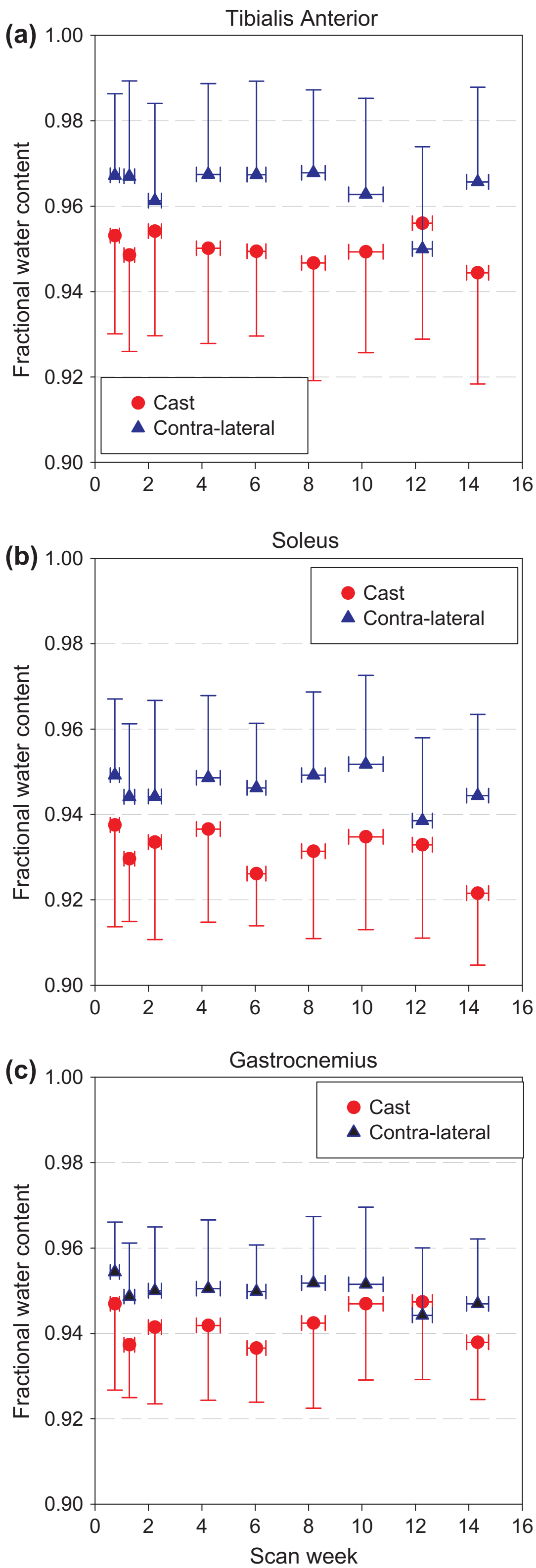


Figure 6. The fractional water content did not change in any muscle over the whole period of the study and cast removal had no effect.
Note: The difference between cast and contralateral legs was only significant for SOL ($p < 0.001$).

during the recovery phase as shown in Figure 7. Linear regression on data from males and females separately enabled calculation of a daily increment from the gradient of the MVC vs. scan day plots for each individual. These showed that there was an average increase in torque generated of 1.10 (0.32) N m day$^{-1}$ in males, 0.74 (0.43) N m day$^{-1}$ in females in plantar-flexion, and 0.36 (0.15) N m day$^{-1}$ in males, 0.28 (0.19) N m day$^{-1}$ in females in dorsi-flexion, although these differences between males and females did not reach significance in either plantar- ($p = 0.092$) or dorsi-flexion ($p = 0.38$). One female showed no improvement over the period of training between days 74 and 130; she could not flex her ankle to 105° at week 6, so no data earlier than week 10 (day 74) are available. Although in absolute terms, male patients thus appeared to regain strength faster than the female patients, expressed as a percentage of final muscle strength, the average daily gain in strength was 0.87% (0.47%) in dorsi-flexion and 1.1% (0.4%) in plantar-flexion in both males and females. The male patients were younger (Table 1) than the females and for the females in dorsi-flexion, there was a significant negative correlation of daily torque increment with age ($r = -0.68$, $p = 0.045$) (Figure 8). A positive correlation was found between muscle cross-sectional area and measured torque for the plantar flexors combined ($r = 0.57$, $p < 0.001$) and for TA ($r = 0.79$, $p < 0.001$). The MVC torque per unit cross-sectional area of muscle increased linearly from 1.41 (0.66) to 2.55 (0.59) N m cm$^{-2}$ in the plantar flexors and 3.78 (0.81) to 5.58 (0.96) N m cm$^{-2}$ in the dorsi flexors over the period from cast off to week 8 of rehabilitation. The ACSA of the dorsi flexor, TA, returned rapidly to baseline values, although dorsi-flexion strength continued to increase throughout the rehabilitation phase.

## Discussion

A number of findings arise from this study. Lower leg muscles showed heterogeneity in the recovery of muscle size during eight weeks of rehabilitation involving strength training. Perhaps not surprisingly, older women recovered more slowly than younger men. Muscles that showed the greatest loss of size during immobilization, such as the soleus muscle, took the longest time to recover. Parameters associated with muscle structural properties, such as $T_2$ relaxation time, appeared to recover before the volume of the muscle was restored.

For this study, we recruited patients attending hospital following a fractured ankle who were treated with a lower leg cast. There was no attempt to match for age, although approximately equal numbers of males and females were sought at recruitment. Overall, most of the factors we measured improved over the course of the rehabilitation period and may represent features that

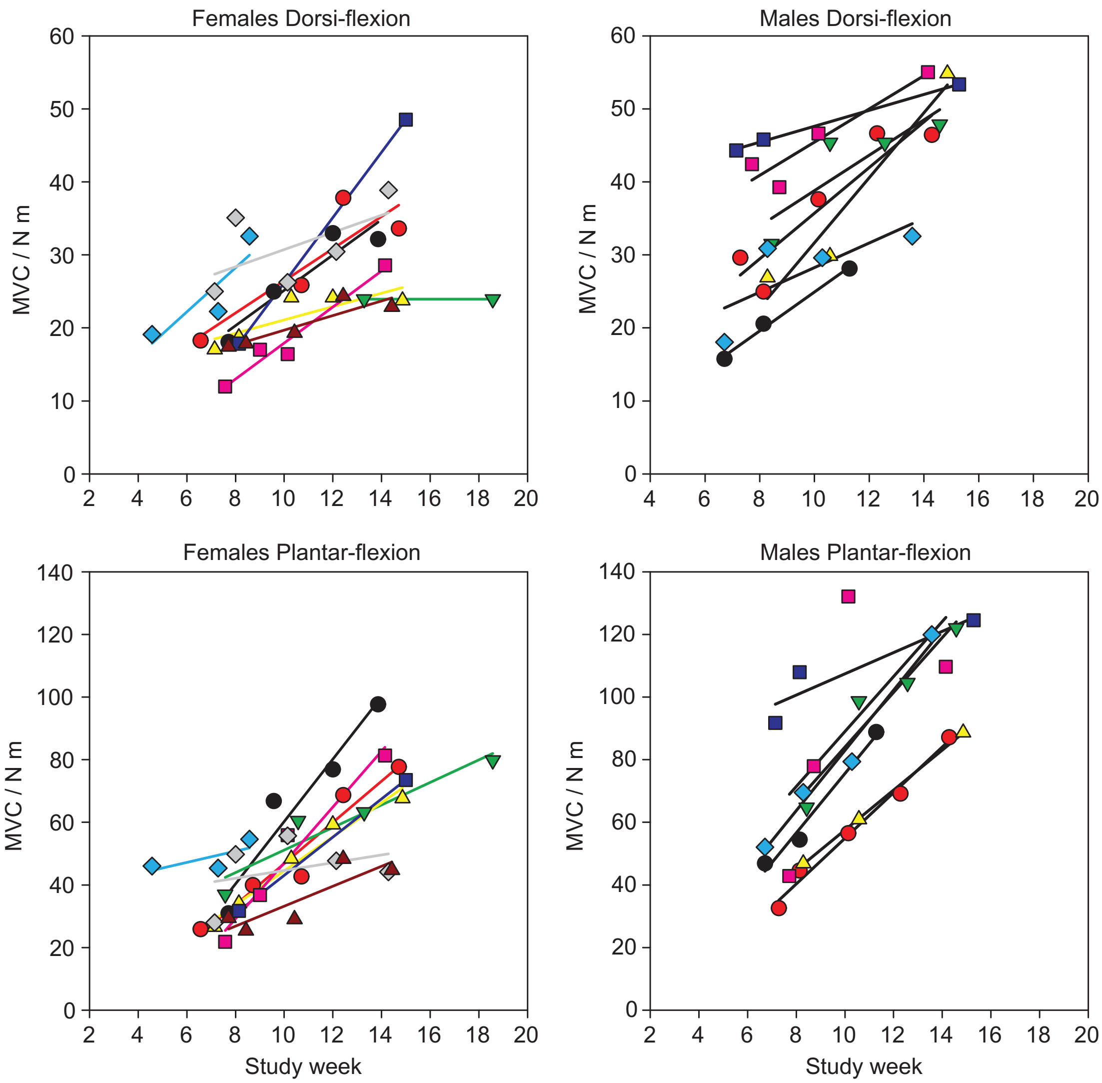


Figure 7. Isometric torque developed in plantar-flexion at 90° and in dorsi-flexion at 105° measured by maximum voluntary contraction (MVC).
Notes: Both increased substantially and significantly during the recovery phase, and linear regression lines are shown for each individual. One female had the cast removed early and another attended late (day 130) for the final session.



could be used as imaging biomarkers to assess muscle quality and quantity. Factors reflecting muscle size and strength, e.g. ACSA, were still changing after eight weeks of rehabilitation, but those more dependent on muscle structure and composition, e.g. $T_2$, had largely returned to baseline values. At the start of the study, we had to take baseline values from scans at five days post-injury due to restrictions imposed by the Ethics Committee on gaining informed consent and the availability of the scanner and the patient. Major changes in size and MRI features may already have occurred by this time, as indicated by the differences between injured and contralateral legs, but data obtained during limb immobilization are included here for completeness to enable these comparisons to be made. The significance of the results from the cast phase, however, is discussed in more detail elsewhere (Psatha et al. 2012). Repeatability was not tested in this study in order not to make further demands on the patients, but measures of $T_2$ and fat/water (Li et al. 2014), ACSA using similar methods (Fortin & Battie 2012), and plantarflexion torque using a Kin-Com dynamometer (Chester et al. 2003) have previously been reported to have excellent reliability (defined as an intra-class correlation coefficient of >0.7).

Limb dominance was not recorded as we believe this to be a secondary effect that our study was not powered to detect. Studies of limb dominance report inconsistent results with some finding a difference in strength of 8.6% between dominant and non-dominant knee extensors (Lanshammar & Ribom 2011) and a statistically

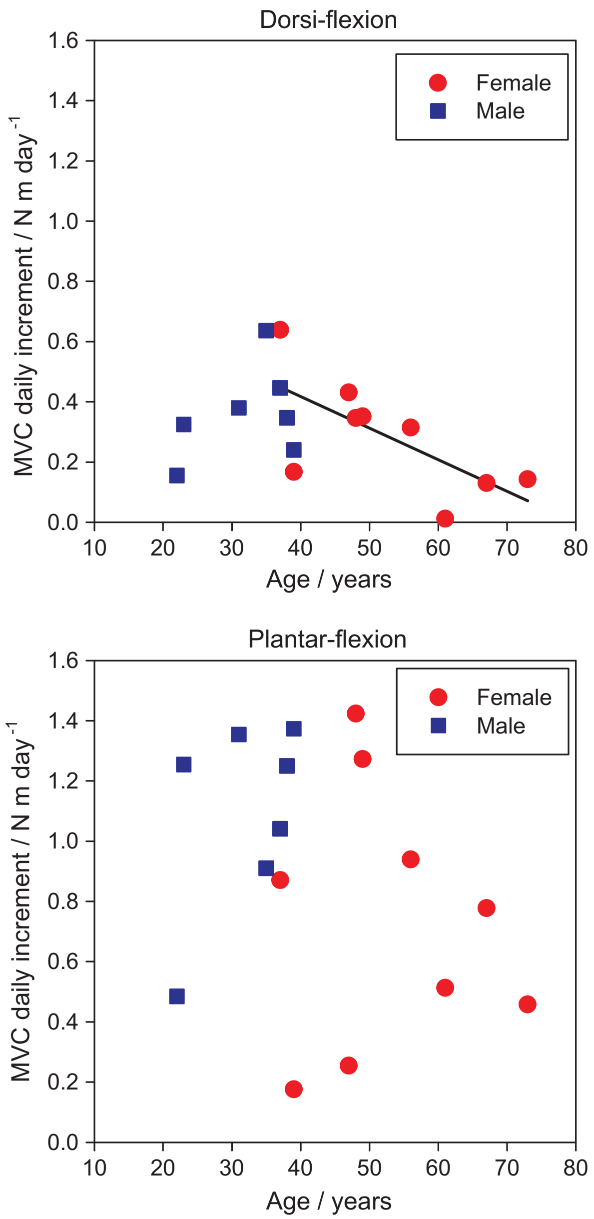


Figure 8. Daily increment in MVC in males and females as a function of age calculated from the gradient of the regression line for each individual in Figure 7.
Note: Males were younger and only in females in dorsi-flexion was there a progressive loss with ageing of the ability to improve muscle strength ($r = -0.68$, $p = 0.045$).

significant, but only 4%, difference in cross-sectional area in medial gastrocnemius in young soccer players (Kearns et al. 2001), while others found no difference in size or physical function (Fahs et al. 2014). It appears that differences may be greater in athletes and in our untrained cohort we would be unlikely to detect any differences.

Muscle volume and its close representative in each separate muscle, the ACSA, were still lower than baseline eight weeks after cast removal. Statistically, this was significant only in soleus, but this is a major plantar flexor of the ankle and may result in a functionally important impairment in muscle function (Ratkevicius et al. 1998). Similar deficits were found by Vandenborne et al. (1998) in a single individual in whom 10 weeks of physical therapy following eight weeks in a cast saw SOL recover to 92% of its initial value, and by Grosset and Onambele-Pearson (2008) again in a single individual following a similar procedure, who recorded deficits of 7% in SOL, 8% in GL and 12% in GM after 10 weeks of physical therapy. A study of a group of 20 individuals undergoing rehabilitation after casting for an ankle fracture showed that muscle hypertrophy was the fastest immediately after cast removal and, although muscle cross-sectional areas had returned almost to normal by the end of the study, there was a still a deficit in plantar specific torque of about −20% (Stevens et al. 2004). Strength recovery in this study was by comparison with a parallel healthy group, whereas we had no baseline values or healthy group; so we cannot determine whether the patients in our study returned to full strength. Our finding of a positive correlation, however, between ACSA and measured plantar-flexion strength indicates that MRI can provide a biomarker for muscle function, and this may be particularly true for longitudinal studies in which progressive changes can then be measured. In dorsi-flexion, muscle strength must arise from factors in addition to muscle cross-sectional area as the ACSA returns to baseline within four weeks of cast removal, whereas strength increases throughout the rehabilitation period in almost all the patients.

Previous studies have found that an increase in pennation angle is involved in muscle hypertrophy (Kawakami et al. 1993). We report here that pennation angles appeared to return within two weeks of cast removal to values comparable with those found in the uninjured leg, which seems overly rapid. A careful comparison, however, with the restoration of gastrocnemius shown in Figure 2(c) suggests that both the ACSA and the pennation angle in GM start to increase before cast removal, presumably due to partial load bearing on the cast leg as the fracture heals. The value of ACSA for GM is not significantly different from the contralateral leg at week 8, so it may be that pennation angle is simply reflecting the overall change in muscle size and architecture occurring over the final period of casting and, more rapidly, following cast removal.

Determining full recovery is difficult. Clearly we could not record strength prior to fracture. The complexity of the study and the demands we were already making on the participants precluded measuring strength in the contralateral leg for comparison. We did, however, find changes in MRI parameters in the contralateral leg, possibly due to disuse soon after fracture and then enhanced use as patients became more mobile but not fully load bearing on the injured leg. This, and possible confounding effects of side dominance, complicate using the contralateral leg as a control. We have also observed a significantly greater increase in muscle strength compared with muscle size during the strength training after immobilization. This is probably associated with an improvement in voluntary activation which can be reduced by more than 40% after lower leg immobilization (Stevens et al. 2006). Thus, changes in muscle strength can be dissociated from changes in muscle size during recovery after ankle injury.

One advantage of MRI is that it can measure chemical and structural changes non-invasively and, by the end of the study, parameters arising from the internal structure of the muscles (pennation angle, $T_2$) had returned to values similar to those in the contralateral leg and/or baseline (Kuno et al. 1990; Hatakenaka et al. 2001; Manini et al. 2007; Marcus et al. 2010; Wall et al. 2015). In a mouse model of reloading following immobilization, $T_2$ values were found to increase during the reloading phase indicating muscle damage (Frimel et al. 2005). This might explain the pattern of increasing $T_2$ values seen in our study in the GM after cast removal. If pennation angle and $T_2$ reflect muscle quality, they suggest that although the muscles may not have recovered their full size, their internal water environment, which gives rise to the MRI signal and reflects something of the structure and composition of the tissue, had recovered essentially to baseline values.

Measurements of $T_2$ are reported to depend on the exercise state of the muscle, increasing with exercise and returning to baseline values with a half-life of around 8 min (Ababneh et al. 2008). This response depends on the dominant fibre type found in the muscle; TA, showed no net response to exercise, whereas soleus and gastrocnemius did show a measurable response. The lack of response of TA may be due to it having too small a change to have been detected. Pereira et al. (2010) found the amplitude of EMG measurements to be lower in TA than gastrocnemius and soleus in heel raising exercises and Segal and Song (2005) found $T_2$ changes in the TA after single-leg heel raises to be significantly less than in gastrocnemius and soleus.

Previous studies have shown that atrophy of the quadriceps through unweighting results in greater than normal $T_2$ changes with exercise (Ploutz-Snyder et al. 1995). In our study, however, $\Delta T_2$ did not change with rehabilitation time after cast removal. A number of issues may underlie this: although the number of heel raises was fixed, the effort was determined by each individual, they may have put in different amounts of effort at each visit as their legs became stronger and that effort may have been shared differently between legs. Whatever the reasons, it does not appear to reflect muscle quality and is unable, therefore, to serve as a biomarker.

Some parameters appeared not to change during training, e.g. fractional water content and $\Delta T_2$. Curiously, fractional water content remained lower than in the contralateral leg throughout the study, but this may indicate that a change occurred very early after fracture. We checked that this was not an artefact of positioning in the scanner by testing for left and right differences but found none, so we are left with a small, albeit mostly non-significant, but oddly consistently one-sided, difference between cast and contralateral legs.

Dynamic exercise resistance training has often been used for strength training of large muscle groups such as knee extensors and flexors (Walker et al. 2014). However, such training is challenging for plantar flexors and extensors when movement isolation as well as control of movement amplitude are required in volunteers after ankle injury. Our results, as well as findings of others (Greenhaff 2006) demonstrate that isokinetic exercise can be an effective means of strength training during recovery from injury. Using the same isokinetic dynamometer for both training and testing minimizes the need for familiarization which would be required for assessment of one repetition maximum.

The male group in this study was younger, reflecting sporting injuries rather than accidents, and although there was no statistical interaction between age and sex, this may explain why the males recovered strength more rapidly than the females, although as a fraction of final strength, which was lower in females, the rate of increase was the same in males and females. Interestingly, in the females in dorsi-flexion, there was a progressive loss of ability to strengthen the muscle with a significant negative correlation between daily strength increment and age.

In conclusion, this study demonstrates significant differences in the patterns of restoration following immobilization among muscles of the lower leg that are detectable using MRI. The measurements reflect both gross morphological changes, such as volume and cross-sectional area, and internal structural features, such as pennation angle and $T_2$ values. The sensitivity of the approach is demonstrated by the detection of changes not only in muscles in the previously immobilized leg but also, to a lesser extent, in the contralateral leg. Full restoration of all features was not found even after eight weeks of rehabilitation including exercise. Thus, MRI may provide a tool to monitor recovery of individual muscle bulk and function. Being non-invasive, it could be used to develop improved rehabilitation exercises directed at individual muscle groups, which may be of particular value in high-performance athletes following injury. New therapies designed to increase speed of recovery following injury could be monitored objectively using this imaging technique.

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The authors have no conflicts of interest to declare.

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